# Elevated hippocampal glutamate levels associated with adverse outcomes in people at clinical high risk for psychosis

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**Key Points**

**Question:** What is the relationship between hippocampal glutamate levels in people at clinical high risk (CHR) for psychosis and subsequent clinical outcomes?

**Findings:** This cross-sectional 3-Tesla proton magnetic resonance spectroscopy ($^{1}$H-MRS) study with a mean clinical follow-up of 18.5 months shows that baseline hippocampal glutamate levels are significantly higher in those CHR subjects who developed psychosis or had poor functional outcome at follow up.

**Meaning:** This association between adverse clinical outcomes in people at CHR for psychosis and increased baseline hippocampal glutamate levels suggests that these measures could contribute to the stratification of CHR subjects according to future clinical outcomes.

**Abstract**

**Importance:** Preclinical and human data suggest that hippocampal dysfunction plays a critical role in the onset of psychosis. Neural hyperactivity in the hippocampus is thought to drive an increase in subcortical dopamine function through glutamatergic projections to the striatum.

**Objective:** To examine the relationship between hippocampal glutamate levels in people at clinical high risk (CHR) for psychosis and subsequent clinical outcomes.

**Design:** Cross-sectional 3-Tesla proton magnetic resonance spectroscopy ($^{1}$H-MRS) study with a mean subsequent clinical follow up of 18.5 months, conducted between November 2011 and November 2017.

**Setting:** Early detection services for CHR individuals in London and Cambridge.

**Participants:** 86 individuals at CHR for psychosis as defined using the Comprehensive Assessment of the At-Risk Mental State (CAARMS) and 30 healthy controls.

**Main Outcomes and Measures:** Concentrations of glutamate and other metabolites were measured in the left hippocampus at first clinical presentation. At follow up, clinical outcomes were assessed in terms of transition/non-transition to psychosis (CAARMS criteria) and the level of overall functioning (Global Assessment of Function scale; GAF).

**Results:** The mean (SD) age of participants was 22.4 (3.5) years in 86 CHR subjects (50 male) and 24.7 (3.8) years in 30 healthy controls (14 male). At follow up, 12 CHR subjects developed a first episode of psychosis and 74 CHR subjects did not; 19 CHR subjects showed good overall functioning (GAF≥65), whereas 38 CHR subjects had a poor functional outcome (GAF<65). Compared to CHR subjects who did not become psychotic, CHR subjects who developed psychosis showed higher hippocampal glutamate levels (p=0.048). They also had higher myo-inositol and creatine levels compared to CHR subjects who did not become psychotic (p=0.002 and p=0.009, respectively), and higher myo-inositol levels than HCs (p=0.005). Higher hippocampal glutamate levels in CHR subjects were also associated with a poor functional outcome (p = 0.015).

**Conclusions and Relevance:** These findings indicate that adverse clinical outcomes in people at CHR for psychosis are associated with an increase in baseline hippocampal glutamate levels, as well as in myo-inositol and creatine levels. This suggests that these measures could contribute to the stratification of CHR subjects according to future clinical outcomes.

## Introduction

Both preclinical and human studies suggest that hippocampal dysfunction plays a critical role in the onset of psychosis. Data from animal models indicate that neural hyperactivity in the hippocampus drives an increase in subcortical dopamine function through glutamatergic projections to the striatum.[1,2] Neuroimaging studies in people at Clinical High Risk (CHR) for psychosis suggest that the subsequent onset of psychosis is associated with changes in several measures of hippocampal integrity, including hypermetabolism,[3] increased resting perfusion,[4] altered activation in response to cognitive tasks,[5] and reduced grey matter volume.[3,6,7] The mechanisms underlying these changes are unclear, but experimental work in rodents suggests that they may be secondary to increases in hippocampal glutamate levels.[3]

A large body of independent research suggests that psychosis involves alterations in glutamate neurotransmission.[8,9] For example, non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine and phencyclidine can induce psychotic symptoms in healthy individuals,[10,11] and exacerbate psychotic symptoms in patients with a psychotic disorder.[12,13] In addition, autoantibodies to the NMDA receptor are present in a proportion of patients with psychosis,[14,15] and several risk genes associated with psychosis code for proteins involved in glutamatergic neurotransmission.[16]

Brain glutamate levels can be measured *in vivo* using Proton Magnetic Resonance Spectroscopy ($^{1}$H-MRS). A meta-analysis on levels of glutamatergic metabolites in patients with psychosis suggests that there are elevations in several brain regions, including increased concentrations of Glx (the combined measure of glutamine and glutamate) in the medial temporal lobe.[17] The few $^{1}$H-MRS studies that examined hippocampal glutamate in CHR subjects did not find differences relative to healthy controls,[18-20] but they did not investigate hippocampal glutamate concentrations in relation to clinical outcomes. However, relationships between glutamate levels and adverse outcomes in CHR subjects have been identified in $^{1}$H-MRS studies of other brain regions. De la Fuente Sandoval and colleagues found that glutamate levels in the striatum were elevated in CHR subjects who developed

psychosis subsequent to scanning, but not in CHR subjects who did not.[21] Furthermore, in the thalamus, low baseline glutamate levels were associated with poor functioning at clinical follow up,[22] and with a failure to achieve symptomatic remission from the CHR state.[23]

