Supplementary Materials for

Longitudinal changes in brain metabolites in healthy controls and patients with first episode psychosis: a 7-Tesla MRS study

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Table S1. Sample size for each metabolite in each brain region.

Abbreviations: GABA, γ-aminobutyric acid; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; and OFR, orbital frontal cortex.

region	metabolite	control	patient
ACC	Glutathione	43	31
	GABA	42	33
	Glutamate	44	36
	Glutamine	43	33
	total Choline	44	33
	Lactate	44	30
	Myo-inositol	45	35
	NAA	47	34
	NAAG	45	25
	total Creatine	46	35
DLPFC	Glutathione	38	29
	GABA	42	30
	Glutamate	45	32
	Glutamine	41	32
	total Choline	40	30
	Lactate	28	19
	Myo-inositol	43	32
	NAA	43	31
	NAAG	29	26
	total Creatine	45	31
Thalamus	Glutathione	45	34
	GABA	44	34
	Glutamate	45	35
	Glutamine	42	30
	total Choline	43	33
	Lactate	28	29
	Myo-inositol	45	36
	NAA	44	35
	NAAG	42	30
	total Creatine	45	34
Centrum semiovale	Glutathione	43	29
	GABA	41	31
	Glutamate	46	30
	Glutamine	42	29
	total Choline	44	31
	Lactate	43	30
	Myo-inositol	45	32
	NAA	44	31
	NAAG	43	31
	total Creatine	46	30
OFR	Glutathione	38	25
	GABA	41	25
	Glutamate	39	26
	Glutamine	37	25
	total Choline	38	26
	Lactate	27	17
	Myo-inositol	41	26
	NAA	39	26
	NAAG	33	16
	total Creatine	38	26

Table S2. Analysis results for attrition bias in metabolite levels of healthy controls.

We compared the metabolite levels in 5 brain regions between the full cohort and the longitudinal cohort. There was not a significant difference observed in any metabolite. This suggests that there is not a bias in the neurometabolic profiles of healthy controls that returned for the longitudinal study. Abbreviations: GABA, γ-aminobutyric acid; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; and OFR, orbital frontal cortex.

region	metabolite	p-value	q-value
ACC	Glutathione	0.52	0.97
ACC	GABA	0.78	0.97
ACC	Glutamate	0.84	0.97
ACC	Glutamine	0.93	0.97
ACC	total Choline	0.26	0.97
ACC	Lactate	0.69	0.97
ACC	Myo-inositol	0.47	0.97
ACC	NAA	0.79	0.97
ACC	NAAG	0.91	0.97
ACC	total Creatine	0.55	0.97
DLPFC	Glutathione	0.62	0.97
DLPFC	GABA	0.81	0.97
DLPFC	Glutamate	0.84	0.97
DLPFC	Glutamine	0.80	0.97
DLPFC	total Choline	0.37	0.97
DLPFC	Lactate	0.98	0.98
DLPFC	Myo-inositol	0.86	0.97
DLPFC	NAA	0.79	0.97
DLPFC	NAAG	0.39	0.97
DLPFC	total Creatine	0.72	0.97
Thalamus	Glutathione	0.70	0.97
Thalamus	GABA	0.92	0.97
Thalamus	Glutamate	0.07	0.97
Thalamus	Glutamine	0.64	0.97
Thalamus	total Choline	0.38	0.97
Thalamus	Lactate	0.97	0.98
Thalamus	Myo-inositol	0.56	0.97
Thalamus	NAA	0.67	0.97
Thalamus	NAAG	0.84	0.97
Thalamus	total Creatine	0.56	0.97

