Magnetic Resonance Imaging Volumetric and Phosphorus 31 Magnetic Resonance Spectroscopy Measurements in Schizophrenia

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The purpose of this study was to examine the relationship between phosphorus magnetic resonance spectroscopy (^{31}P MRS) parameters and left prefrontal volumes in both patients with schizophrenia and healthy subjects. ^{31}P MRS parameters and magnetic resonance imaging (MRI) volumetric data were collected in the left prefrontal region in 10 patients with schizophrenia and 10 healthy subjects of comparable age, handedness, sex, educational level, and parental educational level. No correlations were found between any MRS parameter and grey matter volumes in the combined subjects. Phosphomonoester (PME) and grey matter volumes, however, were both correlated negatively with age. PMEs were found to be decreased, and calculated intracellular magnesium ($[Mg^{2+}]_{intra}$) was found to be increased in the patients with schizophrenia compared with healthy subjects after adjusting for left prefrontal grey and white matter, total brain volume, and age. These findings suggest that cortical grey and white matter volumes are not directly related to PME and $[Mg^{2+}]_{intra}$ abnormalities in schizophrenia patients.

Key Words: schizophrenia, magnetic resonance spectroscopy, magnetic resonance imaging, membrane phospholipids, magnesium, phosphomonoesters

INTRODUCTION

^{31}P MRS allows in vivo calculation of concentrations of different phosphorus-containing compounds in the brains of human subjects. Studies have shown changes in ^{31}P MRS parameters in the dorsolateral prefrontal cortex in patients with schizophrenia compared with healthy subjects. PMEs and phosphodiesters (PDE) are both integral components of neuronal cell membranes, and differences in these measures could reflect changes in cell membrane metabolism. Decreased PME and increased PDE have been reported in never-treated patients with schizophrenia compared with normal subjects (Pettegrew and others 1991; Stanley and others 1995). Elevated $[Mg^{2+}]_{intra}$ levels were also found in

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Table 1

Subject characteristics

		Age (y)				Education[a]		Parental education[a]	
Subjects	Number	Mean	SD	Handedness	Sex	Mean	SD	Mean	SD
Schizophrenia patients	10	35.4	10.5	9 right	8 male	2.6	0.8	1.9	0.9
Healthy controls	10	26.5	16.7	8 right	9 male	2.1	1.7	2.3	1.4

[a]Education levels: 1≤ grade 10; 2 = grades 11 to 13; 3 = 1 to 3 years of college or university; 4 = 3 or more years of college or university.

never-treated patients with schizophrenia compared with healthy subjects (Stanley and others 1995). Patients with chronic schizophrenia have demonstrated decreased PME and increased $[Mg^{2+}]_{intra}$ levels compared with healthy subjects (Stanley and others 1994).

Widespread cortical grey matter deficits have also been found in patients with schizophrenia compared with normal subjects (Zipursky and others 1992). PME and PDE are found in both grey and white matter, although higher levels are measured from cortical white matter (Buchli and others 1994a). $[Mg^{2+}]_{intra}$ levels are about the same in grey and white matter (Buchli and others 1994a). Grey matter deficits in patients with schizophrenia could lead to an increased proportion of white matter in the regions of interest studied with ^{31}P MRS; although this could explain increased PDE levels in patients, it does not explain decreased PME levels, which would be expected to be increased as well. Grey matter deficits in patients, however, could be associated with increased sulcal volumes, which would increase the proportion of cerebrospinal fluid in the region of interest. Cerebrospinal fluid does not contain measurable amounts of PME or PDE (Buchli and others 1994b), so grey matter deficits could account for lower PMEs in patients with schizophrenia. It is also possible that both lower PMEs and grey matter deficits could be related to the same pathophysiological process, such as the loss of neuropil (Pettegrew and others 1991). In this case, correlations between PME levels and grey matter volumes would not necessarily be related to partial volume effects on the PME measure.

This study examined the relationship between ^{31}P MRS parameters and left prefrontal grey and white matter volumes in patients with schizophrenia and healthy subjects. No correlation between PME and left prefrontal grey matter volumes would suggest that these are unrelated findings. As a further test, it was hypothesized that previous findings of decreased PME and increased $[Mg^{2+}]_{intra}$ levels in patients with schizophrenia compared with healthy subjects would remain after adjusting for volumetric parameters.