The primary aim of the present study was to investigate the relationship between hippocampal glutamate levels in CHR subjects and subsequent clinical outcomes. We used $^{1}$H-MRS to examine a sample of CHR subjects and a group of healthy volunteers. CHR subjects were then followed up to determine their clinical outcomes, which were assessed in terms of transition/non-transition to psychosis and level of overall functioning. Our primary hypothesis was that in CHR subjects, elevated hippocampal glutamate levels at baseline would be associated with adverse clinical outcomes: the onset of psychosis and a low level of overall functioning. In view of the evidence of a more general disruption of hippocampal function prior to the onset of psychosis, we also explored the relationship between clinical outcomes and levels of other hippocampal metabolites.

## Materials and Methods

*Participants*

A total of 116 participants took part in the study. The study had National Health Service UK Research Ethics Committee (coREC) approval, and all participants gave written informed consent before taking part.

CHR subjects (n = 86) were recruited via early detection services for people at CHR for psychosis: Outreach and Support in South London (OASIS), the West London Early Intervention service, and Cambridge Early Onset service (CAMEO).[24] The diagnosis was made using the Comprehensive Assessment of the At-Risk Mental State (CAARMS).[25] Subjects met one or more of the following criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (a history of one or more episodes of frank psychotic symptoms that resolved spontaneously within 1 week in the past year), or (c) a recent decline in function, together with either the presence of schizotypal personality disorder or a family history of psychosis in a first-degree relative.

Healthy controls (HC, n=30) were recruited from the local community. All were native English speakers, had no history of psychiatric disorder and none were using prescription medication.

On the day of scanning, symptomatology was assessed using the CAARMS.[26] Psychosocial functioning was examined with the Global Assessment of Function (GAF) scale,[26] and measures of anxiety and depression were obtained using the Hamilton rating scales (HAM-A and HAM-D, respectively).[27,28] Pre-morbid IQ was assessed with the National Adult Reading Test (NART),[29] and handedness was determined using the Annett Handedness Scale.[30] Subjects provided information on tobacco use (cigarettes per day) and cannabis use (0=no use, 1=experimental use, 2=occasional use, 3=moderate use, 4=severe use). Participants were excluded if they reported illicit substance use in the week prior to scanning or alcohol use in the 24 hours prior to scanning, if they met DSM-IV criteria for a

substance misuse or dependence disorder, or had a history of neurological or prior psychotic disorders.

*Clinical follow up*

The CHR sample was followed up to determine clinical outcomes. Fifty-seven subjects underwent a face-to-face clinical re-assessment. The mean interval between the baseline and follow up assessments was 18.5 months (SD= 9.6 months; range 4 - 59 months). Clinical outcome was assessed as a) transition/non-transition to psychosis defined using the criteria in the CAARMS,[25] and b) the level of overall functioning determined using the GAF scale. A minority of the CHR sample could not be re-interviewed (n=29), either because they were too unwell, declined to be seen, or were uncontactable. In these cases, transition to psychosis was determined from information in their clinical records, but it was not possible to rate their overall functioning.

*$^{1}$H-Magnetic Resonance Spectroscopy*

Images were obtained on a General Electric (Milwaukee, Wisconsin) 3.0 Tesla HDx MR system. $^{1}$H-MRS spectra (PRESS - Point RESolved Spectroscopy; TE = 30 ms; TR = 3000 ms; 96 averages) were acquired in the left hippocampus (Figure 1).[19] We employed the standard GE probe (proton brain examination) sequence, which uses a standardised chemically selective suppression (CHESS) water suppression routine. For each metabolite spectrum, unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition. Shimming and water suppression were optimised, with auto-prescan performed twice before each scan. Using standardized protocols, the hippocampal voxel (20x20x15 mm; right-left, anterior-posterior, superior-inferior) was prescribed from the structural T1 scan.

*Structural Magnetic Resonance Imaging*

Structural images were acquired in the same session using a whole-brain three-dimensional sagittal T1-weighted scan, with parameters based on the Alzheimer's Disease Neuroimaging Initiative (ADNI) (TE = 2.85 ms; TR = 6.98 ms; inversion time = 400 ms; flip angle = 11º; voxel size 1.0x1.0x1.2 mm; for full details see http://adni.loni.usc.edu/methods/mri-analysis/mri-acquisition/). Structural T1 images were segmented into grey matter, white matter, and cerebrospinal fluid (CSF) using Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK) to allow correction of the $^{1}$H-MRS data for partial volume CSF contamination.

