Characterizing Acyl-carnitine Biosignatures for Schizophrenia: A Longitudinal Pre- and Post-Treatment Study

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Introduction

➤ Subjects with schizophrenia have high risks of metabolic abnormalities and bioenergetic dysfunction.

➤ Acyl-carnitines involved in bioenergetic pathways provide potential biomarker targets for identifying early changes and onset characteristics in subjects with schizophrenia.

Objective

➤ Compare levels of 29 carnitines between baseline (pretreatment) and after 8 weeks of treatment (posttreatment).

➤ Find potential abnormalities of metabolic pathways involving acyl-carnitines to further explore the connection between schizophrenia and metabolism.

Participants

- 156 subjects with Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria (DSM-IV)-defined schizophrenia

- All subjects were either first psychotic episode and drug-naïve or had recurrent schizophrenia and had not taken any antipsychotic drugs for a minimum of 4 weeks before hospitalization.

- Inclusion criteria: (1) age from 18 to 60 years old; (2) absence of diabetes mellitus, hyperlipidemia, cardiovascular disease, or any other severe or unstable medical illness; (3) absence of comorbidity with other psychiatric disorders, including alcohol and substance use disorders.

Materials

Sample Preparation

- Carnitines were extracted from a 45µL plasma sample through addition of 135µL of methanol solution (1:3, v/v) mixed with 5.4µL isotopically labeled internal standards (v/v=24/1).

Lab Analysis

- Waters BEH Amide column (2.1 × 100 mm, 1.7 µm particles)
- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation liquid chromatography (RSLC) system
- Thermo Scientific™ Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer

Statistical Analysis

- χ^2 test，paired Student's t-test or Wilcoxon signed-rank t-test
- Partial least squares-discriminant analysis (PLS-DA)
- Area under the receiver-operating characteristic curve (AUROC)

Results 1- Basic Characteristics

Variables	Pretreatment(n = 156)	Posttreatment (n = 156)	P value [*]
PANSS scores; mean (SD)			
PANSS total	88.82 (18.23)	50.67 (12.7)	<0.001
PANSS positive	21.78 (8.09)	9.96(4.00)	<0.001
PANSS negative	21.15 (7.99)	13.65 (5.25)	<0.001
General psychopathology	42.84 (12.49)	25.67 (7.94)	<0.001
BMI (kg/m^2); mean (SD)	23.89 (3.96)	24.70 (3.70)	<0.001
Waist (cm); mean (SD)	88.71 (12.19)	90.63 (11.20)	<0.001
FBG (mmol/L); mean (SD)	5.62 (1.80)	5.21 (1.00)	0.005
TG (mmol/L); mean (SD)	1.24 (0.82)	1.96 (1.15)	<0.001
TC(mmol/L); mean (SD)	4.61 (1.08)	4.63 (0.92)	0.821
HDL (mmol/L); mean (SD)	1.42 (0.30)	1.35 (0.32)	0.012
LDL (mmol/L); mean (SD)	2.25 (0.45)	2.29 (0.46)	0.295
VLDL (mmol/L); mean (SD)	0.57 (0.38)	0.91 (0.52)	<0.001

[*]p values were calculated by two-tailed paired-samples. BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; SD, standard deviation.

