

In vivo Evidence for Abnormal Cerebral Bioenergetics in Schizophrenia Measured by ^{31}P Magnetization Transfer Spectroscopy

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Introduction: 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 schizophrenia (1). These include abnormal concentrations of metabolites involved in energy metabolism reported using ^1H - and ^{31}P -magnetic resonance spectroscopy (MRS) (2,3) and dysfunctional oxidative phosphorylation (4) as well as altered mitochondria related gene expression (5) observed in *postmortem* studies. Since mitochondrial energy production is essential for numerous metabolic pathways and for neurotransmitter cycling in the brain, abnormalities in these processes will impact 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. In addition, mitochondrial physiology may provide novel targets for drug development in SZ. Despite suggestions of abnormal mitochondrial and bioenergetic function in the frontal lobe in SZ, creatine kinase (CK) reaction rates have not previously been measured in this condition *in vivo*. We recently implemented the ^{31}P -magnetization transfer (^{31}P -MT) approach on a 4T MRI scanner at our center to accomplish this goal (6). Here, we report the results of our primary measure in this experiment, the CK reaction rate in the human frontal lobe in SZ and age- and sex- matched controls.

Methods: Twenty-six age and sex-matched healthy controls (HC) without any psychiatric or substance use disorders, (14 males and 12 females, 31.9 ± 8.9 years old) and participants with SZ (13 males and 13 females, 34.5 ± 8.4 years old) from the clinical services at McLean Hospital were recruited for these studies. All subjects provided informed consent, and the study procedure was approved by the McLean Hospital Institutional Review Board. HC and SZ participants were screened using a series of standard psychiatric diagnoses and research procedures consisted of consent procedures; a standard clinical evaluation using the SCID-IV; urine toxicology screen; urine pregnancy test, as necessary; The following standardized scales were administered for SZ patients: Positive and Negative Syndrome Scale (PANSS); Young Mania Rating Scale (YMRS); Montgomery-Asberg Depression Rating Scale (MADRS); Edinburgh Handedness inventory; Multnomah Community Ability Scale (MCAS); North American Adult Reading Test (NAART – a putative measure of premorbid IQ); and Fagerstrom Test for Nicotine Dependence. Body mass index (BMI) and educational level were also collected for all subjects as were lifetime number of suicide attempts and hospitalizations for patients. All ^{31}P -MT-MRS study related acquisitions were conducted using a 4T whole-body scanner interfaced with a Varian INOVA console. Brain anatomic imaging and ^{31}P -MRS (see Figs.1~2) were acquired by a specially designed half-helmet head coil with dual-tuned frequency channels. The forward chemical exchange constant (k_f) of creatine kinase (CK) and metabolites concentrations from frontal lobe ($6 \times 6 \times 4 \text{cm}^3$) were measured by ^{31}P -MT approach which has been described in previous publications (6, 7).

Results: There was a substantial (22%) and statistically significant ($p=0.003$) reduction in CK k_f in SZ (0.21 ± 0.07 vs 0.27 ± 0.06). In addition, intracellular pH was significantly reduced (7.00 ± 0.02 vs 7.03 ± 0.01 ; $p=0.007$) in this condition. The concentrations of most phosphate-containing compounds were not substantially altered in SZ, with the exception of a reduction in the PDE indicated by the PDE/ β -ATP ratio (0.91 ± 0.19 vs 1.05 ± 0.19 ; $p=0.015$).

Discussion and Conclusion: Using a novel ^{31}P MRS approach, we provide first direct and compelling *in vivo* evidence for a specific bioenergetic abnormality in SZ. Reduced k_f of the CK enzyme is consistent with an abnormality in ATP production at times of high demand since this enzyme transfers high energy phosphates from storage in PCr to ATP to preserve ATP levels needed for enzymatic reactions. The intracellular pH reduction suggests a shift from oxidative phosphorylation towards glycolysis, providing convergent evidence for bioenergetic abnormalities in SZ. Our findings are supported by several lines of evidence: *postmortem* studies have identified abnormalities in CK enzyme activity (8) as well as oxidative phosphorylation (4) and mitochondria related genes and gene expression (5) in SZ. Taken together, this is a picture of an underlying failure of energy production in schizophrenia.

The relative steady state concentration of ATP and PCr (PCr/ATP ratio) was similar in SZ and healthy subjects, indicating that this is not sensitive measure of the underlying abnormality. And our finding of reduced CK k_f with the relative stable concentrations of HEP at baseline suggests that the machinery of energy metabolism is dysfunctional in SZ but that energy production at baseline is probably sufficiently compensated to approximate healthy levels. But at times of high demand, ATP availability might be compromised. This interpretation is broadly consistent with our previous work, in which we observed that ATP and PCr concentrations were relatively stable with varying depths of anesthesia and changed only in extreme conditions such as ischemia, but CK and $\text{ATP}_{\text{syn}} k_f$ were tightly correlated with brain activity changes (altered depth of anesthesia). The hypothesis of a breakdown in energy production in schizophrenia during times of high demand is testable since the ^{31}P -MRS approach can be coupled with sensory or cognitive stimulation paradigms or neuromodulation therapies such as transcranial magnetic stimulation.

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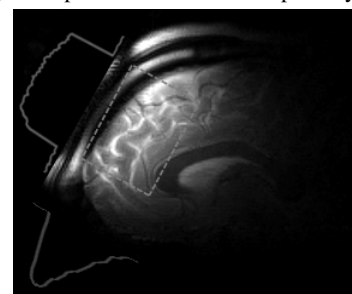


Fig1. T₂-weighted brain anatomic imaging and the sensitivity profile ($6 \times 6 \times 4 \text{cm}^3$) with OVS of the 7-cm ^{31}P surface coil placed over the forehead.

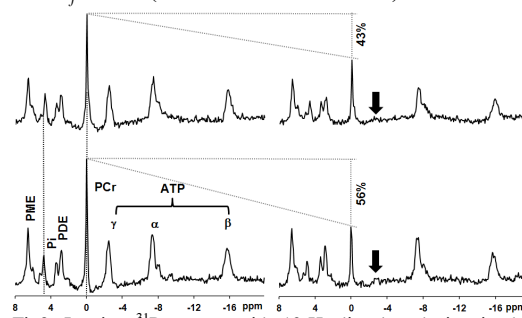


Fig2. *In vivo* ^{31}P spectra with 10-Hz line broadening in the absence and presence of saturating γ -ATP resonance (illustrated by the back arrows) demonstrated in left and right columns, respectively. The spectra on top and bottom rows were acquired from a representative SZ and HC subject, respectively. Saturation time was 12.28 s. All resonance peaks are labeled in the lowest spectrum. The magnetization of PCr was reduced by 56% and 43% for the HC and SZ subject, respectively, indicating a slower chemical cycling rate of CK reaction in SZ. Also note the differential distance between the chemical shifts of PCr and Pi in the SZ and HC groups (aligned by vertical lines across the two spectra), indicating pH reduction in SZ.