*$^{1}$H-MRS Data Processing*

All spectra were analysed with LCModel version 6.3-0A[31] using a standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine (choline), glycine, myo-inositol, L-lactate, N-acetylaspartate (NAA), N-acetylaspartylglutamate, phosphocholine, and taurine), acquired with the same field strength (3 Tesla), localisation sequence (PRESS), and echo time (30 ms). Model metabolites and concentrations used in the basis set are fully detailed in the LCModel manual (http://s-provencher-.com/pages/lcmmanual.shtml). Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds of >20% as reported by LCModel) were excluded from further analysis, and water-scaled glutamate, Glx, myo-inositol, creatine, choline and NAA values were corrected for voxel tissue composition (see Supplementary Methods). See for scan quality parameters and voxel tissue composition Supplementary Tables 1 - 3.

*Statistics*

Group differences in clinical and demographic variables were assessed using two-sample t-tests or chi-squared tests. To examine the relationship between metabolite levels and clinical outcomes, the CHR sample was dichotomised according to: a) transition vs. non-transition to psychosis[25] and b) good overall functioning (GAF≥65) vs. poor overall functioning (GAF<65) at follow-up.[22] As the primary hypothesis related to the relationship between hippocampal glutamate levels and clinical outcomes, general linear models were used to identify group differences in glutamate levels between the respective CHR subgroups and healthy controls, as well as between the total CHR group and controls. Glutamate concentrations were included as the dependent variable with group as the independent variable ($p<0.05$ considered statistically significant). Concentrations of other metabolites (Glx, myo-inositol, creatine, choline and NAA) were also assessed in exploratory general linear models, and were corrected for multiple comparisons (thresholded $p < 0.05/5 = 0.01$). Multiple regression analyses were performed to examine how hippocampal glutamate levels predicted clinical outcomes. Age and tobacco consumption were included as covariates in all analyses because both can influence neurometabolite levels.[32,33] All analyses were performed in SPSS 22. Effect sizes are reported as Hedges' g.

## Results

*Demographic, clinical and medication data*

All CHR participants (n=86) met the Attenuated Psychotic Symptoms diagnostic criteria, with some also fulfilling the BLIPS (n=5) or schizotypy / familial risk criteria (n=2). At the time of scanning, the majority of the CHR sample were antipsychotic naive (72/86). Ten CHR subjects were receiving low doses of antipsychotic medication (less than 1.5 mg haloperidol equivalents per day).

The CHR and HC groups did not differ significantly in terms of gender, handedness, IQ or cannabis use. However, the CHR group was younger, had fewer years of education, and smoked more cigarettes. As expected, they also had higher HAM-A and HAM-D scores and lower levels of functioning at baseline compared to controls (see Table 1 and Supplementary Tables 4 and 5).

At follow up, 12 CHR subjects developed a first episode of psychosis (CHR-Transition, CHR-T) and 74 CHR subjects did not (CHR-Non-Transition, CHR-NT). When dichotomised according to their GAF scores, 19 CHR subjects showed a good overall functioning (GAF≥65; CHR-Good Outcome, CHR-GO), whereas 38 CHR subjects had a poor functional outcome (GAF<65; CHR-Poor Outcome, CHR-PO).

The CHR-T group had higher baseline HAM-A and HAM-D scores than the CHR-NT group, but there were no other significant differences in symptom ratings or demographic measures between these subgroups. There were no significant differences at baseline in any clinical or demographic measure between the CHR-GO and CHR-PO subgroups (see Table 1 and Supplementary Tables 4 and 5).

*Hippocampal metabolite differences*

Transition to psychosis

The CHR-T subgroup had significantly higher hippocampal glutamate levels than the CHR-NT subgroup ($F_{3,81}$=4.03, p=0.048; effect size 0.57), and there was a trend for higher glutamate levels relative to HCs ($F_{3,38}$=3.54, p=0.07; effect size 0.73). There was no difference in glutamate levels between the CHR-NT and HC groups (Figure 2).

Exploratory testing revealed that the CHR-T subjects also had significantly higher hippocampal myo-inositol and creatine levels than the CHR-NT subjects ($F_{3,81}$=10.26, p=0.002 and $F_{3,82}$=7.26, p=0.009, respectively), and higher myo-inositol levels than the HCs ($F_{3,38}$=8.82, p=0.005). The differences in myo-inositol levels were particularly large: in CHR-T subjects, the concentration was 21.8% higher than in CHR-NT subjects (effect size 1.01), and 22.8% higher than in HCs (effect size 0.98). In contrast, there were no significant differences between CHR-NT subjects and HCs in the levels of any hippocampal metabolite (Figure 2).

Functional outcome

CHR subjects with a poor functional outcome had significantly higher glutamate levels than those with a good outcome ($F_{3,52}$ =6.39, p = 0.015; effect size 0.75). There were no other significant differences in metabolite levels. None of the metabolite levels were significantly different between either of the CHR functional outcome subgroups and the HCs (Figure 3).

Prediction of outcome

Results from logistic regression analyses showed that hippocampal glutamate levels significantly predicted clinical outcome, both in terms of transition/non-transition to psychosis (β=0.48, OR=1.61, p=0.05) and overall functioning (β=0.53, OR=1.71, p=0.02).

281 All CHRs vs Healthy controls

282 There were no significant group differences in any of the metabolite concentrations

283 between the total CHR group (independent of outcomes) and HCs (Table 2).