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Centrum semiovale	Glutathione	0.48	0.97
Centrum semiovale	GABA	0.85	0.97
Centrum semiovale	Glutamate	0.58	0.97
Centrum semiovale	Glutamine	0.75	0.97
Centrum semiovale	total Choline	0.70	0.97
Centrum semiovale	Lactate	0.13	0.97
Centrum semiovale	Myo-inositol	0.64	0.97
Centrum semiovale	NAA	0.90	0.97
Centrum semiovale	NAAG	0.92	0.97
Centrum semiovale	total Creatine	0.78	0.97
OFR	Glutathione	0.16	0.97
OFR	GABA	0.30	0.97
OFR	Glutamate	0.76	0.97
OFR	Glutamine	0.49	0.97
OFR	total Choline	0.75	0.97
OFR	Lactate	0.76	0.97
OFR	Myo-inositol	0.91	0.97
OFR	NAA	0.62	0.97
OFR	NAAG	0.89	0.97
OFR	total Creatine	0.67	0.97

Table S3. Analysis results for attrition bias in metabolite levels of first episode psychosis patients.

We compared the metabolite levels in 5 brain regions between the full cohort and the longitudinal cohort. There was not a significant difference observed in any metabolite. This suggests that there is no bias in the neurometabolic profiles of patients that returned for the longitudinal study. Abbreviations: GABA, γ-aminobutyric acid; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; and OFR, orbital frontal cortex.

region	metabolite	p-value	q-value
ACC	Glutathione	0.19	0.96
ACC	GABA	0.66	0.96
ACC	Glutamate	0.32	0.96
ACC	Glutamine	0.53	0.96
ACC	total Choline	0.67	0.96
ACC	Lactate	0.35	0.96
ACC	Myo-inositol	0.57	0.96
ACC	NAA	0.52	0.96
ACC	NAAG	0.74	0.96
ACC	total Creatine	0.80	0.96
DLPFC	Glutathione	0.67	0.96
DLPFC	GABA	0.95	0.96
DLPFC	Glutamate	0.71	0.96
DLPFC	Glutamine	0.86	0.96
DLPFC	total Choline	0.89	0.96
DLPFC	Lactate	0.88	0.96
DLPFC	Myo-inositol	0.92	0.96
DLPFC	NAA	0.75	0.96
DLPFC	NAAG	0.62	0.96
DLPFC	total Creatine	0.89	0.96
Thalamus	Glutathione	0.36	0.96
Thalamus	GABA	0.55	0.96
Thalamus	Glutamate	0.62	0.96
Thalamus	Glutamine	0.58	0.96
Thalamus	total Choline	0.84	0.96
Thalamus	Lactate	0.36	0.96
Thalamus	Myo-inositol	0.37	0.96
Thalamus	NAA	0.71	0.96
Thalamus	NAAG	0.66	0.96
Thalamus	total Creatine	0.49	0.96

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Centrum semiovale	Glutathione	0.91	0.96
Centrum semiovale	GABA	0.60	0.96
Centrum semiovale	Glutamate	0.50	0.96
Centrum semiovale	Glutamine	0.53	0.96
Centrum semiovale	total Choline	0.84	0.96
Centrum semiovale	Lactate	0.70	0.96
Centrum semiovale	Myo-inositol	0.74	0.96
Centrum semiovale	NAA	0.39	0.96
Centrum semiovale	NAAG	0.87	0.96
Centrum semiovale	total Creatine	0.85	0.96
OFR	Glutathione	0.79	0.96
OFR	GABA	0.66	0.96
OFR	Glutamate	0.52	0.96
OFR	Glutamine	0.35	0.96
OFR	total Choline	0.88	0.96
OFR	Lactate	0.74	0.96
OFR	Myo-inositol	0.79	0.96
OFR	NAA	0.39	0.96
OFR	NAAG	0.70	0.96
OFR	total Creatine	0.97	0.97

Table S4. Analysis results for tissue fractions

We examined annual percentage changes (denoted as % in the table) of tissue fractions and didn't observe significant changes over time. Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; OFR, orbital frontal cortex.

