In Vivo Evidence for Cerebral Bioenergetic Abnormalities in Schizophrenia Measured Using $^{31}$P Magnetization Transfer Spectroscopy

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**IMPORTANCE** Abnormalities in neural activity and cerebral bioenergetics have been observed in schizophrenia (SZ). Further defining energy metabolism anomalies would provide crucial information about molecular mechanisms underlying SZ and may be valuable for developing novel treatment strategies.

**OBJECTIVE** To investigate cerebral bioenergetics in SZ via measurement of creatine kinase activity using in vivo $^{31}$P magnetization transfer spectroscopy.

**DESIGN, SETTING, AND PARTICIPANTS** Cross-sectional case-control study in the setting of clinical services and a brain imaging center of an academic psychiatric hospital. Twenty-six participants with chronic SZ (including a subgroup diagnosed as having schizoaffective disorder) and 26 age-matched and sex-matched healthy control subjects (25 usable magnetic resonance spectroscopy data sets from the latter).

**INTERVENTION** $^{31}$P magnetization transfer spectroscopy.

**MAIN OUTCOMES AND MEASURES** The primary outcome measure was the forward rate constant ($k_f$) of the creatine kinase enzyme in the frontal lobe. We also collected independent measures of brain intracellular pH and steady-state metabolite ratios of high-energy phosphate-containing compounds (phosphocreatine and adenosine triphosphate [ATP]), inorganic phosphate, and the 2 membrane phospholipids phosphodiester and phosphomonoester.

**RESULTS** A substantial (22%) and statistically significant ($P = .003$) reduction in creatine kinase $k_f$ was observed in SZ. In addition, intracellular pH was significantly reduced (7.00 in the SZ group vs 7.03 in the control group, $P = .007$) in this condition. The phosphocreatine to ATP ratio, inorganic phosphate to ATP ratio, and phosphomonoester to ATP ratio were not substantially altered in SZ, but a significant ($P = .02$) reduction was found in the phosphodiester to ATP ratio. The abnormalities were similar between SZ and schizoaffective disorder.

**CONCLUSIONS AND RELEVANCE** Using a novel $^{31}$P magnetization transfer magnetic resonance spectroscopy approach, we provide direct and compelling evidence for a specific bioenergetic abnormality in SZ. Reduced $k_f$ of the creatine kinase enzyme is consistent with an abnormality in storage and use of brain energy. The intracellular pH reduction suggests a relative increase in the contribution of glycolysis to ATP synthesis, providing convergent evidence for bioenergetic abnormalities in SZ. The similar phosphocreatine to ATP ratios in SZ and healthy controls suggest that the underlying bioenergetics abnormality is not associated with change in this metabolite ratio.

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Schizophrenia (SZ) is a common and severe brain disorder associated with poor functional outcome. Several lines of evidence suggest that mitochondrial and bioenergetic abnormalities are associated with SZ. These include the following: (1) abnormal levels of metabolites involved in energy metabolism (phosphocreatine [PCr] and adenosine triphosphate [ATP]) reported using $^{31}$P magnetic resonance spectroscopy (MRS), (2) creatine reported using proton MRS, (3) dysfunctional oxidative phosphorylation, and (4) altered mitochondria-related gene expression observed in postmortem studies. Because energy production is essential for numerous metabolic pathways and for neurotransmitter cycling in the brain, abnormalities in these processes will affect all aspects of brain function. In vivo probes of mitochondrial function and energy metabolism would provide crucial information to characterize the exact bioenergetic abnormalities in SZ and delineate their relationship to pathophysiology and symptom formation.