## Discussion

To our knowledge, this is the largest $^{1}$H-MRS study of metabolite levels in CHR subjects conducted to date. The overall finding was that adverse clinical outcomes in these subjects were associated with increases in hippocampal glutamate levels, as well as in the levels of a number of other metabolites. Thus, the subsequent onset of psychosis was linked to higher baseline levels of glutamate, myo-inositol, and creatine at first clinical presentation, while a low level of functioning at follow up was associated with increased glutamate levels. In contrast to the differences within the CHR group, there were no differences in metabolite levels between the total CHR sample and healthy controls, or between CHR subjects who did not have adverse clinical outcomes and controls.

In line with our main hypothesis, increased hippocampal glutamate levels at baseline were associated with adverse clinical outcomes at follow up: both the onset of psychosis and a low level of overall functioning. These observations are consistent with preclinical and human data implicating hippocampal dysfunction and glutamate transmission in the onset of psychosis. In preclinical models, neural hyperactivity of the hippocampus drives an increase in subcortical dopamine activity through glutamatergic projections to the striatum.[1,2] Neuroimaging data from CHR samples indicate that the subgroup of subjects who subsequently develop psychosis have increased resting hippocampal metabolism[3] and perfusion,[4] altered hippocampal response to cognitive tasks,[5] and smaller hippocampal volumes.[3,6,7] As previously suggested by experimental work in rodents,[3] one possibility is that these alterations are secondary to increases in hippocampal glutamate levels. Consistent with data from previous $^{1}$H-MRS studies,[18-20] there were no differences in hippocampal glutamate levels between the CHR-NT or total CHR group (independent of clinical outcomes) and controls. This is also in line with previous studies using other neuroimaging modalities, which showed differences within the CHR group rather than between the total CHR group and controls in terms of hippocampal volume,[7] brain activity patterns,[5] and dopamine synthesis capacity.[34] However, adverse clinical outcomes in CHR subjects have been linked to altered glutamate metabolite levels in other brain regions. De la

Fuente Sandoval and colleagues demonstrated increased baseline glutamate levels in the striatum of those CHR subjects who went on to develop a first episode of psychosis.[21] Allen et al found that a poor functional outcome in CHR subjects was linked to lower glutamate concentrations in the thalamus at baseline,[22] while Egerton and colleagues reported that lower thalamic glutamate levels were associated with a failure to achieve symptomatic remission from the CHR state.[23]

Our second main finding was that adverse clinical outcomes were also associated with elevations in the levels of myo-inositol and creatine in the hippocampus. For both these metabolites and for glutamate, the pattern of group differences was strikingly similar, with higher levels in CHR subjects who developed psychosis relative to those who did not become psychotic (Figure 2). This consistent pattern across different metabolites suggests that the onset of psychosis was associated with a more general increase in hippocampal metabolite levels, as opposed to a change that was specific to glutamate. Such a widespread change in metabolites is consistent with previous evidence that the subsequent onset of psychosis in CHR subjects is linked to an overall change in hippocampal integrity, as indicated by hypermetabolism,[3] increased resting perfusion,[4] and reduced grey matter volume.[3,6,7] Although previous $^{1}$H-MRS studies in CHR subjects have not reported associations between clinical outcomes and changes across multiple metabolites, higher levels of glutamate, myo-inositol and choline have been described in the striatum in medication-naïve first episode patients relative to controls.[35,36]

In the present study, the elevation in myo-inositol levels was relatively large, with concentrations around 22% higher (and effect sizes around 1.0) in the CHR subjects who developed psychosis than in both those who did not and healthy controls. Myo-inositol is regarded as a marker for glial activation,[37] and independent data from PET studies of glial activity have reported that this is increased in the hippocampus (and in other regions) in both CHR subjects and in patients with psychosis.[38-40] $^{1}$H-MRS studies of myo-inositol and creatine levels in the hippocampus in patients with chronic psychosis have not found a consistent pattern of differences in comparison to controls.[41-44] However, inconsistencies in

$^{1}$H-MRS findings in patients with chronic psychosis may be related to confounding effects of age, duration of illness and treatment,[17] and alterations in metabolite levels may be more marked in the early than the later stages of the disorder.[17,45]

Because the number of CHR subjects that went on to develop psychosis was modest (n=12), we cannot exclude the possibility that additional findings were undetected because of limited statistical power. This issue could be addressed by studying larger CHR samples, which can be achieved by combining $^{1}$H-MRS data from multiple centres.[46] Although the mean time of clinical follow up was 18.5 months, the variance in duration of follow up intervals was fairly high (range 4 - 59 months). The main reason for this was that follow up times were not a priori defined. Importantly, a recent study of transitions in our early intervention service showed that about 60% of the transitions occurred in the first 18 months, with the rate strongly decreasing thereafter.[47] Our findings could be confounded by effects of antipsychotic treatment. This is unlikely, however, because the vast majority of the CHR subjects (72/86) were naive to antipsychotic drugs, and if treated, low doses of antipsychotics were prescribed. Moreover, there were no significant differences between medicated and unmedicated CHR subjects for any of the hippocampal metabolites. Residual effects of illicit substance use cannot be excluded because this was checked by self-report rather than by urine toxicology screening. Given the dimensions and orientation of our $^{1}$H-MRS voxel, other medial temporal lobe regions than the hippocampus, such as the parahippocampal gyrus, are also included in the voxel, which may have confounded our results. Consequently, although $^{1}$H-MRS values were corrected for CSF volume, we cannot exclude the possibility that increased metabolite concentrations are related to changes in hippocampal volume. Finally, using conventional $^{1}$H-MRS, it is not possible to determine whether differences in glutamate levels are related to neurotransmission or metabolism, an issue which may be addressed by using more sophisticated MRS protocols.[48]