region	tissue fraction	% (patient)	% (control)	p-value (patient *vs* control)	q-value (patient *vs* control)
ACC	Grey matter	0.38	-0.31	0.60	0.82
ACC	White matter	-0.56	0.47	0.30	0.82
ACC	Cerebrospinal fluid	0.19	-0.16	0.57	0.82
Centrum semiovale	Grey matter	1.09	-0.88	0.31	0.82
Centrum semiovale	White matter	-1.18	0.96	0.31	0.82
Centrum semiovale	Cerebrospinal fluid	0.09	-0.07	0.54	0.82
DLPFC	Grey matter	0.23	-0.18	0.72	0.90
DLPFC	White matter	-0.70	0.55	0.43	0.82
DLPFC	Cerebrospinal fluid	0.47	-0.37	0.48	0.82
OFR	Grey matter	0.03	-0.03	0.98	0.98
OFR	White matter	0.05	-0.04	0.97	0.98
OFR	Cerebrospinal fluid	-0.09	0.07	0.41	0.82
Thalamus	Grey matter	-0.45	0.35	0.58	0.82
Thalamus	White matter	-0.09	0.07	0.92	0.98
Thalamus	Cerebrospinal fluid	0.54	-0.43	0.26	0.82

Table S5. Analysis results for group comparison of longitudinal changes between patients with non-affective and affective psychosis

We compared the annual percentage changes and didn't observe any significant differences between patients with non-affective and affective psychosis in any brain metabolites in any brain region. Abbreviations: GABA, γ-aminobutyric acid; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; and OFR, orbital frontal cortex.

region	metabolite	p-value	q-value
ACC	Glutathione	0.32	0.90
ACC	GABA	0.82	0.96
ACC	Glutamate	0.82	0.96
ACC	Glutamine	0.35	0.90
ACC	total Choline	0.31	0.90
ACC	Lactate	0.59	0.96
ACC	Myo-inositol	0.95	0.98
ACC	NAA	0.04	0.79
ACC	NAAG	0.49	0.96
ACC	total Creatine	0.46	0.96
DLPFC	Glutathione	0.79	0.96
DLPFC	GABA	0.81	0.96
DLPFC	Glutamate	0.63	0.96
DLPFC	Glutamine	0.09	0.79
DLPFC	total Choline	0.93	0.98
DLPFC	Lactate	0.13	0.79
DLPFC	Myo-inositol	0.22	0.90
DLPFC	NAA	0.12	0.79
DLPFC	NAAG	0.40	0.90
DLPFC	total Creatine	0.65	0.96
Thalamus	Glutathione	0.84	0.96
Thalamus	GABA	0.94	0.98
Thalamus	Glutamate	0.82	0.96
Thalamus	Glutamine	0.35	0.90
Thalamus	total Choline	0.97	0.98
Thalamus	Lactate	0.87	0.97
Thalamus	Myo-inositol	0.25	0.90
Thalamus	NAA	0.82	0.96
Thalamus	NAAG	0.63	0.96
Thalamus	total Creatine	0.41	0.90

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Centrum semiovale	Glutathione	0.25	0.90
Centrum semiovale	GABA	0.71	0.96
Centrum semiovale	Glutamate	0.79	0.96
Centrum semiovale	Glutamine	0.58	0.96
Centrum semiovale	total Choline	0.37	0.90
Centrum semiovale	Lactate	0.30	0.90
Centrum semiovale	Myo-inositol	0.12	0.79
Centrum semiovale	NAA	0.80	0.96
Centrum semiovale	NAAG	0.81	0.96
Centrum semiovale	total Creatine	0.53	0.96
OFR	Glutathione	0.34	0.90
OFR	GABA	0.41	0.90
OFR	Glutamate	0.18	0.90
OFR	Glutamine	0.99	0.99
OFR	total Choline	0.08	0.79
OFR	Lactate	0.63	0.96
OFR	Myo-inositol	0.28	0.90
OFR	NAA	0.71	0.96
OFR	NAAG	0.01	0.23
OFR	total Creatine	0.07	0.79

Table S6. **Impact of confounding factors on annual percentage changes of metabolites in the anterior cingulate cortex (ACC) in the first episode psychosis patient group.**