Adenosine triphosphate, a high-energy phosphate (HEP) compound, is essential for all physiological mechanisms that require energy in living tissues. In the human brain, most ATP is used to restore cell membrane ion gradients and to regulate enzyme activity and signaling pathways. Adenosine triphosphate is formed from adenosine diphosphate (ADP) and inorganic phosphate (Pi) in mitochondria primarily through oxidative phosphorylation catalyzed by the enzyme ATP synthase (ATP$_{syn}$). This process is tightly coupled to the reversible creatine kinase (CK) reaction, which transfers HEP moieties from ATP to creatine to generate a storage of HEP bonds in PCr or draws on PCr to restore levels of ATP. Therefore, PCr acts as an HEP reservoir and maintains stable ATP levels during altered neuronal activity. The chemical exchange of phosphate moieties between PCr $\leftrightarrow$ ATP $\leftrightarrow$ Pi has a fundamental role in cerebral bioenergetics and brain function. In principle, these chemical exchange rates can be measured explicitly and noninvasively using in vivo $^{31}$P magnetization transfer spectroscopy ($^{31}$P-MT-MRS). This dynamic MRS approach relies on saturating the signal from one HEP containing metabolite (eg, either PCr or ATP) and observing the loss of signal in the other metabolite with progressive MRS acquisitions. The rate of this signal loss is related to the rate of HEP transfer via the CK reaction. This approach reports the overall CK reaction rate and cannot distinguish CK signal from the mitochondria and cytosol.

Despite suggestions of abnormal mitochondrial and bioenergetic function in the frontal lobe in SZ, CK and ATP$_{syn}$ reaction rates have not previously been measured in this condition in vivo to our knowledge. This approach examines specific biological processes directly involved in bioenergetics, as opposed to generic glucose or oxygen metabolic rates available through other methods such as positron emission tomography. Therefore, the information to be gleaned may be especially relevant to molecular pathophysiology and treatment development for neuropsychiatric conditions. A $^{31}$P-MT-MRS approach was recently implemented on a 4-T magnetic resonance imaging system at McLean Hospital to accomplish this goal. Herein, we report the results of our primary outcome measure in this experiment, the forward rate constant ($k_f$) of the CK enzyme in the frontal lobe in SZ and in age-matched and sex-matched control subjects.

We hypothesized that we would find a reduced CK reaction rate in SZ, consistent with the literature on mitochondrial and bioenergetics abnormalities in this condition. We focused on the prefrontal cortex because this is where most bioenergetic abnormalities in SZ are reported. Although the ATP$_{syn}$ reaction is also of interest, it is more challenging to quantify and we could not measure this reaction reliably in the present clinical study, in which the imaging time was shorter and the volume of interest smaller than in our previous work (F.D. and D.O., unpublished data, May 2013). As part of the $^{31}$P-MT-MRS experiment, we also measured intracellular pH, magnesium ion concentration, intrinsic longitudinal $T_1$ relaxation time in the absence of chemical exchange of PCr, and steady-state ratios of HEP-containing metabolites as secondary measures. We hypothesized that we would see a reduction in pH reflecting elevated lactic acid levels due to higher relative glycolysis rates compensating for bioenergetic dysfunction. We could not entertain a directional hypothesis on metabolite ratio levels because of discrepancies in the past literature.

**Methods**

**Participants**

The eMethods in the Supplement provide details of our institutional review board-approved human participants procedures. Informed consent was obtained from all participants at study entry. Table 1 summarizes their characteristics.

**Magnetic Resonance Imaging and In Vivo $^{31}$P-MT-MRS Experiments**

The diagnostic imaging was performed in a 3-T system (Trion; Siemens); details have been published previously. All $^{31}$P-MT-MRS study-related acquisitions were conducted using a 4-T whole-body imaging system (Unity/Inova; Varian NMR Instruments). Brain anatomic imaging and $^{31}$P-MT-MRS were acquired by a specially designed half-helmet head coil with dual-tuned frequency channels (proton quadrature surface coil and phosphate 7-cm surface coil) placed on the forehead. Each channel has independent transmission and receiver functions with dedicated decoupling.

