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Elevated Myo-Inositol, Choline, and Glutamate Levels in the Associative Striatum of Antipsychotic-Naive Patients With First-Episode Psychosis: A Proton Magnetic Resonance Spectroscopy Study With Implications for Glial Dysfunction

Eric Plitman[1,2], Camilo de la Fuente-Sandoval[*,3,4], Francisco Reyes-Madrigal[3], Sofia Chavez[1,5], Gladys Gómez-Cruz[3], Pablo León-Ortiz[3,6], and Ariel Graff-Guerrero[1,2,5,7,8]

[1]Multimodal Imaging Group, Research Imaging Centre, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada; [2]Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; [3]Laboratory of Experimental Psychiatry, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico; [4]Neuropsychiatry Department, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico; [5]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; [6]Department of Education, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico; [7]Geriatric Mental Health Division, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada; [8]Campbell Institute Research Program, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada

*To whom correspondence should be addressed; Laboratory of Experimental Psychiatry & Neuropsychiatry Department, Instituto Nacional de Neurología y Neurocirugía, Insurgentes Sur 3877, La Fama, Tlalpan, 14269 Mexico City, Mexico; tel: +52 55 5606 3822; ext. 5059, fax: +52 55 5171 6456, e-mail: fcamilo@unam.mx

Glial disturbances are highly implicated in the pathophysiology of schizophrenia and may be linked with glutamatergic dysregulation. Myo-inositol (mI), a putative marker of glial cells, and choline (Cho), representative of membrane turnover, are both present in larger concentrations within glial cells than in neurons, and their elevation is often interpreted to reflect glial activation. Proton magnetic resonance spectroscopy (^{1}H-MRS) allows for the evaluation of mI, Cho, glutamate, glutamate + glutamine (Glx), and *N*-acetylaspartate (NAA). A collective investigation of these measures in antipsychotic-naive patients experiencing their first nonaffective episode of psychosis (FEP) can improve the understanding of glial dysfunction and its implications in the early stages of schizophrenia. 3-Tesla ^{1}H-MRS (echo time = 35 ms) was performed in 60 antipsychotic-naive patients with FEP and 60 age- and sex-matched healthy controls. mI, Cho, glutamate, Glx, and NAA were estimated using LCModel and corrected for cerebrospinal fluid composition within the voxel. mI, Cho, and glutamate were elevated in the FEP group. After correction for multiple comparisons, mI positively correlated with grandiosity. The relationships between mI and glutamate, and Cho and glutamate, were more positive in the FEP group. These findings are suggestive of glial activation in the absence of neuronal loss and may thereby provide support for the presence of a neuroinflammatory process within the early stages of schizophrenia. Dysregulation of glial function might result in the disruption of glutamatergic neurotransmission, which may influence positive symptomatology in patients with FEP.

Key words: MRS/schizophrenia/neuroinflammation/glutamatergic/positive symptoms/astrocyte

Introduction

Glial disturbances are highly implicated in the pathophysiology of schizophrenia.[1,2] Myo-inositol (mI) and choline-containing compounds (Cho) act as markers of glial cells and membrane metabolism, respectively.[3–6] Both mI and Cho are present in higher concentrations within glial cells than in neurons[7–9] and have been investigated in patients with schizophrenia using proton magnetic resonance spectroscopy (^{1}H-MRS).[10–12] Elevated levels of these neurometabolites have been proposed to reflect glial activation and have been observed in several neuroinflammatory disorders.[4]

Astrocytes (a subtype of glial cells) contribute to the regulation of glutamatergic neurotransmission.[13,14] Glutamatergic dysregulation is thought to be involved in the schizophrenia disease process[15–18] and has been evaluated in patients with schizophrenia using ^{1}H-MRS through the measurement of glutamate, glutamine, and glutamate + glutamine (Glx).[19–23] ^{1}H-MRS has also been used to ascertain levels of *N*-acetylaspartate (NAA), which serves as an index of neuronal integrity.[24,25]