In the analysis comparing patients and controls, we adjusted for the confounding effects of age, gender, race, and smoking status. The patient group may be influenced by intrinsic factors (such as duration of illness, and positive and negative symptoms) and extrinsic factors (such as medications), which cannot be adjusted in two-group comparisons since they don't apply to controls. To evaluate the impact of these factors, including demographic variates, we performed linear regressions with gender and race (which don't change over time) as covariates. Q values (p values corrected for multiple comparison correction) were used to measure the statistical significance of impacts. Abbreviations: GABA, γ-aminobutyric acid; NAA, N-acetylaspartate; DOI, duration of illness; CPZ, chlorpromazine equivalent dose; SANS, the scale of the assessment for negative symptoms; and SAPS, the scale for the assessment of positive symptoms.

(A) associations between confounding factors and APCs of glutamate in ACC

Confounding factors	t-value	p-value	q-value
DOI	-2.24	0.04	0.38
mood stabilizer (baseline)	-1.99	0.06	0.38
CPZ (cumulative)	1.74	0.10	0.40
SANS	-1.53	0.15	0.43
CPZ (baseline)	1.30	0.21	0.43
smoke	1.28	0.22	0.43
antidepressants (baseline)	1.20	0.25	0.43
SAPS	-0.84	0.41	0.62
mood stabilizer (change)	0.61	0.55	0.67
age	-0.60	0.56	0.67
diagnosis	-0.44	0.67	0.73
antidepressants (change)	-0.23	0.82	0.82

(B) associations between confounding factors and APCs of GABA in ACC

Confounding factors	t-value	p-value	q-value
antidepressants (baseline)	1.88	0.08	0.54
DOI	-1.75	0.10	0.54
CPZ (cumulative)	1.26	0.23	0.54
SANS	-1.25	0.23	0.54
smoke	1.14	0.27	0.54
SAPS	-1.06	0.31	0.54
diagnosis	-1.04	0.32	0.54
antidepressants (change)	-0.88	0.39	0.59
mood stabilizer (baseline)	-0.64	0.53	0.67
age	-0.60	0.56	0.67
mood stabilizer (change)	-0.44	0.66	0.73
CPZ (baseline)	-0.17	0.87	0.87

(C) associations between confounding factors and APCs of NAA in ACC

Confounding factors	t-value	p-value	q-value
CPZ (cumulative)	3.04	0.01	0.05
DOI	-3.01	0.01	0.05
antidepressants (baseline)	1.36	0.19	0.64
SANS	-1.05	0.31	0.64
smoke	0.92	0.37	0.64
CPZ (baseline)	-0.90	0.38	0.64
mood stabilizer (baseline)	-0.87	0.40	0.64
mood stabilizer (change)	0.80	0.44	0.64
diagnosis	-0.72	0.48	0.64
age	0.43	0.67	0.80
antidepressants (change)	0.26	0.80	0.83
SAPS	-0.22	0.83	0.83

(D) associations between confounding factors and APCs of myo-inositol in ACC

Confounding factors	t-value	p-value	q-value
mood stabilizer (baseline)	-2.90	0.01	0.13
SANS	-2.12	0.05	0.30
antidepressants (change)	1.19	0.25	0.61
age	-1.06	0.30	0.61
antidepressants (baseline)	-1.01	0.33	0.61
diagnosis	1.00	0.33	0.61
smoke	-0.96	0.35	0.61
CPZ (cumulative)	0.66	0.52	0.77
CPZ (baseline)	-0.26	0.80	0.97
SAPS	-0.20	0.84	0.97
DOI	0.07	0.95	0.97
mood stabilizer (change)	-0.04	0.97	0.97