A rapid 2-dimensional gradient-recalled echo image was initially used to acquire single images in 3 dimensions. This permitted rapid determination of the position of the participant; the individual was repositioned if necessary. Manual global shimming of unsuppressed water signal was then undertaken, yielding a global water linewidth of 24 Hz or less. High-contrast $T_2$-weighted sagittal and axial images were acquired to serve as an anatomical guide to position MRS voxels. Localized shimming with a voxel of $6 \times 6 \times 4$ cm$^3$ on the prefrontal lobe was performed manually to further minimize local field inhomogeneity for $^{31}$P-MT-MRS.
Table 1. Demographic and Clinical Characteristics of the Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HC (n = 26)</th>
<th>SZ (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>31.9 (8.9)</td>
<td>34.5 (8.4)</td>
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<td>Sex, No.</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>14</td>
<td>13</td>
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<tr>
<td>Female</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
<td>24.4 (3.7)</td>
<td>28.5 (4.8)</td>
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<tr>
<td>Education, mean (SD), y</td>
<td>6.5 (1.8)</td>
<td>5.1 (1.5)</td>
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<tr>
<td>Parental socioeconomic status, mean (SD)</td>
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<td>6.1 (2.3)</td>
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<tr>
<td>Age at onset, mean (SD)</td>
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<tr>
<td>Lifetime No. of suicide attempts, mean (SD)</td>
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<tr>
<td>Lifetime hospitalizations, mean (SD), No.</td>
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<td>5.5 (3.9)</td>
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<td>Test score, mean (SD)</td>
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<td>Montgomery-Åsberg Depression Rating Scale</td>
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<tr>
<td>Young Mania Rating Scale</td>
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<td>9.2 (6.0)</td>
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<tr>
<td>Multnomah Community Ability Scale</td>
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<td>Positive and Negative Syndrome Scale</td>
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<tr>
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<td>Chlorpromazine equivalent, mean (SD), mg/d</td>
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<tr>
<td>Benzodiazepine use, No.</td>
<td>...</td>
<td>11</td>
</tr>
</tbody>
</table>

Abbreviations: HC, healthy control; SZ, schizophrenia; ellipsis, not applicable.

* P = .27.
* * P = .077, P = .78.
* Calculated as weight in kilograms divided by height in meters squared (P = .001).
* Range 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) (P = .005).
* Calculated according to the Hollingshead Scale (P = .82).
* P = .36.

The 31P signal was acquired using a 31P surface coil with outer-volume saturation (Figure 1). The 31P-MT pulse sequence and experimental design have been described previously, and additional information is given in the eMethods in the Supplement.

31P Spectrum Processing and Quantification of pH

The 31P spectra were analyzed in the time domain using the AMARES algorithm within a software package (jMRUI; http://mrui.uab.es/mrui/). Details are given in the eMethods in the Supplement. Brain pH was estimated based on the chemical shift difference in parts per million between Pi and PCr.
rium, respectively. $T_1$ is the intrinsic spin-lattice relaxation time of PCr. Therefore, the $k_f$ of the CK reaction and the $T_1$ of PCr can be determined by fitting the experimental data to a single exponential decay. Seven saturation time points (0, 0.48, 1.89, 3.78, 6.61, 8.50, and 12.28 seconds) were applied in the present study. Last, the chemical reaction flux ($F$) is calculated by equation 5, where $[M]$ is the metabolite concentration (in micromoles per milliliter) of PCr, determined via the PCr:β-ATP ratio assuming the ATP concentration is 3.0 mM.29 In this instance, we assumed a fixed ATP concentration. Everywhere else herein, we have used metabolite ratios instead. The chemical reaction fluxes were converted to the well-accepted units of micromoles per gram per minute using an assumed brain tissue density of 1.1 g/mL.17–18

Statistical Analysis
The eMethods in the Supplement provide details of our statistical approach. All analyses were performed using commercially available software (SPSS version 17; SPSS, Inc).

Results
Forward Rate Constants and Fluxes
The mean (SD) quality control measure of linewidth (in hertz) of the PCr resonance with 10-Hz line broadening in the magnetization transfer experiment did not differ for the first or last acquired spectra between the healthy control (HC) and SZ groups (19.2 [1.8] and 20.8 [5.7], respectively, $P = .17$; and 20.3 [2.2] and 22.0 [5.8], respectively, $P = .16$). Likewise, the mean (SD) PCr signal-to-noise ratio (SNR) did not differ between the SZ and HC groups (16.4 [7.6] and 19.2 [6.6], respectively, $P = .20$). The principle of the magnetization transfer experiment is shown in Figure 2. The intrinsic $T_1$, $k_f$, and flux parameters were determined as previously described (Table 2). The $F$ in the present study did not differ between the SZ and HC groups (16.4 [7.6] and 19.2 [6.6], respectively, $P = .20$).