Existing ^{1}H-MRS literature is heterogeneous in terms of voxel placement, stage of illness, and medication status; antipsychotic treatment has been suggested to influence the assessment of mI, Cho, glutamatergic markers, and NAA levels.[22,26–28] Our group has previously investigated neurometabolic differences in antipsychotic-naive patients experiencing their first nonaffective episode of psychosis (FEP) within the right associative striatum, an area rich in dopamine afferents and dopamine D_2 receptors, which is involved in the pathophysiology of schizophrenia[29,30] and is often included in the quantification of in vivo occupancy studies of antipsychotics.[31,32] Our first study found higher Cho and glutamate levels in the FEP group in comparison to controls.[20] We replicated these findings in a longitudinal study, in which we also found elevated baseline mI and Glx levels in the FEP group.[33]

In the present study, we used a larger sample to compare mI, Cho, glutamate, Glx, and NAA levels in the associative striatum between antipsychotic-naive patients with FEP and a group of age- and sex-matched healthy controls. We also explored the associations between neurometabolite levels and clinical measures, as well as the relationships amongst levels of neurometabolites. We hypothesized that neurometabolites predominantly present in glial cells would be elevated in the FEP group along with levels of glutamatergic compounds, in accordance with our previous findings. Our additional hypotheses were exploratory. We hypothesized that dysregulated neurometabolite levels would correlate with clinical symptom severity. Also, we hypothesized that abnormal levels of glial neurometabolites would be linked with disrupted glutamatergic levels in the patient group, such that the relationships between these measures would differ from healthy controls. The assessment of unmedicated patients is vital towards characterizing the pathophysiology of schizophrenia in that the confounding effects of medication are eliminated.[34] To the best of our knowledge, this is the largest sample to date of antipsychotic-naive patients with FEP in which ^{1}H-MRS was performed.

Methods

Participants

This study received approval from the Ethics and Scientific Committees of the National Institute of Neurology and Neurosurgery of Mexico (INNN). Individuals were included after providing informed written consent, which was obtained from both parents for participants under 18 years old. Participants did not receive a stipend.

Sixty-four patients were recruited during their FEP from inpatient or outpatient services at the INNN between 2008 and 2013. The Structured Clinical Interview for DSM-IV was utilized to determine inclusion. Patients met inclusion criteria if they were antipsychotic naive; all but 3 patients had less than 2 years of psychotic symptoms. Exclusion criteria included a concomitant medical or neurological illness, current substance abuse or history of substance dependence (excluding nicotine), comorbidity with other Axis I disorders, a high risk for suicide, and psychomotor agitation. Sixty-three age- and sex-matched healthy controls were also enrolled and assessed in the same manner as the patients. Controls with a history of psychiatric illness or a family history of psychosis were excluded.

Each participant was screened for drugs of abuse, including cannabis, cocaine, heroin, opioids, and benzodiazepines at the time of inclusion and 1 hour prior to the magnetic resonance imaging (MRI) scan. The current sample included a subset of participants (FEP: $n = 35$; controls: $n = 35$) previously reported upon[20,33]; additional subjects were added to increase statistical power.

Clinical Assessment

Patients' psychopathology was assessed by research psychiatrists (C.d.l.F.-S., F.R.-M., P.L.-O.) using the Positive and Negative Syndrome Scale (PANSS).[35]

Magnetic Resonance Studies

Participants were scanned at the INNN in a 3T GE whole-body scanner (Signa Excite HDxt; GE Healthcare) with a high-resolution 8-channel head coil. The participant's head was positioned along the canthomeatal line and immobilized using a forehead strap. Each participant was scanned using a T_1-weighted spoiled gradient-echo 3-dimensional axial acquisition (SPGR, echo time [TE] = 5.7 ms, repetition time [TR] = 13.4 ms, inversion time = 450 ms, flip angle = 20°, field of view = 25.6 cm, ≥256 × ≥256 matrix, slice thickness ≤ 1.2 mm), oriented above and parallel to the anterior-posterior commissure line. These T_1-weighted SPGR images were reformatted to sagittal and coronal views and were subsequently used for ^{1}H-MRS voxel localization.