Results 2 – PLS-DA
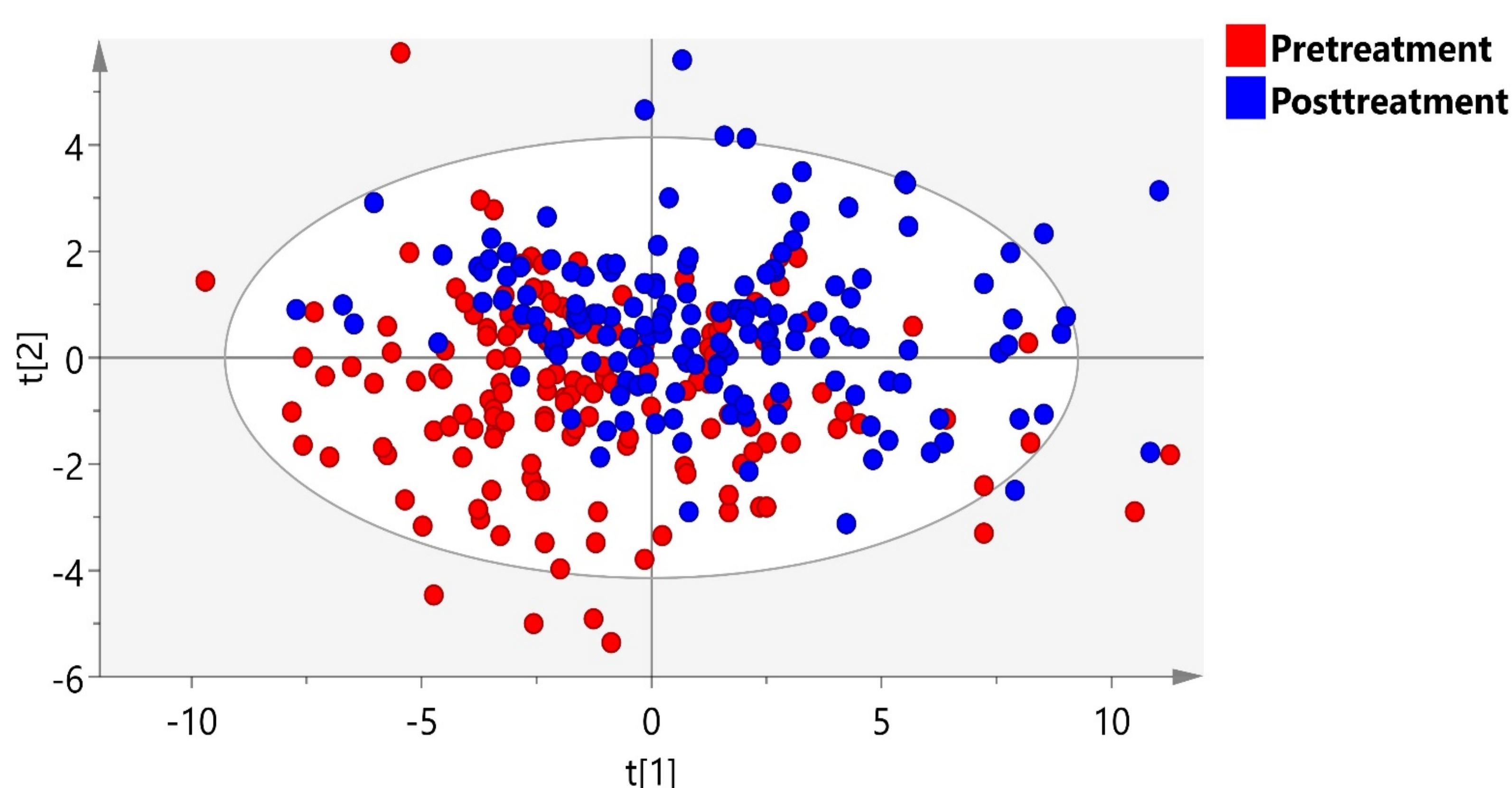
Figure 1. Score plot of the PLS-DA model for the differentiation of pretreatment and posttreatment subjects. R^{2}X (cum) = 0.744，R^{2}Y (cum) = 0.333，Q^2 (cum) = 0.277