The spectra on the top and bottom rows were acquired from a representative patient in the schizophrenia (SZ) group and a participant in the healthy control (HC) group, respectively. The saturation time was 12.28 seconds. All resonance peaks are labeled in the lowest spectrum. The magnetization of phosphocreatine (PCr) was reduced by 43% and 56% for the SZ and HC participants, respectively. The pH was calculated via equation 6, where $\delta$ is the distance between the chemical shifts of PCr and inorganic phosphate (Pi). This distance is different in the SZ and HC participants (aligned by vertical lines across the 2 spectra), indicating intracellular pH reduction in SZ. GPC indicates glycerophosphocholine; GPE, glycerophosphoethanolamine; and PME, phosphomonoester.
in this measure was observed between patients diagnosed as having SZ vs schizoaffective disorder \((P = .19)\). We also calculated the flux through this reaction, although this was not our primary measure. This parameter was decreased by 19% in SZ \((F_{50,4} = 6.264, P = .007)\) (Table 2). This reduction of 0.03 pH units corresponds to an elevation of about 7% in proton concentration.

**Phosphate Metabolite Ratios**

The ratios of metabolites to ATP are given in Table 2. We reported metabolite-level results using \(\beta\)-ATP as an internal reference to the control for participant-specific sources of variance. No between-group differences were observed in any metabolite ratio except for the significant reduction in the ratio of phosphodiester (PDE, which includes GPC and GPE) to \(\beta\)-ATP \((F_{50,4} = 6.348, P = .02)\) in the SZ group compared with the HC group. In addition, magnesium ion concentrations, deduced from the chemical shift of \(\beta\)-ATP, were calculated and were similar in the SZ and HC groups (Table 2).

**Additional Analyses**

No significant correlations were found between spectroscopic parameters and demographic variables for both groups or among clinical variables for the patient group. The negative correlation between BMI and intracellular pH approached the significance threshold \((R = −0.496)\) when including both groups. Therefore, BMI was included as a covariate in the model involving intracellular pH, as described above. In addition, we created a correlation matrix between the independent parameters \(k_f\), intracellular pH, and the 4 metabolite ratios given in Table 2 but found only one correlation with \(R > 0.5\) in this matrix in either the patient or control groups. Specifically, a negative correlation was found between intracellular pH and the PCr:γ-ATP ratio in the HC group \((R = −0.583)\) but not in the SZ group \((R = 0.100)\) (Figure 4).

**Discussion**

Using a novel \(^{31}\)P-MT-MRS approach, we report abnormalities in the reaction rate and flux through the CK enzyme system in chronically ill patients with SZ compared with matched HCs. We also report a reduction in intracellular pH in the same patients, suggesting a relative increase in the contribution of glycolysis to ATP synthesis, with resultant buildup of lactic acid. Most important, we do not see a change in relative PCr or ATP levels (quantified as PCr:Pi, ATP:Pi, ATP to phosphomonoester [PME], and PCr:PME ratios). This is important because changes in enzyme reaction rate are not necessarily accompanied by changes in levels of substrate or product. Therefore, the reaction rate measure provides an additional, complementary approach.

This study is a direct in vivo demonstration of bioenergetic abnormalities in SZ. The subgroups of patients with SZ and schizoaffective disorder had comparable abnormalities, suggesting no diagnostic specificity to our findings for these 2 conditions. In the healthy brain, energy use primarily supports glutamatergic neurotransmitter cycling.\(^{31}\) Therefore, bioenergetic abnormalities in SZ are likely to have implications for neuronal and circuit activity. Our present work does not provide information about brain regions other than the prefrontal cortex nor about specific contributions from white matter or gray matter. With future technical improvements (eg, localization to smaller voxels to enable white matter–dominant or gray matter–dominant voxels, as well as chemical shift imaging to collect data from other brain regions\(^{32}\)), we expect to probe these issues more deeply. Because the present data come from both white matter and gray matter, we expect that abnormalities may exist in both.