(E) associations between confounding factors and APCs of total choline in ACC

Confounding factors	t-value	p-value	q-value
SANS	-2.13	0.05	0.64
diagnosis	-1.16	0.27	0.80
antidepressants (baseline)	1.00	0.33	0.80
DOI	-0.82	0.43	0.80
mood stabilizer (baseline)	-0.66	0.52	0.80
antidepressants (change)	-0.60	0.56	0.80
age	0.58	0.57	0.80
SAPS	0.54	0.60	0.80
CPZ (cumulative)	0.53	0.60	0.80
CPZ (baseline)	-0.31	0.77	0.90
smoke	0.22	0.83	0.90
mood stabilizer (change)	-0.13	0.90	0.90

(F) associations between confounding factors and APCs of total creatine in ACC

Confounding factors	t-value	p-value	q-value
SANS	-1.64	0.12	0.85
mood stabilizer (baseline)	-1.51	0.15	0.85
CPZ (cumulative)	0.82	0.42	0.85
DOI	-0.81	0.43	0.85
age	-0.75	0.46	0.85
antidepressants (baseline)	-0.53	0.61	0.85
smoke	-0.49	0.63	0.85
CPZ (baseline)	0.43	0.67	0.85
SAPS	-0.31	0.76	0.85
antidepressants (change)	-0.29	0.77	0.85
mood stabilizer (change)	0.22	0.83	0.85
diagnosis	0.20	0.85	0.85

Figure legends

Figure S1. Boxplots of the metabolite levels in the Anterior Cingulate Cortex.

Significant longitudinal reductions were observed in glutamate **(A)** in both patients and controls, while for γ-aminobutyric acid (GABA) **(B)**, N-acetylaspartate (NAA) **(C)**, *myo*-inositol **(D)**, total choline **(E)**, and total creatine (**F)**, significant longitudinal reductions were observed only in patients, but not in controls.

Metabolite levels were calculated relative to the unsuppressed voxel water and are expressed in institutional units (IU, approximately millimolar). The box represents the standard deviation and the solid line in the middle of the box shows the mean value of the metabolite level. The x-axis is visits, where V1, V2, and V3 are the first (baseline), second, and third annual visits, respectively.

Figure S2. Boxplots of the metabolite levels in Glutathione.

We didn't observe significant longitudinal changes in glutathione in anterior cingulate cortex (ACC) **(A)**, thalamus **(B)**, dorsolateral prefrontal cortex (DLPFC) **(C)**, centrum semiovale **(D)**, or orbital frontal cortex (OFR) **(E)**.

Metabolite levels were calculated relative to the unsuppressed voxel water and are expressed in institutional units (IU, approximately millimolar). The box represents the standard deviation and the solid line in the middle of the box shows the mean value of the metabolite level. The x-axis is visits, where V1, V2, and V3 are the first (baseline), second, and third annual visits, respectively.