METHODS

Subjects

All diagnoses were established by the Structured Clinical Interview for DSM-III-R (SCID) (Spitzer and others 1990). No subject had a history of head injury, drug or alcohol abuse, or serious medical illness. ^{31}P MRS results for some of these subjects have been reported previously (Stanley and others 1995). Four subjects were in their 1st episode of illness and had not received antipsychotic medication prior to the study. One had received 1 mg of lorazepam in the 24 h before the study. The other subjects with schizophrenia received an average chlorpromazine-equivalent dose of 917 mg/d (SD = 499). Four patients were taking an anticholinergic medication on a regular basis; 6 were classified as paranoid, 3 as residual, and 1 as undifferentiated. The average length of illness from the onset of positive symptoms was 12.2 y (SD = 8.6). Ten healthy subjects were recruited by advertisement or were contacted after participating in other studies. Demographic features of the subjects are shown in Table 1. Parental education was unavailable in 1 normal subject because of adoption. Informed consent was obtained from all subjects.

MR spectra

In vivo ^{31}P MR spectra were acquired with a 5-cm diameter transmit–receive surface coil switchable between ^{1}H and ^{31}P frequencies on a whole-body magnetic resonance imager (Helicon SP System, Siemens AG, Erlangen, Germany) operating at a static field of 2.0 T. The surface coil was placed over the left dorsolateral prefrontal cortex, with positioning confirmed with the surface coil ^{1}H MR sagittal images. The ^{31}P MRS procedure, spectral quantification, and calculation of $[Mg^{2+}]_{intra}$ concentrations have been described elsewhere (Stanley and others 1994). Figure 2 shows the location of the approximate excitation volume from the ^{31}P surface coil.

MRI scans

3D FLASH MRI scans were performed at 1.5 T with the head coil on the same imager and day immediately prior to ramping to 2.0 T. The MRI volumetric images (1.25 mm ×