Mitochondrial and other bioenergetic abnormalities have been suggested by previous genetic, postmortem, and neuro-
imaging studies provide strong and converging evidence in this regard. However, the exact abnormalities are uncertain, and discrepancies exist among neuroimaging studies in the literature. For example, elevations in ATP levels have been reported in drug-naive first-episode SZ, but other investigators have found no significant difference. Likewise, reported PCR levels have been variable, with both increases and no change being observed. Alterations in PME and PDE in SZ are also debated, because these 2 metabolites are precursors and breakdown products of cell membrane metabolism, respectively, they may also reflect bioenergetic abnormalities related to cell membrane metabolism. Freely mobile PME levels may be reduced or normal in chronic and first-episode patients with SZ, while PDE levels may be elevated or normal in the same groups. Most studies report reduced PME and elevated PDE in SZ, consistent with accelerated phospholipid metabolism in this condition. The discrepancies likely arise because of differences in MRS methods, study participant selection, phases of illness, and medication regimens.

**CK Reaction**

The 31P MRS studies of bioenergetic dysfunction in neuropsychiatric disorders, including SZ, typically measure steady-state levels of HEP metabolites. By contrast, we assessed the reaction rate for a key enzyme in bioenergetics in the present study. This approach may focus attention on specific molecular targets and processes in the pathophysiology of SZ that may lead to the development of new treatment interventions. On the other hand, the MRS signal we used cannot pinpoint CK abnormalities in mitochondria vs cytosol, and subcellular localization needs to be probed further in future work. In addition, the relationship between forward and reverse reaction rates and between the CK reaction and other systems may complicate interpretation of our findings. It is reassuring to note that previous work showed that the CK and adenosine triphosphatase reactions are in approximate equilibrium in the human brain. In addition, the adenosine triphosphatase reaction rate is approximately equal to the ATP oxidative synthesis rate. Finally, the CK and adenosine triphosphatase reaction rates are correlated with brain activity levels across a wide range. These findings suggest that our MRS measures reflect meaningful indexes of brain activity at a “macro” level. Our finding of reduced CK k_f suggests that the machinery of energy metabolism is dysfunctional in SZ. Therefore, ATP availability might be compromised, especially at times of high demand, such as during brain activation. The hypothesis of a breakdown in energy production in SZ is testable because the 31P-MT-MRS approach can be coupled with sensory or cognitive stimulation paradigms or neuromodulation therapies, such as transcranial magnetic stimulation.

What explains the 22% reduction in the k_f of CK in SZ? As described in equation 2, this parameter is determined by proton and ADP concentrations, as well as a constant k, which describes the intrinsic activity of the CK enzyme. The reduction of 0.03 pH units we observe in SZ corresponds to a 7% elevation in proton concentration, which could account for about one-third of the CK enzyme abnormality. The concentration of ADP (typically reported as 0.3 mM) is too low to be measured directly. However, β-ADP makes a minor contribution to the γ-ATP resonance we quantified. Therefore, substantial changes in β-ADP would be reflected as minor differences in calculated γ-ATP and β-ATP levels (these 2 would normally be identical). However, we did not observe any difference between the PDE:γ-ATP ratio vs the PDE:β-ATP ratio or any of the other metabolite:γ-ATP ratios vs metabolite:β-ATP ratios. Therefore, we suggest that abnormal CK enzyme activity in SZ may at least partially be a result of alterations in k, the constant reflecting enzyme concentration or molecular structure. This conclusion is supported by several lines of evidence: postmortem studies have identified abnormalities in CK enzyme activity, as well as oxidative phosphorylation and mitochondria-related genes and gene expression, in SZ. Taken together, this is a picture of an underlying failure of energy production in SZ.

One potential shortcoming of this framework is that reductions in CK k_f may be compensated for by other sys-
tems (eg, glycolysis and the adenylate cyclase reaction \([2\text{ADP} \leftrightarrow \text{ATP} + \text{adenosine monophosphate}]\)). We cannot rule this out in the present work, and future studies may be needed to identify whether compensation is taking place.

**pH Findings**

Furthermore, the observation of reduction in intracellular pH in the present study is consistent with bioenergetic abnormalities in SZ. Most important, intracellular pH was correlated negatively with BMI in our study. This relationship suggests that abnormal peripheral metabolism (manifesting as elevated BMI) may in fact be associated with abnormal brain metabolism (manifesting as reduced intracellular pH). Parallel evidence supports this intriguing finding from other systems.\(^{49}\) This notable result needs to be pursued in future studies. Because BMI differed significantly between the SZ and HC groups in this study, we added it to our analyses as a co- variate. The reduced intracellular pH in the SZ group remained significant even after adjusting for BMI.