^{1}H-MRS spectra were obtained using point-resolved spectroscopy (PRESS, TE = 35 ms, TR = 2000 ms, spectral width = 5000 Hz, 4096 data points used, 128 water-suppressed, and 16 water-unsuppressed averages) centered on the right dorsal-caudate nucleus in volume elements (voxels) of 8 ml (2 × 2 × 2 cm). The lower end of the dorsal-caudate voxel (associative striatum) was located 3 mm dorsal to the anterior commissure to include maximum gray matter (GM) and with a dorsal extension (thickness) of 2 cm. Voxel placement is identified in supplementary figure 1. During the acquisition, ^{1}H-MRS spectra were shimmed to achieve a full-width at half maximum (FWHM) of 12 Hz or less, measured on the unsuppressed water signal from the voxel. Spectra with larger FWHM were excluded from ensuing analyses.[23]

^{1}H-MRS Data Analysis

All water-suppressed spectra were analyzed using LCModel version 6.3-0E.[36] Spectra were normalized to the unsuppressed water signal, allowing for neurometabolite quantification, expressed in institutional units. A standard basis set of metabolites, delineated within the supplementary material, was used for analysis. In this study, Cho is the sum of glycerophosphocholine + phosphocholine, NAA is the sum of NAA + *N*-acetylaspartylglutamate, and creatine-containing compounds (Cr) is the sum of creatine + phosphocreatine. One analyzed spectrum is included in supplementary figure 2.

Spectra with %SD values of 20% or greater for neurometabolites of interest were considered poor quality and excluded from subsequent analyses.[37,38] Four patients and 3 controls were excluded due to either rejection by LCModel analysis or a FWHM greater than 12 Hz, resulting in the inclusion of 60 patients and 60 healthy controls; of the 7 participants/spectra removed in total, 1 was previously reported upon. Glutamine was not analyzed because of poor spectra fitting. To control for correlations introduced by the LCModel fitting procedure, the triangular table of correlation coefficients was used. All reported metabolite correlations did not show strong negative pairwise correlations in the triangular table: no correlational coefficients were less than −0.5.[38]

T1-weighted MRI scans used for voxel localization were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Imaging Neurosciences, University College London, UK). The size and location of each area were extracted from the spectra file headers to calculate the percentage of GM, WM, and CSF content within the voxel using an in-house software, allowing for the correction of the CSF fraction of the spectroscopic values.[20]

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 20 (IBM Corporation). Demographic and clinical characteristics, Cramer-Rao lower bounds (CRLBs), FWHM values, signal-to-noise ratios, GM, WM, and CSF percentages, and GM/(GM + WM) were compared between groups using independent-sample *t* tests. Frequency data were analyzed using χ^2 or Fisher's exact tests. Neurometabolite levels were compared between groups using analyses of variance. To check for confounders, tobacco use, GM content, GM/(GM + WM), and age were each investigated as covariates. Outliers were defined as greater than 3 times the interquartile range and were removed in a neurometabolite-specific manner; 2 mI outliers, 1 glutamate outlier, and 1 Cr outlier were removed. Due to a priori hypotheses, neurometabolite level group comparisons were conducted with a significance level of $P < .05$.

Pearson correlations were performed to investigate the association between PANSS subscale total scores and neurometabolite levels that differed significantly between groups. If any correlation reached an uncorrected $P < .05$, Pearson correlations between the neurometabolite and items within the specific PANSS subscale were also examined. All investigations were corrected for multiple comparisons and a statistical threshold of $P < .05 \div n$ was used, where n = # of comparisons ($n = 16$; 3 neurometabolites with 3 subscale total scores and 1 neurometabolite with 7 subscale items).

The relationships amongst levels of neurometabolites that differed significantly between groups were assessed using Pearson correlations. A statistical threshold of $P < .05 \div n$ was used, where n = # of comparisons ($n = 6$; 3 neurometabolites investigated separately for each group).

Group differences in correlational coefficients were evaluated by converting correlational coefficients with Fisher's transformation (Equation 1) and comparing them using Fisher's z test (Equation 2), which allowed for z score acquisition. Here, comparisons were conducted with a significance level of $P < .05$.

$$r' = (0.5)\log e\left|\frac{1+r}{1-r}\right| \quad (1)$$

$$z = \frac{r_1' - r_2'}{\sqrt{\frac{1}{n_1 - 3} + \frac{1}{n_2 - 3}}} \quad (2)$$

Above, r represents the sample correlational coefficient, r' is the transformed value of r, n indicates sample size, and z refers to z score.