In conclusion, our study suggests that clinical outcomes in people at CHR for psychosis are related to baseline hippocampal metabolite concentrations. While the findings require

replication, they raise the possibility that measuring hippocampal metabolite levels could contribute to the stratification of CHR subjects according to future clinical outcomes.

## Author Contributions

Drs Bossong and Antoniades had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Howes, Stone, Allen, McGuire

Acquisition, analysis, or interpretation of data: All authors

Drafting of the manuscript: Bossong, Antoniades, McGuire

Critical revision of the manuscript for important intellectual content: All authors

Statistical analysis: Bossong, Antoniades

Obtained funding: Bossong, Howes, Stone, Allen, McGuire

Administrative, technical, or material support: Stone

Study supervision: Allen, McGuire

## Conflict of Interest Disclosures

No disclosures were reported.


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The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Information on previous presentation of the information reported in the manuscript**

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## Figure Legends

**Figure 1. Example of $^{1}$H-MRS voxel placement and spectrum.**

Example of $^{1}$H-MRS voxel placement in the left hippocampus (left), and $^{1}$H-MRS spectrum obtained from this voxel (black line) and the overlay of the spectral fit (red line) (right).

**Figure 2. Hippocampal metabolite concentrations and the transition to psychosis.**

Left hippocampal metabolite concentrations in healthy controls (HC; n=30), clinical high risk subjects who did not make a transition to psychosis (CHR-NT; n=74), and clinical high risk subjects who made a transition to psychosis (CHR-T; n=12). At first presentation, the CHR-T subgroup showed significantly higher hippocampal levels of glutamate, myo-inositol and creatine than the CHR-NT subgroup, and higher concentrations of myo-inositol than HCs. Glx, combined measure of glutamine and glutamate; NAA, N-acetylaspartate. * Significant difference between groups.

**Figure 3. Hippocampal metabolite concentrations and functional outcome.**

Left hippocampal metabolite concentrations in healthy controls (HC; n=30), clinical high risk subjects with a good functional outcome (CHR-GO; n=19), and clinical high risk subjects with a poor functional outcome (CHR-PO; n=38). At first presentation, the CHR-PO subgroup showed significantly higher hippocampal glutamate levels than the CHR-GO subgroup. Glx, combined measure of glutamine and glutamate; NAA, N-acetylaspartate. * Significant difference between groups.

560 **Table 1** Baseline demographic, clinical and medication data.

| **Measure** | **HC**<br>**N=30** | **CHR**<br>**N=86** | **p** | **CHR-NT**<br>**N=74** | **CHR-T**<br>**N=12** | **p** | **CHR-GO**<br>**N=19** | **CHR-PO**<br>**N=38** | **p** |
|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 24.7 (3.8) | 22.4 (3.5) | 0.005 | 22.5 (3.7) | 22.1 (2.8) | 0.71 | 22.1 (3.3) | 23.3 (3.9) | 0.24 |
| NART IQ | 104.8 (13.6) | 103.9 (12.2) | 0.75 | 104.7 (12.0) | 99.3 (12.5) | 0.17 | 106.3 (9.2) | 105.2 (13.3) | 0.76 |
| Years of education | 15.8 (3.3) | 14.5 (2.2) | 0.021 | 14.6 (2.1) | 14.3 (2.5) | 0.71 | 14.8 (2.3) | 14.3 (2.1) | 0.45 |
| CAARMS | | | | | | | | | |
| Positive score | _ | 10.2 (4.2) | _ | 10.0 (4.4) | 11.3 (3.3) | 0.33 | 10.2 (4.2) | 10.5 (4.3) | 0.75 |
| Negative score | _ | 5.5 (4.2) | _ | 5.4 (4.1) | 6.1 (4.9) | 0.63 | 6.4 (3.9) | 5.5 (4.3) | 0.44 |
| Total score | _ | 43.6 (21.8) | _ | 42.8 (21.7) | 48.9 (22.9) | 0.39 | 45.5 (19.9) | 42.7 (20.8) | 0.63 |
| Baseline GAF score | 93.0 (5.1) | 57.7 (9.4) | <0.001 | 58.4 (9.5) | 53.6 (7.6) | 0.11 | 56.8 (8.9) | 54.8 (9.6) | 0.48 |
| HAM-A score | 3.6 (4.2) | 18.4 (11.0) | <0.001 | 17.1 (10.3) | 27.3 (12.1) | 0.01 | 18.3 (12.8) | 20.6 (11.8) | 0.57 |
| HAM-D score | 1.7 (3.6) | 17.4 (11.0) | <0.001 | 16.4 (11.1) | 24.4 (8.2) | 0.05 | 15.5 (10.3) | 19.6 (11.9) | 0.28 |
| Tobacco (cigarettes/day) | 1.9 (3.3) | 5.5 (8.5) | 0.024 | 6.1 (8.9) | 1.83 (3.6) | 0.11 | 6.7 (10.0) | 5.7 (8.5) | 0.70 |
| Alcohol (units/day) | 1.6 (2.2) | 1.5 (3.1) | 0.82 | 1.6 (3.4) | 0.83 (0.72) | 0.44 | 1.4 (1.0) | 1.5 (4.0) | 0.91 |
| Cannabis (median)[a] | 0 | 0 | 0.71 | 0 | 0 | 0.81 | 1 | 0 | 0.18 |
| | | | | | | | | | |