Figure S1

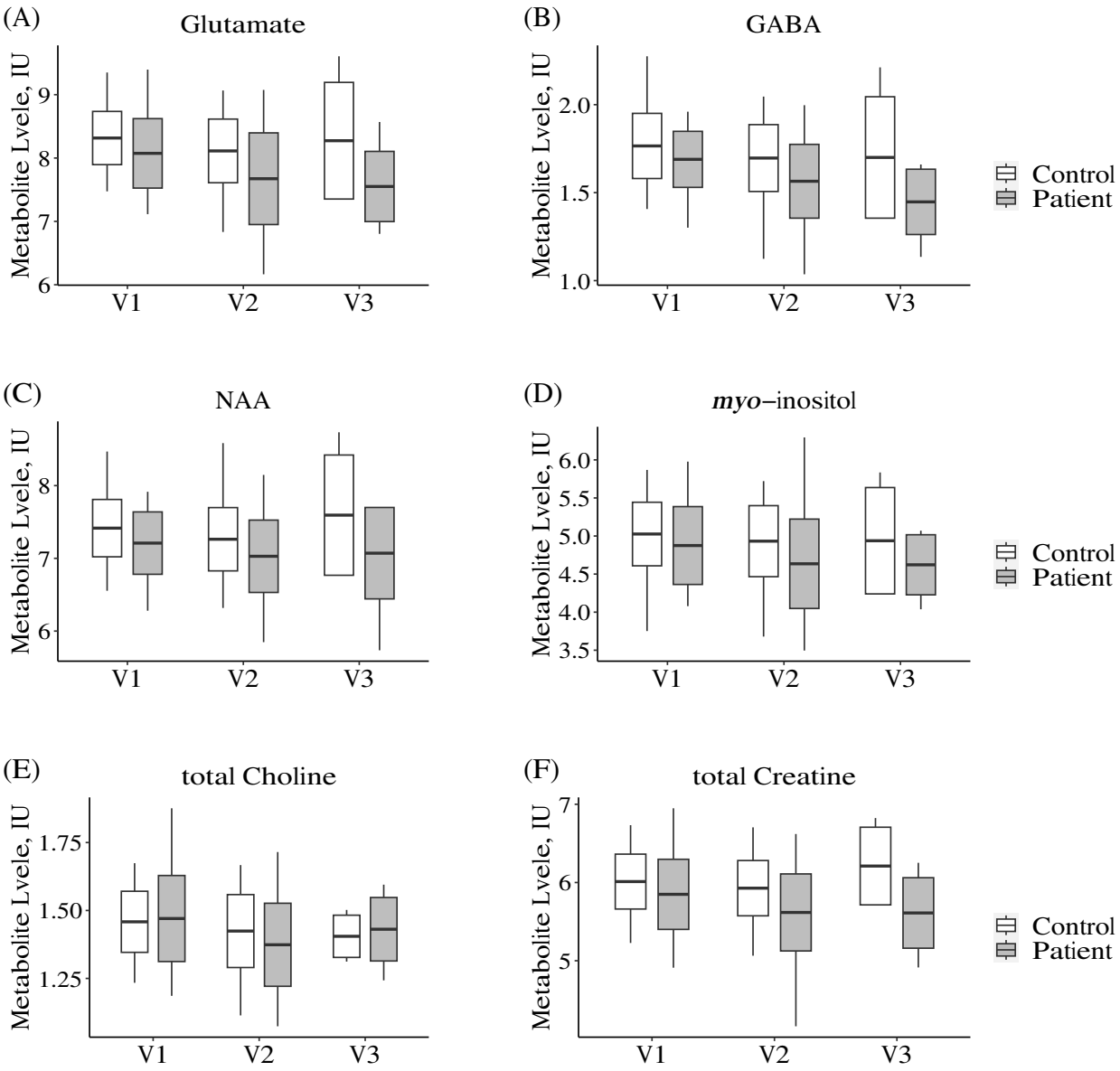
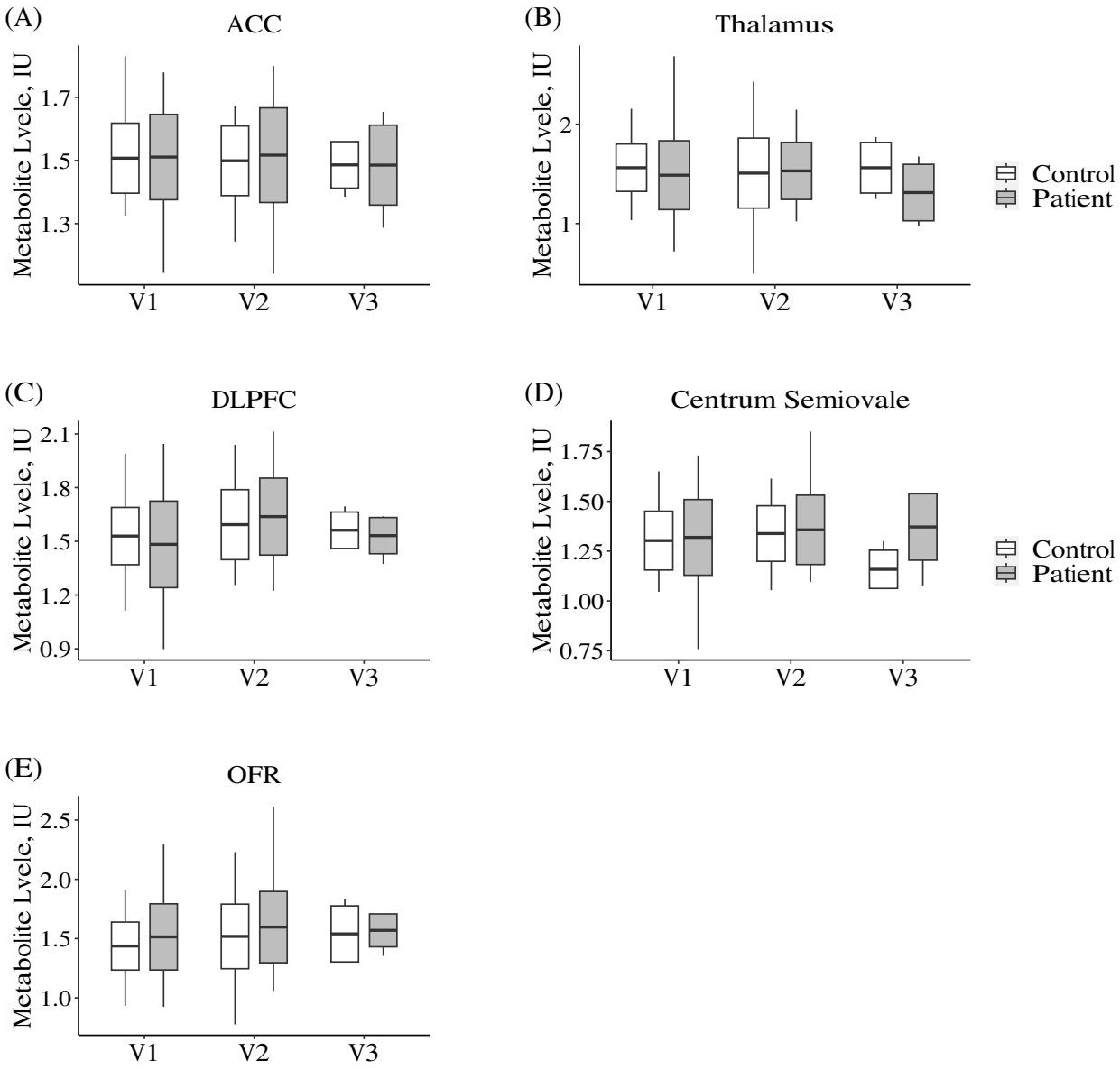