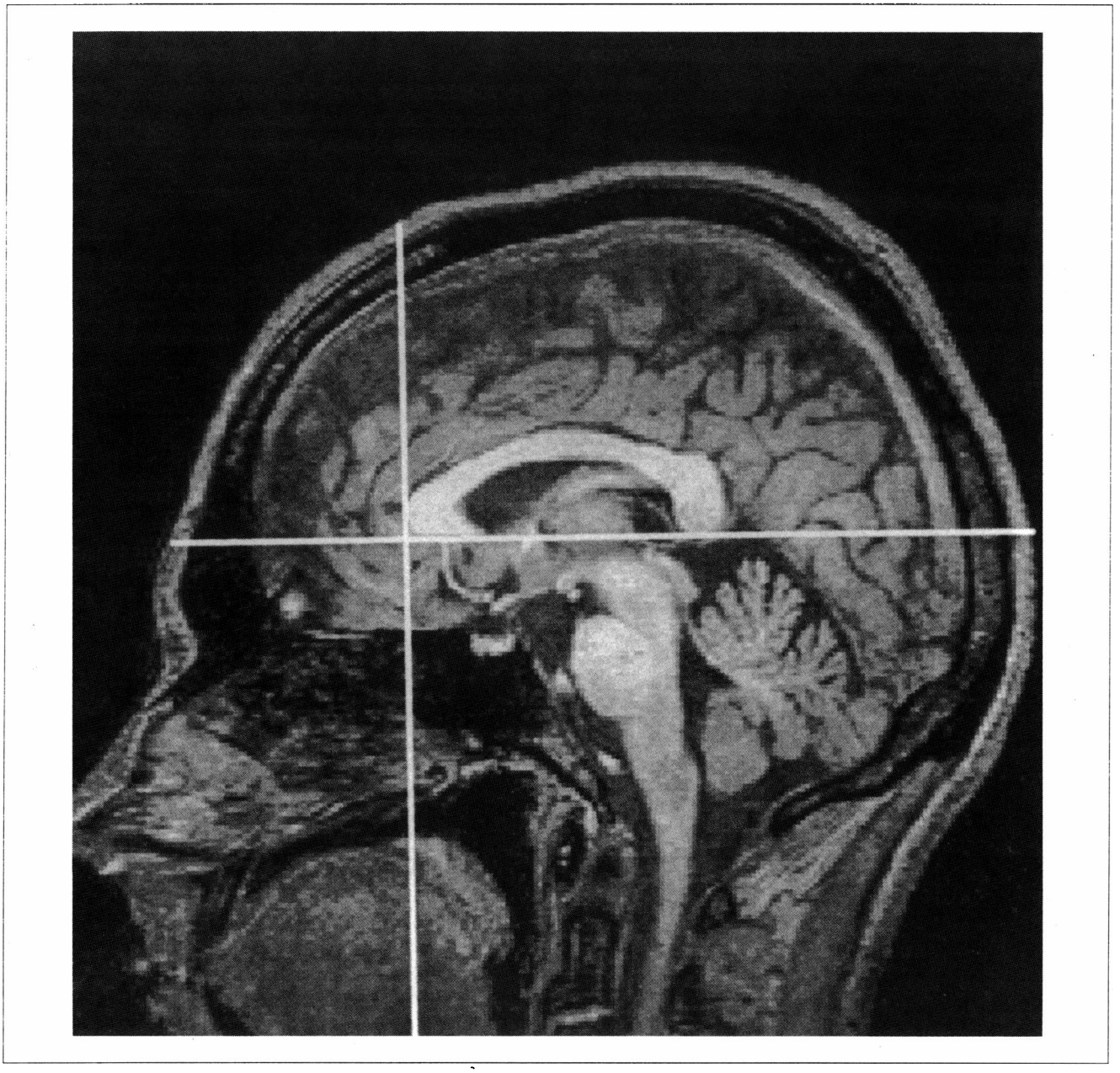

Figure 1. An MRI sagittal image showing a line that passes through the inferior margins of the corpus callosum. The oblique slices were all parallel to this line. The perpendicular line at the rostral edge of the genu of the corpus callosum demonstrates the posterior boundary of the volume of interest for calculating prefrontal volumes.

0.94 mm resolution) consisted of 64 contiguous sagittal slices, 2.50-mm thick, and were reformatted on a SUN Workstation using ANALYZE software (Robb 1990) into oblique slices parallel to a plane passing through the most inferior margins of the corpus callosum. Total brain volumes were calculated after editing away cerebrospinal fluid, blood vessels, cerebellum, and medulla. Prefrontal lobe volumes were calculated for areas rostral to a perpendicular cut at the rostral edge of the genu of the corpus callosum as in Zipursky and others (1992). Figure 1 shows the location of the oblique plane in relation to the corpus callosum and the location of the cut to demarcate the prefrontal lobe volumes. Left and