| | N (%) | N (%) | p | N (%) | N (%) | p | N (%) | N (%) | p |
|---|---|---|---|---|---|---|---|---|---|
| Antipsychotic medication | 0 (0) | 10 (12) | _ | 10 (13) | 1 (0.08) | 0.63 | 3 (16) | 2 (5) | 0.19 |
| Male | 14 (47) | 50 (58) | 0.28 | 43 (58) | 7 (58) | 0.95 | 9 (47) | 23 (61) | 0.35 |
| Right-handed | 27 (90) | 70 (81) | 0.13 | 60 (80) | 11 (92) | 0.33 | 17 (90) | 29 (76) | 0.24 |

[a] 0=never, 1=experimental use, 2=occasional use, 3=moderate use, 4=severe use.

CAARMS, Comprehensive Assessment for the At-Risk Mental State; CHR, clinical high risk; CHR-GO, clinical high risk good outcome; CHR-NT, clinical high risk non-transition; CHR-PO, clinical high risk poor outcome; CHR-T, clinical high risk transition; GAF, Global Assessment of Functioning scale; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; HC, healthy controls; NART, National Adult Reading Test.

**Table 2** Mean (SD) hippocampal metabolite levels in healthy controls (HC; n=30) and clinical high risk subjects (CHR; n=86).

| | **HC** **N=30** | **CHR** **N=86** | **Analysis** | |
|---|---|---|---|---|
| | | | **F** | **p** |
| Glutamate | 8.31 (1.12) | 8.45 (1.48) | 0.49 | 0.48 |
| Glx | 11.61 (2.23) | 11.57 (2.45) | 0.01 | 0.92 |
| Myo-inositol | 6.19 (1.51) | 6.43 (1.42) | 2.11 | 0.15 |
| Creatine | 7.42 (1.10) | 7.43 (1.08) | 0.20 | 0.66 |
| Choline | 2.30 (0.40) | 2.41 (0.42) | 1.95 | 0.17 |
| NAA | 9.34 (1.43) | 9.36 (1.13) | 0.002 | 0.97 |

CHR, clinical high risk; Glx, combined measure of glutamine and glutamate; HC, healthy controls; NAA, N-acetylaspartate.