(A)
Glutamate
Metabolite Lvele, IU
9
8
7
6
V1
V2
V3
(B)
GABA
Metabolite Lvele, IU
2.0
1.5
1.0
V1
V2
V3
Control
Patient
(C)
NAA
Metabolite Lvele, IU
8
7
6
V1
V2
V3
(D)
myo−inositol
Metabolite Lvele, IU
6.0
5.5
5.0
4.5
4.0
3.5
V1
V2
V3
Control
Patient
(E)
total Choline
Metabolite Lvele, IU
1.75
1.50
1.25
V1
V2
V3
(F)
total Creatine
Metabolite Lvele, IU
7
6
5
V1
V2
V3
Control
Patient

Figure S2


(A)
ACC
Metabolite Lvele, IU
1.7
1.5
1.3
V1
V2
V3
(B)
Thalamus
Metabolite Lvele, IU
2
1
V1
V2
V3
Control
Patient
(C)
DLPFC
Metabolite Lvele, IU
2.1
1.8
1.5
1.2
0.9
V1
V2
V3
(D)
Centrum Semiovale
Metabolite Lvele, IU
1.75
1.50
1.25
1.00
0.75
V1
V2
V3
Control
Patient
(E)
OFR
Metabolite Lvele, IU
2.5
2.0
1.5
1.0
V1
V2
V3