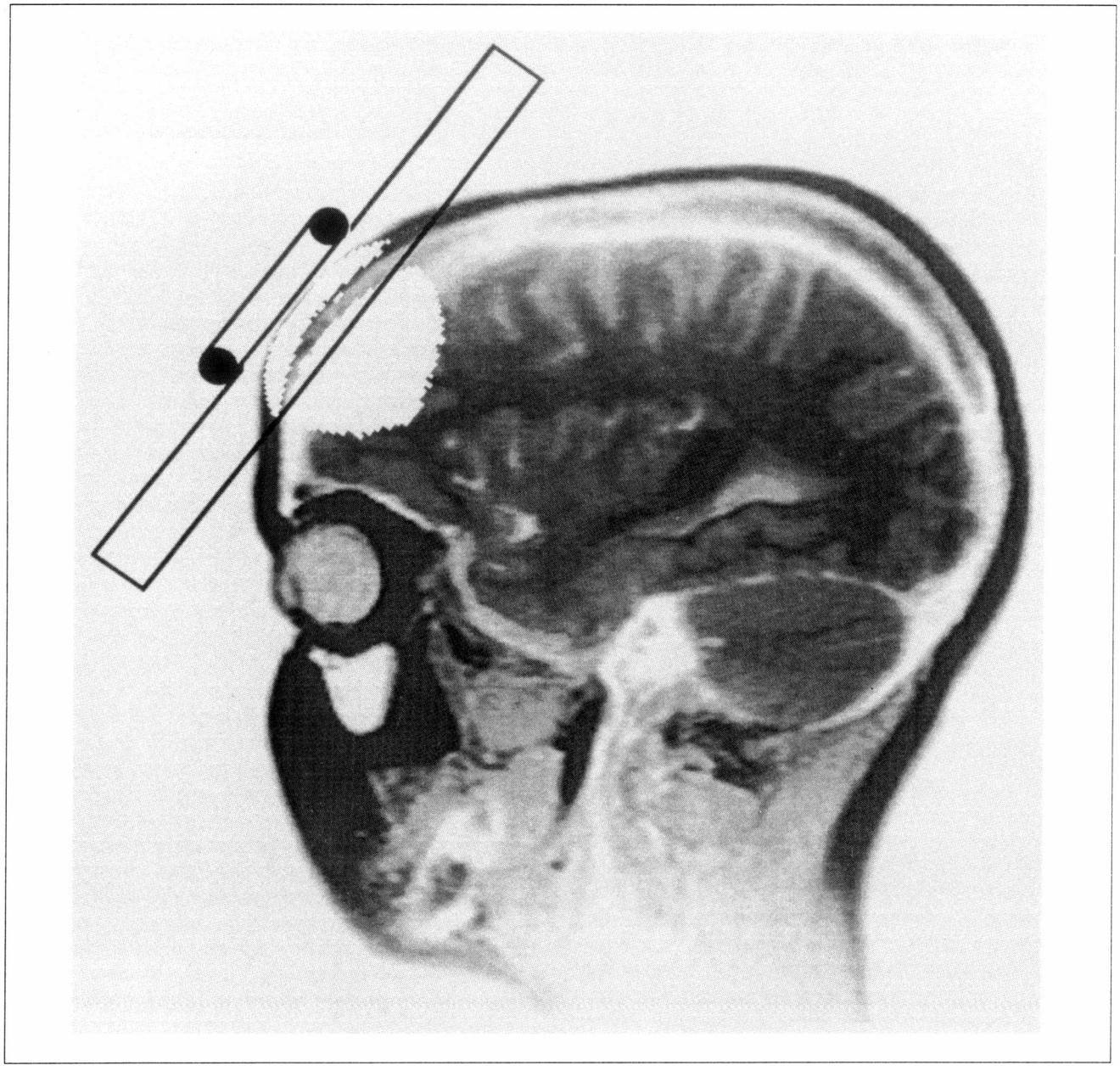

Figure 2. An illustration of the surface coil and saturation slice, generated to suppress signal from scalp and skull, is superimposed on a ^{1}H MR sagittal image. The shaded area below the saturation slice approximates the region of interest for ^{31}P MRS measurements.

right prefrontal lobes were separated using the interhemispheric fissure. Grey and white matter were separated using a semiautomated histogram technique in Xstatpak (Davis, unpublished observations). Ten subjects had volumes calculated twice on the same scan to determine test–retest reliability. All volume measurements were performed by the same rater without knowledge of diagnosis or MRS measures.

Statistics

Repeated-measure stability was assessed with intraclass correlation coefficients. MRS data from the patient group and healthy subjects were compared using an analysis of covariance after adjusting for differences in age, left prefrontal grey and white matter, and total brain volumes. Variances for

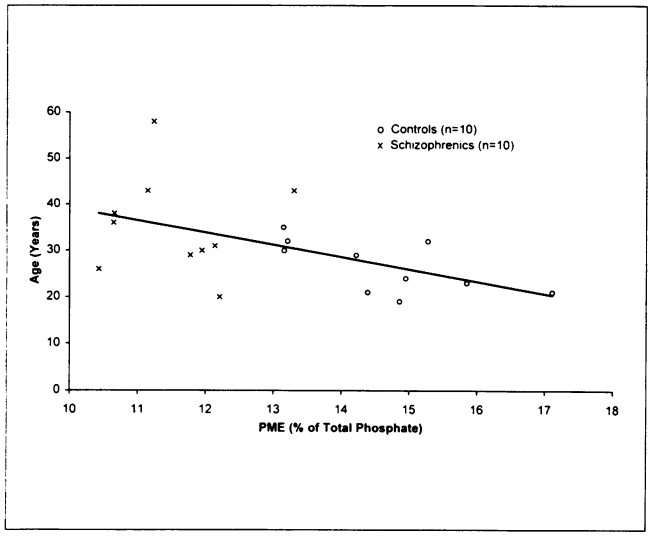

Figure 3. PME levels expressed as a percentage of total observed ^{31}P MR spectrum plotted against age in patients with schizophrenia and healthy subjects.