Results 3- Carnitines Concentrations

Carnitines	Pretreatment (n = 156)	Posttreatment (n = 156)	q [a]	FC [b]	VIP [c]	ROC	95% CI
Concentration (µmol/L), median (IQR)							
C0	45.74 (34.22-60.09)	38.51 (28.57-56.16)	0.03	0.84	0.97	0.574	0.511, 0.638
C2	10.29 (6.92-15.18)	7.62 (5.98-9.78)	3.44×10^{-08}	0.74	0.9	0.677	0.618, 0.736
C3	0.42 (0.31-0.58)	0.55 (0.40-0.77)	1.74×10^{-09}	1.31	1.65	0.669	0.610, 0.729
C4	0.17 (0.13-0.23)	0.25 (0.18-0.40)	4.54×10^{-11}	1.47	1.82	0.708	0.651, 0.766
C4-OH(C3-DC) (10^{-2})	3.62 (1.95-7.81)	1.83 (1.34-2.79)	2.53×10^{-13}	0.51	1.54	0.734	0.678, 0.790
C5 (10^{-2})	8.30 (6.16-11.65)	9.94 (7.33-12.99)	1.93×10^{-03}	1.2	1.67	0.591	0.528, 0.654
C6 (10^{-2})	7.11 (4.02-10.68)	5.49 (3.57-8.00)	7.05×10^{-04}	0.77	0.9	0.621	0.559, 0.683
C6:1 (10^{-2})	0.67 (0.48-1.02)	0.58 (0.43-0.85)	0.002	0.87	0.61	0.581	0.518, 0.644
C8 (10^{-2})	13.78 (7.13-24.22)	9.11 (6.19-16.04)	0.004	0.66	0.77	0.605	0.542, 0.668
C10 (10^{-2})	17.9 (7.83-28.41)	9.61 (6.58-18.74)	7.05×10^{-04}	0.54	0.72	0.622	0.560, 0.684
C10:1 (10^{-2})	19.78 (10.51-31.22)	13.07 (8.35-21.66)	2.43×10^{-04}	0.66	0.75	0.632	0.570, 0.693
C10:2 (10^{-2})	1.97 (1.24-2.9)	1.71 (1.06-2.66)	0.04	0.87	0.92	0.562	0.499, 0.626
C12 (10^{-2})	4.70 (2.31-7.5)	3.35 (2.05-5.62)	0.01	0.71	0.96	0.596	0.532, 0.659
C12:1 (10^{-2})	8.38 (3.97-11.83)	4.67 (2.75-8.13)	1.63×10^{-04}	0.56	0.78	0.64	0.578, 0.701
C14 (10^{-2})	1.36 (0.93-2.01)	1.17 (0.88-1.61)	0.008	0.86	1.14	0.588	0.525, 0.651
C14:1 (10^{-2})	6.85 (3.63-10.23)	4.01 (2.3-7.23)	1.59×10^{-05}	0.59	0.76	0.655	0.594, 0.715
C14:1-OH (10^{-2})	0.59 (0.28-0.83)	0.36 (0.2-0.57)	4.30×10^{-05}	0.61	0.74	0.646	0.585, 0.708
C14:2 (10^{-2})	7.62 (3.68-11.4)	4.50 (2.48-8.41)	1.06×10^{-04}	0.59	0.77	0.641	0.580, 0.703
C14:2-OH (10^{-2})	0.28 (0.14-0.45)	0.19 (0.09-0.34)	7.05×10^{-04}	0.68	0.77	0.618	0.556, 0.681
C16 (10^{-2})	9.32 (7.31-11.47)	7.64 (6.2-9.52)	8.04×10^{-07}	0.82	0.83	0.655	0.595, 0.716
C16:1 (10^{-2})	2.92 (1.97-3.98)	1.99 (1.34-2.77)	1.14×10^{-08}	0.68	0.78	0.696	0.637, 0.754
C16:1-OH (10^{-2})	0.19 (0.11-0.3)	0.13 (0.07-0.21)	9.36×10^{-05}	0.68	0.77	0.64	0.579, 0.701
C16:2 (10^{-2})	1.37 (0.83-1.9)	0.81 (0.48-1.4)	4.34×10^{-07}	0.59	0.82	0.677	0.618, 0.736
C16:2-OH (10^{-2})	0.48 (0.3-0.65)	0.34 (0.22-0.53)	2.20×10^{-04}	0.71	0.77	0.632	0.571, 0.694
C16-OH (10^{-2})	0.15 (0.09-0.23)	0.13 (0.08-0.18)	0.02	0.87	1.21	0.583	0.520, 0.646
C18 (10^{-2})	1.96 (1.62-2.6)	1.69 (1.28-2.11)	9.99×10^{-08}	0.86	0.64	0.641	0.580, 0.701
C18:1 (10^{-2})	12.23 (9.84-15.07)	8.20 (5.62-11.20)	2.53×10^{-13}	0.67	1.08	0.734	0.678, 0.790
C18:1-OH (10^{-2})	0.21 (0.12-0.30)	0.12 (0.06-0.19)	1.37×10^{-06}	0.57	0.74	0.688	0.629, 0.746
C18:2 (10^{-2})	13.31 (11.01-16.46)	9.91 (7.95-14.44)	2.19×10^{-07}	0.74	0.69	0.665	0.603, 0.727

[a] q values were FDR corrections for p values which were calculated from two-tailed paired t-test of log10-transformed data.
[b] Fold changes were calculated as the ratios of median metabolite levels (posttreatment patients/pretreatment patients).
[c] Variable importance in the projection (VIP) values were obtained from cross-validated PLS-DA models with a threshold of 1.0.