Results

Demographic and Clinical Characteristics

Participants' demographic and clinical characteristics are reported in supplementary table 1. Patients' DSM-IV diagnoses were: brief psychotic disorder ($n = 14$), schizophreniform disorder ($n = 21$), and schizophrenia ($n = 25$). Education years were higher in the control group ($t(118) = 6.40$, $P < .001$), while tobacco use was greater in the FEP group ($\chi^2 = 5.21$, $P = .039$). Age, sex, handedness, and cannabis use did not differ between groups. The FEP group had a mean duration of untreated psychosis of 33.03 ± 52.70 weeks, and mean PANSS positive, negative, and general psychopathology subscale total scores of 24.13 ± 4.97, 24.33 ± 5.66, and 48.75 ± 8.38, respectively.

Neurometabolite Levels

Neurometabolite levels are reported in table 1 and displayed in figure 1. mI, Cho, and glutamate levels were higher in the FEP group ($F(1,116) = 5.66$, $P = .019$; $F(1,118) = 10.66$,

Table 1. Neurometabolite Levels in Patients With First-Episode Psychosis and Healthy Controls

	Mean (SD)					
	mI	Cho	Glu	Glx	NAA	Cr
FEP group	5.81 (1.10)*	2.53 (0.29)**	13.10 (1.31)***	16.61 (1.69)	11.00 (1.08)	8.61 (0.74)
HC group	5.31 (1.19)	2.36 (0.28)	12.39 (0.95)	16.21 (1.50)	10.90 (0.96)	8.49 (0.75)

Note: Cho, choline-containing compounds; Cr, creatine-containing compounds; FEP, first-episode psychosis; Glu, glutamate; Glx, glutamate + glutamine; HC, healthy control; mI, myo-inositol; NAA, *N*-acetylaspartate.
*$P < .05$.
**$P < .01$.
***$P < .001$.

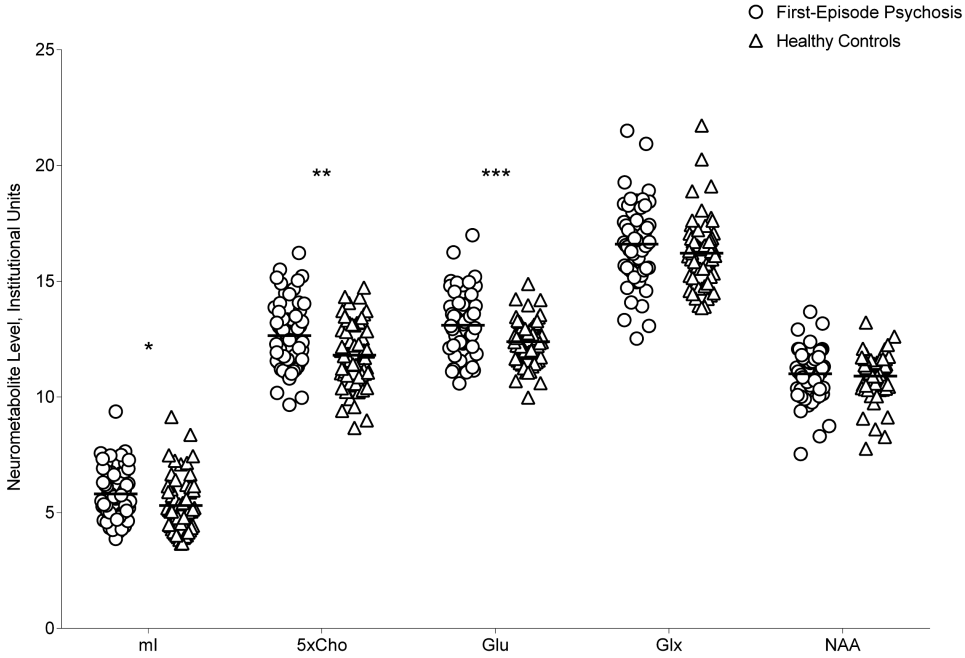

Fig. 1. Neurometabolite levels in patients with first-episode psychosis and healthy controls. Cho, choline-containing compounds; Glu, glutamate; Glx, glutamate + glutamine; mI, myo-inositol; NAA, *N*-acetylaspartate; *$P < .05$; **$P < .01$; ***$P < .001$.

$P = .001$; $F(1,117) = 11.63$, $P < .001$, respectively). Glx and NAA levels did not differ between groups ($F(1,118) = 1.84$, $P = .18$; $F(1,118) = 0.29$, $P = .59$, respectively). Results were unaffected when tobacco use, GM content, GM/(GM + WM), and age were included as covariates.