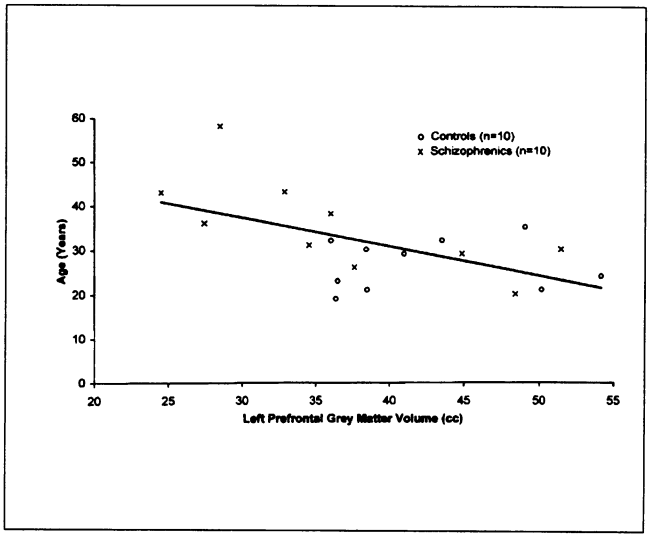

Figure 4. Left prefrontal grey matter in cubic centimeters (cc) plotted against age in patients with schizophrenia and healthy subjects.

$[Mg^{2+}]_{intra}$ were not equal between groups; the data, therefore, were reciprocally transformed prior to analysis for $[Mg^{2+}]_{intra}$ values only. (Reciprocally transformed $[Mg^{2+}]_{intra}$ values were expected to be lower in the schizophrenic group.) Correlations between MRS, volumetric, and age parameters were done with Pearson correlations.

RESULTS

Intraclass correlation coefficients for test–retest reliability were 0.994 for total brain volumes, 0.857 for left prefrontal white matter, and 0.983 for left prefrontal grey matter volumes.

Figure 3 shows the relationship between age and PME. In the total subject group (N = 20), PME decreased significantly with age ($r = -0.53$, $P < 0.02$), but no other ^{31}P MRS measure showed any significant correlation with age. It is interesting to note that in the normal subgroup, PME was significantly negatively correlated with age ($r = -0.63$, $P < 0.05$), but no significant correlation was found between age and PME in the patient subgroup ($r = -0.06$, $P < 0.9$).

Figure 4 shows the relationship between left prefrontal grey matter and age. In the total subject group (N = 20), age was significantly correlated with left prefrontal grey matter ($r = -0.57$, $P < 0.009$) and left prefrontal white matter ($r = -0.47$, $P < 0.04$). There was a trend toward a negative correlation between age and total brain volume ($r = -0.43$, $P < 0.06$).

In the total subject group, there was a significant negative correlation between phosphocreatine (PCr) and total brain volume ($r = -0.57$, $P < 0.01$). There were no other significant correlations between any ^{31}P MRS measure and volume measure. PME levels were not significantly correlated with left prefrontal grey matter in the total subject group ($r = 0.29$, $P < 0.3$), the patient subgroup ($r = 0.31$, $P < 0.4$), or the normal subgroup ($r = -0.34$, $P < 0.4$). Figure 5 shows the relationship between left prefrontal grey matter and PME levels.

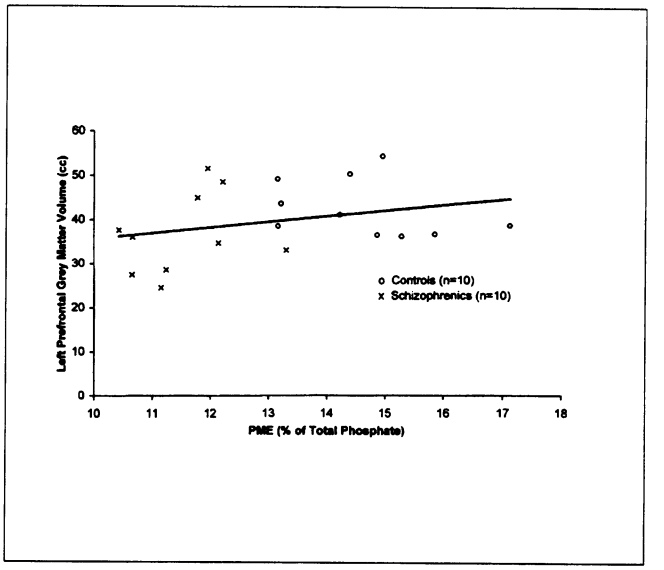

Figure 5. PME levels expressed as a percentage of total observed ^{31}P MR spectrum plotted against left prefrontal grey matter in cubic centimeters (cc) in patients with schizophrenia and healthy subjects.