Reduced intracellular pH indicates that oxidative phosphorylation is compromised in SZ, leading to a relative increase in the contribution of glycolysis to ATP synthesis, with subsequent buildup of lactic acid.\(^{51}\) Intracellular pH is independent of the CK reaction; therefore, this finding suggests that bioenergetic abnormalities are widespread in SZ. One intriguing suggestion is the coupling between glycolysis and the adenylate cyclase reaction (\(2\text{ADP} \leftrightarrow \text{ATP} + \text{adenosine monophosphate}\)) that is upregulated when the CK reaction and oxidative phosphorylation are failing.\(^{54}\) Because HEP metabolite ratios were normal in the present study, this pattern suggests that despite a higher reliance on glycolysis, a less efficient means of energy production, the brain is able to maintain baseline levels of important metabolites in SZ. Our observation of reduced intracellular pH agrees with a prior study\(^{55}\) of elevated cerebrospinal fluid lactic acid in SZ; however, several other groups have reported decreased,\(^{56,57}\) elevated,\(^{58,59}\) or normal\(^{54,58}\) pH in SZ in the prefrontal cortex or other brain regions. In the present study, we also observed a negative correlation of intracellular pH with the ratio of PCr to \(\gamma\)-ATP in the HC group but not in the SZ group. This correlation would be expected on the basis of equation 3. The fact that it is not found in SZ may suggest subtle abnormalities in data quality and measurement error in this group or perhaps an abnormal \(K_{\text{syn}}\) for the CK reaction.

**Metabolite Levels**

The only statistically significant change we observed in metabolite ratios was a reduction in the PDE:ATP ratio in SZ (approximately 12%). The magnitude of this reduction was smaller than that of abnormalities in CK \(K_a\) and intracellular pH, and it was not accompanied by changes in PME. In addition, most previous studies report PDE elevations, not reductions. This pattern has led to the proposal of an accelerated phospholipid metabolism hypothesis in SZ because PDE is a breakdown product of membrane phospholipids. Our finding of reduced PDE without a change in PME is not consistent with this literature, although it is not the first to be discrepant.\(^{59}\) Reductions in PDE in this study may arise from global atrophy in SZ or from changes in gray matter and white matter composition in the voxel of interest. In vivo \(^{31}\)P-MT-MRS provides an attractive noninvasive approach for directly studying bioenergetics and mitochondrial function associated with brain activity changes.\(^{16-18,60}\) However, low SNR is a limitation of this approach because of the intrinsically low nuclear gyromagnetic ratio of \(^{31}\)P and the low concentration of some of the metabolites studied (eg, Pi at approximately 1mM). Therefore, we had to collect data from a large brain region using a dedicated surface coil to achieve sufficient SNR. As shown in Figure 2, most acquired signal comes from a \(6 \times 6 \times 4\) cm\(^3\) region in the frontal lobes. Outer-volume suppression ensured exclusion of signal from HEP-rich extra-cranial muscle. It was previously shown that rates for the CK and ATP\(_{\text{syn}}\) or adenosine triphosphatase reactions can be calculated in the human frontal lobe noninvasively at 4 T.\(^{20}\) However, we found in this study that we could not achieve sufficient SNR to quantify the less sensitive ATP\(_{\text{syn}}\) reaction because of smaller voxels and short imaging times. In addition, we do not have interassay reliability calculations available.