Relationships With Clinical Measures

The relationships between neurometabolites and PANSS subscale total scores are presented in table 2. After correction for multiple comparisons, mI levels were positively correlated at a trend-level significance with PANSS positive total score ($r(57) = .31$, *P-uncorrected* = .017) (figure 2a) and PANSS item P3 (Hallucinatory Behavior) score ($r(57) = .37$, *P-uncorrected* = .004) (figure 2b). mI levels were also positively correlated with PANSS item P5 (Grandiosity) score ($r(57) = .49$, *P-uncorrected* < .001) (figure 2c). mI levels were not related to PANSS negative or general psychopathology total scores. Removing a potential outlier (mI > 8) did not alter findings. Cho and glutamate levels were not related to any PANSS subscale total scores. Including tobacco use, GM content, GM/(GM + WM), and age as covariates did not affect results.

Relationships Between Neurometabolites

mI levels positively correlated with Cho levels in both groups (FEP: $r(57) = .58$, *P-uncorrected* < .001; control: $r(57) = .69$, *P-uncorrected* < .001) (figure 3a). mI levels positively correlated with glutamate levels in the FEP group only (FEP: $r(57) = .29$, *P-uncorrected* = .024; control: $r(56) = -.13$, *P-uncorrected* = .32) (figure 3b), though this relationship did not survive correction for multiple comparisons. Cho levels positively correlated with glutamate in the FEP group only (FEP: $r(58) = .48$, *P-uncorrected* < .001; control: $r(57) = .13$, *P-uncorrected* = .32) (figure 3c). Results were unaffected by the addition of tobacco use, GM content, GM/(GM + WM), and age as covariates.

r-to-*Z* transformations identified that the relationships between levels of mI and glutamate, and Cho

Table 2. Relationships Between Neurometabolite Levels and PANSS Subscale Total Scores

Variable	Correlational Coefficient (*r*)		
	mI	Cho	Glu
PANSS subscale			
Positive	$r(57) = .31$, $P = .017$*	$r(58) = .11$, $P = .40$	$r(58) = -.05$, $P = .71$
Negative	$r(57) = -.04$, $P = .78$	$r(58) = .03$, $P = .82$	$r(58) = -.01$, $P = .91$
General psychopathology	$r(57) = .08$, $P = .56$	$r(58) = .06$, $P = .65$	$r(58) = .001$, $P = .99$

Note: Cho, choline-containing compounds; Glu, glutamate; mI, myo-inositol; PANSS, Positive and Negative Syndrome Scale.
**P-uncorrected* < .05.

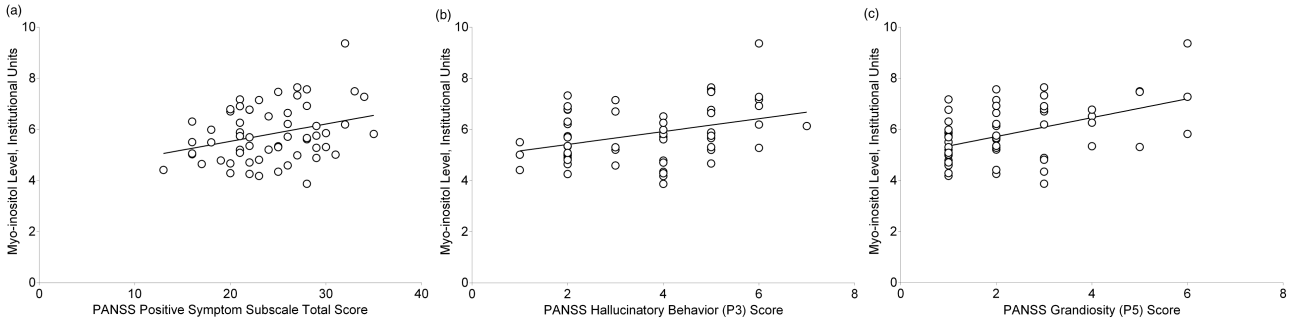

Fig. 2. Relationships between myo-inositol levels and positive symptom subscale total (a), hallucinatory behavior (b), and grandiosity (c) scores. PANSS, Positive and Negative Syndrome Scale.