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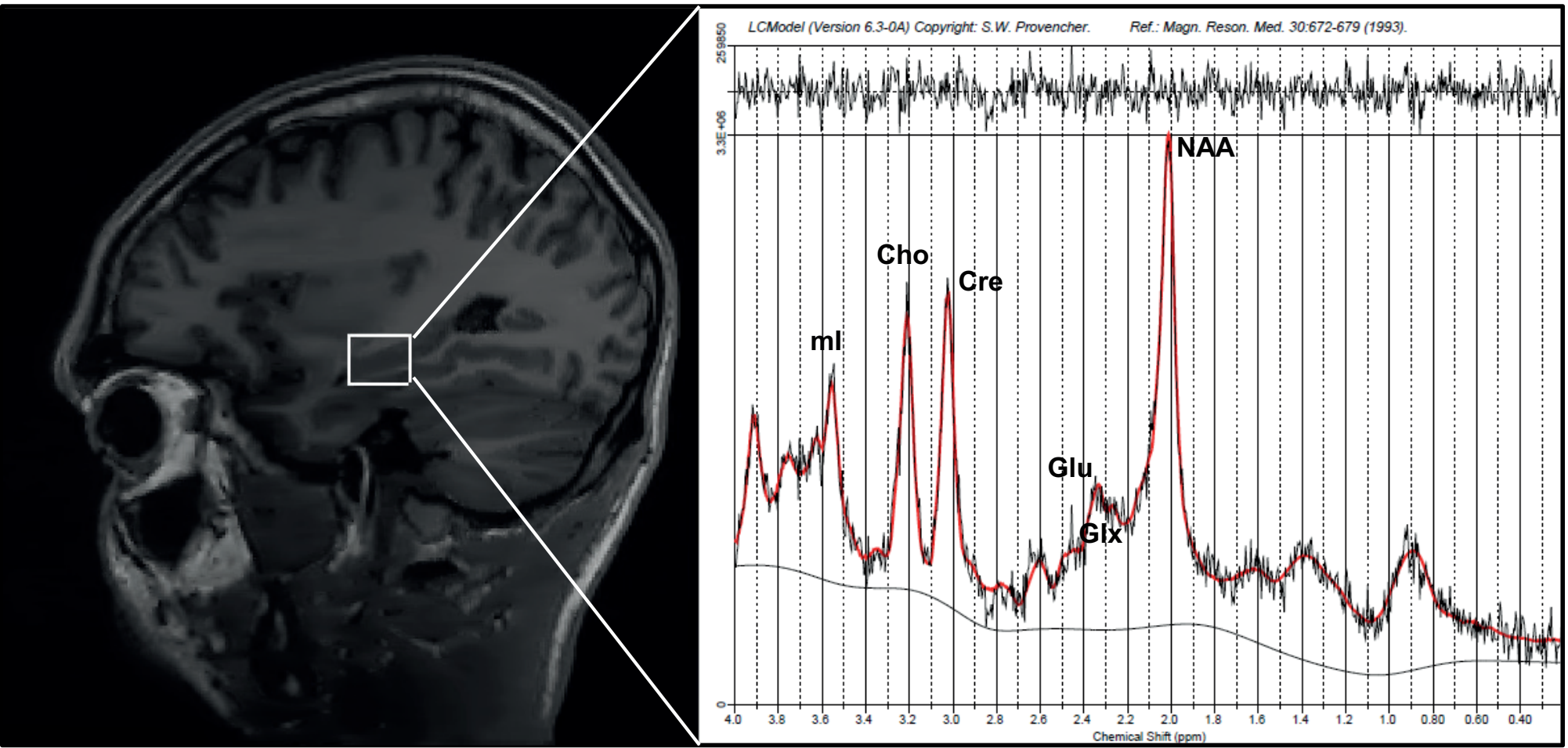
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25.9850
3.3E+06
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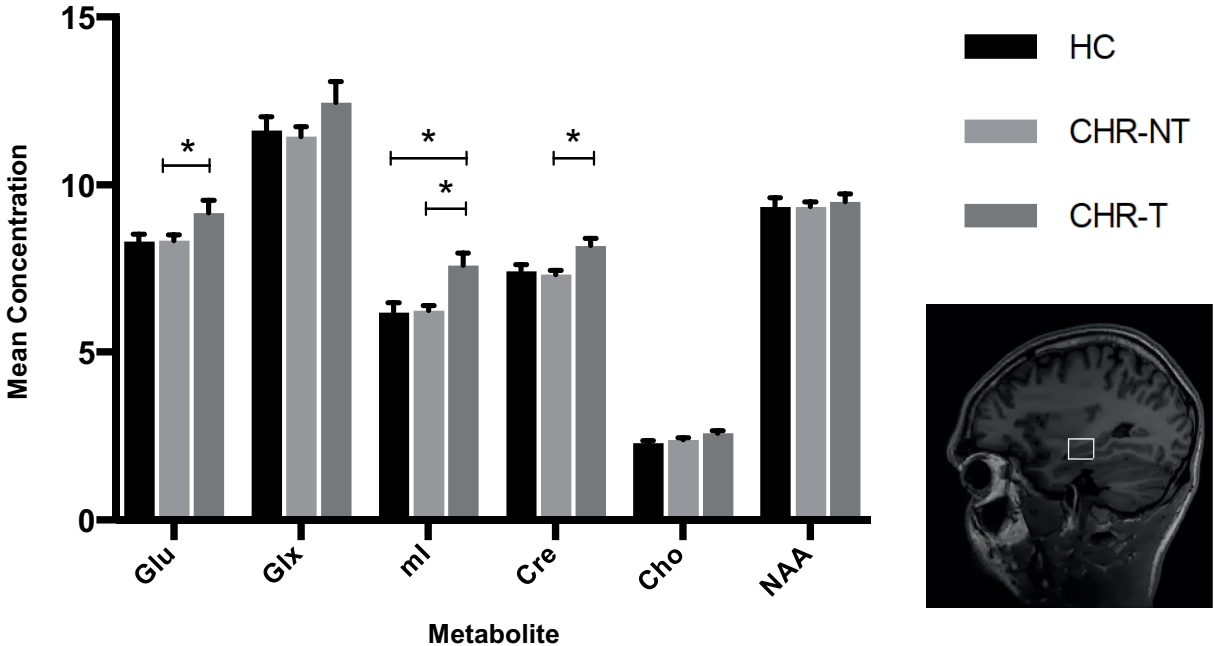
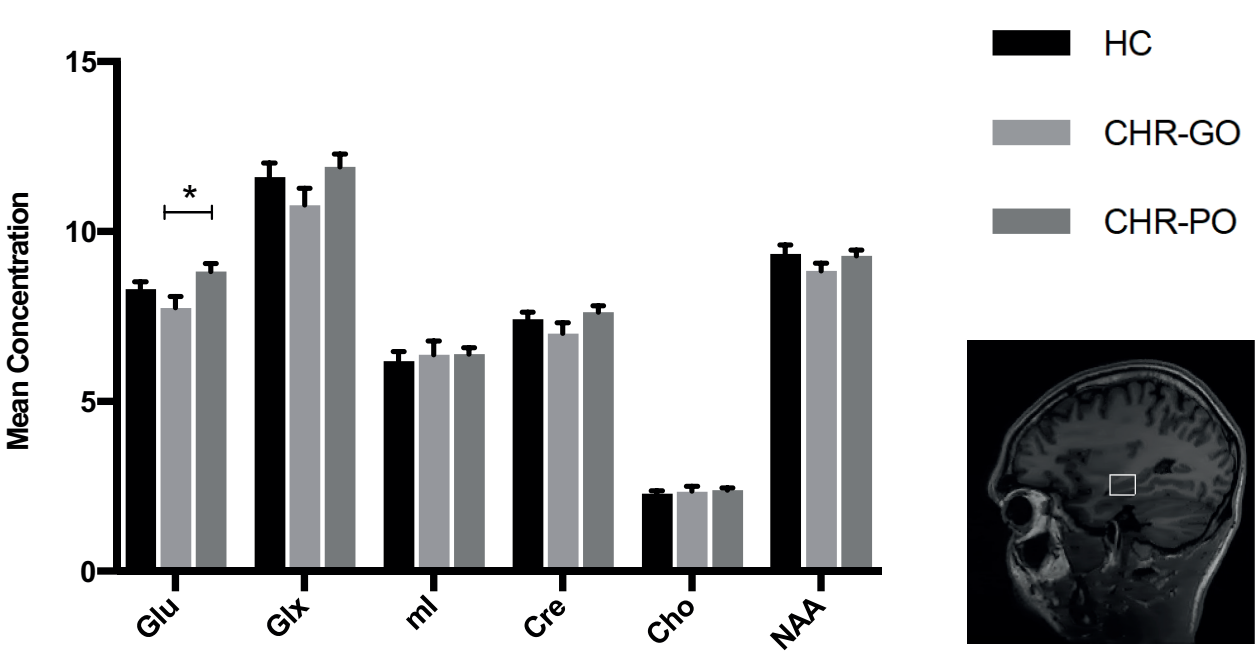
Chemical Shift (ppm)