A related limitation is the long acquisition time. Current measurements were performed with a 14-second repetition time at approximately fully relaxed conditions to minimize the confounding effects of \(B_1\) inhomogeneity of a \(^{31}\)P surface coil. Some novel \(^{31}\)P-MT-MRS approaches, such as 4-angle saturation transfer and triple repetition time saturation transfer, aim to measure the same chemical reaction fluxes.\(^{61,62}\) Recently, a novel \(^{31}\)P-MT- MRS approach (\(T_1^{*}\) maps) was developed, aimed at rapidly mapping energy-ATP metabolic fluxes.\(^{53,64}\) Using this approach, only 2 spectra are needed to calculate CK and ATP\(_{\text{syn}}\) reaction rates, as long as the intrinsic \(T_1\) relaxation time of PCr is known and is constant across groups and times. Acquisition time is significantly shorter with this approach, enabling improved SNR, increased spatial and temporal resolution, and greater reliability.\(^{60,64}\) In the present work, we showed that the intrinsic \(T_1\) relaxation time is not apparently different between patients with SZ and HCs, laying the groundwork for rapid-acquisition \(^{31}\)P-MT-MRS in future studies.

A related limitation is the fact that we did not correct the results for voxel composition of gray matter and white matter because of our use of a surface coil for excitation. The resulting \(B_1\) inhomogeneity creates uncertainty as to where in the region the optimized 90° flip angle is found, leading to differential contribution of signal from different regions. However, this does not affect our measurement of the CK kinetics and relative metabolite ratios because \(^{31}\)P data were collected at fully relaxed condition. All signal acquired from regions within our volume of interest will follow the same decay curve with increased saturation time because this does not depend on absolute signal intensity. Likewise, the peak area ratios will be the same from all different regions at fully relaxed condition. Calculating contributions from each region within the volume of interest (\(6 \times 6 \times 4\) cm\(^3\)) to each of the spectra is possible from our measured \(^{31}\)P sensitivity 3-dimensional profiles. However, further voxel segmentation would be complicated because imaging was acquired by proton quadrature surface coil. While it is possible to calculate signal contribution from inhomogeneous fields,\(^{65,66}\) we would need to compile data acquired using 2 different coils for our purposes, and this has not been previously validated to date. An added complication is the loss of both gray matter and
white matter in SZ, which would lead to unpredictable voxel compositions. Loss of gray matter may have more influence on our findings, but given the current limitations we are not able to ascertain the specific effect from each tissue type.

Another limitation of the study is that most patients with SZ in this study were taking medication. We cannot exclude the possibility that our results are secondary to medication effects. Future studies with adequate-sized groups of patients taking and not taking medication are needed to fully address this issue. However, we did not observe correlations between any of our neuroimaging measures and chlorpromazine equivalents. In addition, the effects of antipsychotic medication on brain bioenergetics are complex and dependent on the type of medication. The eMethods in the Supplement give a detailed discussion of this important topic.

One final limitation is that we report metabolite concentration ratios, not absolute metabolite concentrations, which could be obtained using an external reference with known concentration and matched electromagnetic properties with the human brain. However, this is time consuming and can be confounded by factors such as radiofrequency coil loading, especially at high field. We chose a more reliable and popularly used method, internal referencing. We considered reporting ratios of PCr and of ATP to total 31P signal, but we chose ATP ratios because we were particularly interested in the more biologically relevant PCr:ATP and Pi:ATP ratios. However, this approach meant that we did not separately examine ATP concentrations in patients with SZ and in HCs in this study. This is not ideal because ATP itself is a HEP, and there is concern that ATP may be abnormal in conditions of bioenergetic compromise. Still, the pattern of our findings (no abnormalities in PCr:ATP, Pi:ATP, or PME:ATP ratios, accompanied by a reduced PDE: ATP ratio in SZ) suggests no major abnormalities in ATP. The literature is conflicting on this point, and additional studies dedicated to quantifying ATP concentrations are needed to settle the issue. This is relevant only for metabolite concentration quantification and not for CK $K_p$, intracellular pH, and $T_1$ relaxation time calculations.

In summary, we measured CK reaction rates, intracellular pH, and HEP metabolite levels in the human frontal lobe in patients with SZ and HCs using a novel 31P-MR spectroscopy approach. The forward rate constant and flux for the CK enzyme were significantly reduced, as was intracellular pH in patients with SZ, but no metabolite concentration ratio abnormalities were observed except for a modest reduction in PDE. Our findings suggest abnormalities in the CK reaction and in glycolysis in SZ, and these processes may underlie abnormal neurotransmission and information processing in this disorder.
Schizophrenia Cerebral Bioenergetic Abnormalities

Original Investigation Research