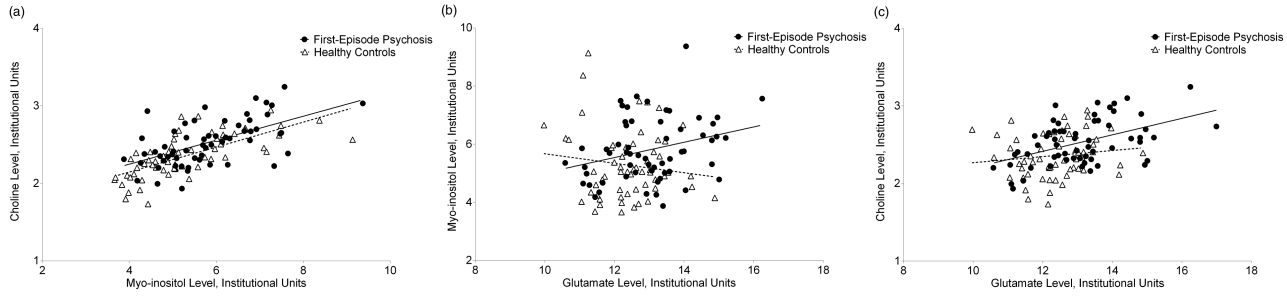

Fig. 3. Relationships between levels of myo-inositol and choline (a), glutamate and myo-inositol (b), and glutamate and choline (c) in patients with first-episode psychosis and healthy controls.

and glutamate, were more positive in the FEP group ($Z = 2.26$, $P = .024$; $Z = 2.08$, $P = .038$, respectively). The relationships between levels of mI and Cho were not different between groups ($Z = .98$, $P = .33$).

CRLB, FWHM, Signal-to-Noise Ratios, and Tissue Heterogeneity

CRLBs, FWHM values, signal-to-noise ratios, GM, WM, and CSF percentages, and GM/(GM + WM) did not differ between groups (supplementary tables 2 and 3).

Discussion

The present study investigated neurometabolite levels in the associative striatum within the largest sample of antipsychotic-naive patients with FEP to date and a group of age- and sex-matched healthy controls. We found elevations in mI, Cho, and glutamate levels in the FEP group. Additionally, mI levels positively correlated with grandiosity, while positive symptom total score and hallucinatory behavior positively correlated with mI levels at a trend-level significance. Lastly, the correlations between levels of mI and glutamate, and Cho and glutamate, were more positive in the FEP group.

We previously reported increases in mI and Cho levels within the associative striatum of antipsychotic-naive patients with FEP.[20,33] Though most ^{1}H-MRS studies have found unaltered levels of mI and Cho,[10–12,39] others have reported deviations in these neurometabolites within several brain regions.[40–44] To the best of our knowledge, no previous ^{1}H-MRS study has observed statistical differences in mI levels within the striatum of patients with schizophrenia. However, in terms of Cho, one study found increased levels in the caudate nucleus of antipsychotic-naive patients with schizophrenia,[45] while others

have reported increases in the basal ganglia, encompassing caudate and lenticular nucleus regions, within medicated patients.[46,47]

Both mI and Cho are present in greater concentrations within glial cells than in neurons.[7–9] The strong correlation between mI and Cho levels in both groups suggests that these neurometabolites are linked, although these results must be interpreted with caution due to the potential for spurious correlations.[48] Elevated levels of mI and Cho are often interpreted as glial activation, which is commonly associated with a neuroinflammatory response[4]; accordingly, mI and Cho levels are elevated in several neuroinflammatory disorders.[4,49–52] Recently, Chiappelli et al reported that mI levels within frontal WM were negatively correlated with fractional anisotropy of WM in both patients with schizophrenia and in healthy controls[53]; in support of the link between mI and neuroinflammation, this finding was interpreted by the authors as evidence for a general effect of inflammation on WM microstructure. Further, previous literature has suggested that schizophrenia may have a neuroinflammatory component and that antipsychotics may have anti-inflammatory effects.[54–56] Additionally, anti-inflammatory agents might have beneficial effects on symptomatology as adjunctive therapies.[57,58] In our study, the concomitant elevation of mI and Cho levels in the patient group may provide an ^{1}H-MRS finding in support of early neuroinflammation that either accompanies or precedes the FEP in schizophrenia.