Table 2

MRI volumetric and ^{31}P MRS parameters in patients with schizophrenia and healthy subjects

	Patients		Healthy subjects		
Parameters	Mean	SD	Mean	SD	Healthy subjects versus patients
PME[a]	11.55	0.89	14.62	1.28	$P < 0.002$†
Inorganic orthophosphate[a]	3.62	0.71	3.81	0.72	ns†
PDE[a]	38.92	2.45	38.08	1.67	ns†
PCr[a]	9.36	0.84	9.25	0.65	ns†
βATP (β-adenosine triphosphate)[a]	10.54	1.39	10.37	1.19	ns†
$[Mg^{2+}]_{intra}$[b]	2.54	0.39	3.26	0.27	$P < 0.002$†
Left prefrontal grey matter volume[c]	36.60	9.08	42.36	6.56	ns‡
Left prefrontal white matter volume[c]	23.56	5.80	24.64	4.84	ns‡
Total brain volume[c]	1247.22	158.95	1269.43	93.75	ns‡

[a]Expressed as metabolite percentage of total observed ^{31}P MR spectrum.
[b]Expressed as μmol reciprocally transformed values.
[c]Expresssed in cubic centimeters (cc).
P values after adjusting for age (†), left frontal lobe grey and white matter, and total brain volumes and age (‡).

Table 2 shows the volumes and ^{31}P parameters for the 2 subgroups of participants. There were no differences in left prefrontal grey or white matter or total brain volume between healthy subjects and patients before or after adjusting for age. After adjusting for left prefrontal grey and white matter and total brain volume, the patient group still had significantly lower PME levels ($P < 0.001$) and lower reciprocally transformed $[Mg^{2+}]_{intra}$ values ($P < 0.001$) than healthy subjects. When the data were adjusted for age, patients still had lower PME levels ($P < 0.002$) and lower reciprocally transformed $[Mg^{2+}]_{intra}$ values ($P < 0.002$) than healthy subjects. It was not possible to evaluate differences in PDE levels previously reported in drug-naive patients because of the small number of these patients in this study.

DISCUSSION

PME levels were not correlated with left prefrontal grey matter volumes in either group or the groups combined, which suggests that these 2 parameters are not directly related. In keeping with previous studies (Panchalingam and others 1990; Pettegrew and others 1991), both of these parameters were found to correlate with age. Consequently, the difference in age between the groups could have an effect on this relationship. This seems unlikely because PME values did not correlate with age in the patient group, but healthy subjects did show decreasing levels of both grey matter and PME levels with age. It is possible, however, that a decrease in PME levels could precede grey matter losses since the patients with schizophrenia in the study showed significantly reduced PME levels but did not yet show the significant volume reductions reported in schizophrenia (Zipursky and others 1992).

Although the patients with schizophrenia tended to have smaller left prefrontal grey and white matter and total brain volumes than healthy subjects, none of these differences was statistically significant. The inclusion of 1st-episode patients, who have not yet experienced the chronic deterioration often seen in schizophrenia, may have diminished the differences in volume between subject groups. In addition, the small number of subjects in each group decreased the power of this study to detect significant differences.

Our findings indicate that significant grey matter atrophy is not a necessary prerequisite for abnormally low PME levels to be found in subjects with schizophrenia. Even after correcting for age and volume differences, PME levels and reciprocally transformed $[Mg^{2+}]_{intra}$ levels were lower in the patient group than in healthy subjects, consistent with previous reports (Stanley and others 1994, 1995). It is not possible to assess whether differences in PDE in never-treated patients were affected by volumetric measurements because only 4 never-treated patients were included in this study.

It is acknowledged that our volumes did not completely correspond to the regions studied with ^{31}P MRS. The purpose of this study, however, was to examine the relationship between these 2 parameters in the same subjects. Future studies will examine partial volume problems with both ^{31}P MRS and volumetric measurements from the same volume.

While differences in PME and $[Mg^{2+}]_{intra}$ do not seem to be related to grey or white matter volumes, it is still not clear what explains the differences. Membrane phospholipid or other metabolic anomalies are a possibility (Pettegrew and others 1991; Stanley and others 1994, 1995). Spin-lattice relaxation time constant saturation effects have also been considered (Stanley and others 1995). Alternatively, differences could be related to loss of dendritic proliferation yielding increased neuronal density (Selemon and others 1995), which has recently been demonstrated on postmortem examination.

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