| Metabolite | HC N=30 | CHR-NT N=74 | CHR-T N=12 | CHR-NT vs CHR-T p (effect size) | CHR-T vs HC p (effect size) | CHR-NT vs HC p (effect size) |
|---|---|---|---|---|---|---|
| Glutamate | 8.31 (1.12) | 8.33 (1.48) | 9.16 (1.28) | **0.048** (0.57) | 0.07 (0.73) | 0.70 (0.01) |
| Glx | 11.61 (2.23) | 11.43 (2.48) | 12.44 (2.16) | 0.18 (0.41) | 0.32 (0.38) | 0.89 (0.07) |
| Myo-Inositol | 6.19 (1.51) | 6.24 (1.36) | 7.60 (1.23) | **0.002** (1.01) | **0.005** (0.98) | 0.43 (0.04) |
| Creatine | 7.42 (1.10) | 7.32 (1.09) | 8.18 (0.74) | **0.009** (0.82) | 0.035 (0.75) | 0.90 (0.09) |
| Choline | 2.30 (0.40) | 2.40 (0.43) | 2.59 (0.21) | 0.06 (0.47) | 0.020 (0.81) | 0.35 (0.23) |
| NAA | 9.34 (1.43) | 9.34 (1.18) | 9.49 (0.80) | 0.63 (0.13) | 0.66 (0.12) | 0.92 (0.07) |




| Metabolite | HC N=30 | CHR-GO N=19 | CHR-PO N=38 | CHR-GO vs CHR-PO p (effect size) | CHR-PO vs HC p (effect size) | CHR-GO vs HC p (effect size) |
|---|---|---|---|---|---|---|
| Glutamate | 8.31 (1.12) | 7.76 (1.40) | 8.83 (1.43) | **0.015** (0.75) | 0.19 (0.40) | 0.35 (0.45) |
| Glx | 11.61 (2.23) | 10.78 (2.11) | 11.90 (2.38) | 0.09 (0.49) | 0.59 (0.13) | 0.21 (0.38) |
| Myo-Inositol | 6.19 (1.51) | 6.37 (1.70) | 6.39 (1.17) | 0.80 (0.01) | 0.36 (0.15) | 0.35 (0.11) |
| Creatine | 7.42 (1.10) | 7.01 (1.31) | 7.63 (1.18) | 0.08 (0.51) | 0.55 (0.18) | 0.62 (0.35) |
| Choline | 2.30 (0.40) | 2.36 (0.56) | 2.40 (0.37) | 0.90 (0.09) | 0.48 (0.26) | 0.28 (0.13) |
| NAA | 9.34 (1.43) | 8.84 (0.97) | 9.28 (1.06) | 0.16 (0.43) | 0.65 (0.05) | 0.41 (0.39) |