Furthermore, consistent with our previous reports,[20,33] we found elevated glutamate levels in the FEP group. Notably, this result was also identified in the subjects not included in previous reports ($F(1,48) = 13.53$, $P < .001$; Cohen's $d = 1.05$). This finding is in accordance with previous ^{1}H-MRS literature suggesting increased levels of glutamatergic markers in antipsychotic-naive patients with schizophrenia, minimally treated patients with schizophrenia, and individuals at ultra-high risk for psychosis who later transitioned to psychosis[21–23,59–61]—levels that may subsequently normalize to or decrease below those of healthy controls following antipsychotic treatment.[22,23,27,62–64] While some previous ^{1}H-MRS studies investigating the basal ganglia (including lenticular nucleus, putamen, and substantia nigra regions) in patients with schizophrenia have failed to find differences in glutamatergic markers,[44,65] our findings are comparable to those of Goto et al, who reported increased basal ganglia Glx in patients with first-episode schizophrenia[66]; however, it is important to distinguish between glutamate and Glx levels in this comparison, especially since only the former was found to be elevated in our study and the latter is a composite measure of both glutamate and glutamine levels.

The present study was also supplemented by the investigation of the relationships between levels of glutamate and levels of mI and Cho. Studies localizing mI and Cho to glial cells specifically found elevated concentrations of these neurometabolites within astrocytes.[7–9] Typically, synaptic glutamate is taken up by astrocytes and converted to glutamine.[13,14] Thus, the parallel increase of mI and Cho levels in patients with FEP may support a mechanism wherein astrocytic function is abnormally altered in response to a pathological process and glutamatergic neurotransmission is consequently disturbed, as suggested by our observation of increased glutamate levels. This notion is reinforced by our findings of positive correlations between levels of mI and glutamate, and Cho and glutamate, in the FEP group only, and the fact that the correlational coefficients of these relationships were more positive in the FEP group. Previous studies have observed increases in S100B, a marker for astrocyte function, in patients with schizophrenia during acute psychosis stages, in addition to concomitant increases in mI levels, supporting the notion that astrocytic activation with associated mI elevation may exist in schizophrenia.[67] Likewise, elevated Cho levels have been interpreted to represent increased astrocytic turnover of glutamatergic compounds.[19]

Though the exact mechanism by which astrocytic dysfunction might lead to glutamatergic dysregulation has not been characterized, evidence in patients with schizophrenia has suggested a role for astrocytic overproduction of kynurenic acid, an endogenous *N*-methyl-D-aspartate receptor (NMDAR) antagonist.[68,69] Given that the administration of exogenous NMDAR antagonists leads to increased levels of glutamatergic compounds,[70,71] elevated kynurenic acid may connect the aforementioned phenomena.[72–74] Additionally, astrocyte dysfunction might disturb glutamate transporter function, preventing the reuptake of extracellular glutamate[75–78] and thereby contributing to dysregulated glutamatergic neurotransmission.

The relationships between mI levels and positive symptom total score, hallucinatory behavior, and grandiosity also warrant discussion. Though 2 of these correlations did not retain significance after correction for multiple comparisons, our results provide some suggestion that mI levels may be linked with positive symptomatology, reinforcing the notion that the group difference in mI levels is related to illness pathophysiology. We believe this is the first study to suggest that mI might be related to positive symptomatology, while associations with other symptom domains have been observed. Our group previously reported trend-level reductions in mI levels following clinically effective antipsychotic treatment (PANSS total score reduction of at least 30%) of antipsychotic-naive patients with FEP.[33] In medicated patients with schizophrenia, Homan et al found a negative relationship between mI levels in Broca's area and total PANSS scores.[79] Furthermore, Chiappelli et al found a negative correlation between trait depressive symptoms and anterior cingulate cortex mI levels in patients with schizophrenia spectrum disorders and in healthy controls.[80] The authors also observed lower mI levels in patients with at least one major depressive episode, suggesting that mI may be a biomarker of depressive

symptoms in this patient population. In the present study, we propose that within the associative striatum, mI is related to positive symptomatology through astrocytic dysregulation of glutamatergic neurotransmission.[81] However, despite the elevation in glutamate levels within the FEP group, glutamate was unexpectedly not associated with symptomatology. Thus, the exact mechanism connecting increased mI levels and positive symptoms remains elusive and necessitates further investigation.