Results 4- FC and AUROC

Posttreatment/Pretreatment
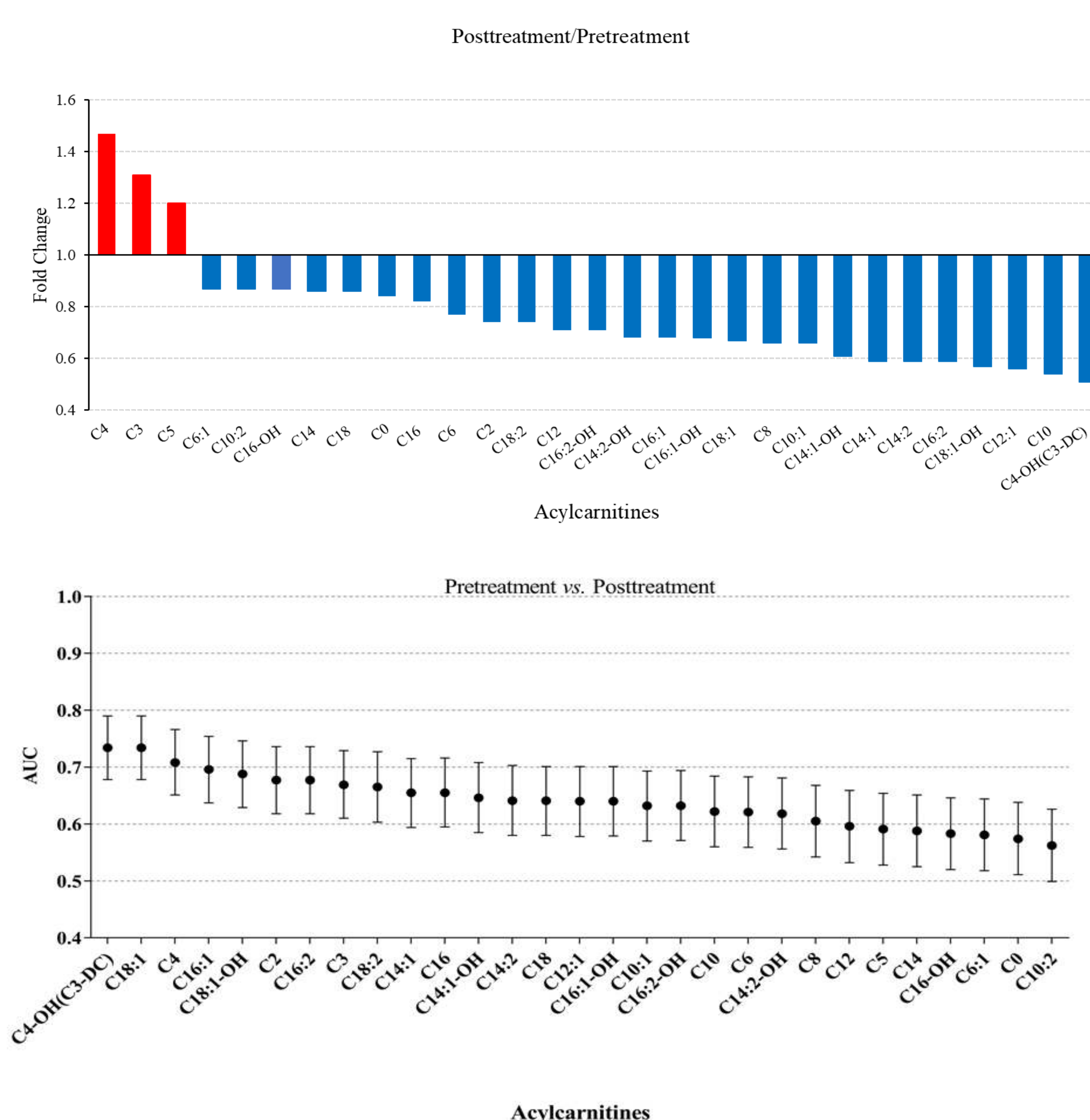
Figure 2. Fold change(FC) of carnitineas between from subjects with posttreatment and pretreatment patients. The FC was calculated as the ratio of the median carnitine levels between two groups. FC>1.0 indicated a relatively higher concentration of carnitines while FC<1.0 indicated a relatively lower concentration in subjects with posttreatment as compared to pretreatment.

Pretreatment *vs.* Posttreatment

Figure 3. The AUROCs and 95% CIs of carnitines for the comparison between posttreatment with pretreatment patients.

Conclusion

- **Pretreatment > Posttreatment**: C4-OH (C3-DC), C6:1, C16 and C16:1
- **Pretreatment < Posttreatment**: C3, C4, C5, C8, C10:1, C10: 2 and C18
- Acyl-carnitines with abnormalities in cellular bioenergetics of schizophrenia.
- Acyl-carnitines can be potential targets for future investigations into their roles in the pathoetiology of schizophrenia.
- Acyl-carnitines present novel treatment and screening opportunities for patients with schizophrenia.

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