In terms of NAA levels, we failed to find group differences, contrasting previous ^{1}H-MRS studies that report reductions.[10,82] Our finding suggests preserved neuronal integrity in the associative striatum at an early stage of schizophrenia. We posit that neuronal loss occurs later in the illness, resulting from either glutamate-mediated excitotoxicity or the advancement and chronicity of neuroinflammation and glial activation.[4,83] These processes would align with literature suggesting progressive NAA reductions in schizophrenia.[12]

One methodological consideration is that neurometabolite concentrations were referenced relative to water. While mI, Cho, and glutamate levels were elevated in the FEP group, it deserves emphasis that Glx and NAA levels did not differ between groups. Thus, even though neurometabolite concentrations were corrected for CSF, decreased water content likely did not drive group differences, as further evidenced by the similar voxel CSF content between groups. Notably, when referenced to Cr levels, which importantly did not differ between groups ($F(1,117) = 0.70$, $P = .41$),[10] results did not differ for analyses concerning group differences in neurometabolite levels (mI: $F(1,116) = 4.45$, $P = .037$; Cho: $F(1,118) = 6.06$, $P = .015$; glutamate: $F(1,118) = 4.82$, $P = .030$; Glx: $F(1,118) = 0.02$, $P = .90$; NAA: $F(1,118) = 0.40$, $P = .53$; supplementary table 4) and their relationships with clinical symptoms (supplementary table 5), whereas findings related to the relationships among neurometabolite levels were not identical (supplementary table 6).

Our study is not without limitations. First, the functions assigned to neurometabolites do not comprehensively delineate their physiological roles. Particularly, the involvement of mI and Cho extend beyond glial cells and neuroinflammation to include an extensive range of other structural and signaling functions. Second, the wide age range in our sample is a source of biographic inhomogeneity. While age has been shown to influence neurometabolite levels,[41,42,53,84,85] it was not presently a primary focus, though analyses concerning age are included in supplementary table 7. Of note, in the full sample, mI levels were positively correlated with age at a trend-level significance ($r(116) = .17$, $P = .066$), a finding consistent with past work.[53] Third, ^{1}H-MRS cannot distinguish between extracellular and intracellular measurements and does not directly assess neurotransmission. Fourth, using a TE of 35 ms at 3T renders glutamate and glutamine difficult to distinguish; thus, the glutamate peak may be contaminated by glutamine. This was evidenced by the correlational coefficients between glutamate and glutamine in the triangular table, which were close to or less than −0.5. Fifth, at a TE of 35 ms, Glx levels may contaminate the NAA peak.[86] Sixth, not all patients with FEP progress to schizophrenia, affecting generalizability, although 73% of the FEP group (44 patients) received a follow-up diagnosis of schizophrenia. Seventh, only the right associative striatum was studied to reduce imaging time in patients with active psychosis. However, previous ^{1}H-MRS studies did not observe laterality differences in levels of mI, Cho, or glutamatergic markers in patients with schizophrenia.[42,87] Eighth, the group difference in nicotine smoking presents an important limitation. Ninth, chemical shift artifacts were not specifically addressed, though it is noteworthy that with PRESS at 3T, they could potentially account for a reduced water signal in the patient group. Tenth, simulated macromolecular resonances may not be representative of the true macromolecular spectrum. Finally, since all neurometabolite levels are relative to water, spurious correlations may exist between pairs of neurometabolites. However, similar to the reasoning provided by Kraguljac et al,[88] our hypothesis was initially formulated in terms of ratios and primarily concerns a difference in correlations between patients and controls, which we were able to assess using *r*-to-*Z* transformations.

Taken together, our findings are suggestive of glial activation in the absence of neuronal loss and may thereby provide support for the presence of a neuroinflammatory process within the early stages of schizophrenia. Astrocytic dysfunction might disrupt glutamatergic neurotransmission, which may subsequently influence positive symptomatology in patients with FEP and may have an excitotoxic effect in later stages of the illness. Considering that approximately 20% to 35% of patients have unremitting positive symptoms following antipsychotic treatment,[89,90] the development of a fuller picture of schizophrenia and its neurochemical underpinnings is vital towards understanding the pathophysiology of the illness and improving treatment interventions. Future research should continue to investigate neuroinflammation and glial abnormalities in schizophrenia, as well as their impact on glutamatergic neurotransmission.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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