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Profiling of Acylcarnitines in First Episode Psychosis before and after Antipsychotic Treatment

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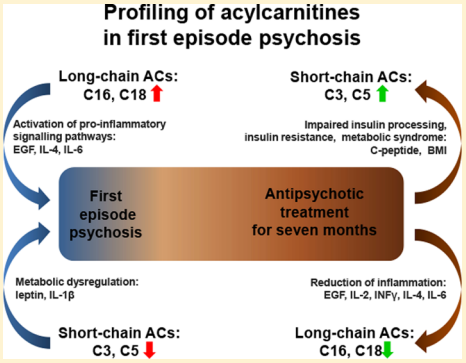
Supporting Information

ABSTRACT: Acylcarnitines (ACs) have been shown to have a potential to activate pro-inflammatory signaling pathways and to foster the development of insulin resistance. The first task of the current study was to study the full list of ACs (from C2 to C18) in first episode psychosis (FEP) patients before and after antipsychotic treatment. The second task was to relate ACs to inflammatory and metabolic biomarkers established in the same patient cohort as in our previous studies. Serum levels of ACs were determined with the AbsoluteIDQ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry ([FIA]−MS/MS) as well as liquid chromatography ([LC]−MS/MS) technique. Identification and quantification of the metabolites was achieved using multiple reactions monitoring along with internal standards. The comparison of ACs in antipsychotic-naïve first-episode psychosis (FEP) patients (N = 38) and control subjects (CSs, N = 37) revealed significantly increased levels of long-chain ACs (LCACs) C14:1 (p = 0.0001), C16 (p = 0.00002), and C18:1 (p = 0.000001) in the patient group. These changes of LCACs were associated with augmented levels of CARN palmitoyltransferase 1 (CPT-1) (p = 0.006). By contrast, the level of short-chain AC (SCAC) C3 was significantly reduced (p = 0.00003) in FEP patients. Seven months of antipsychotic drug treatment ameliorated clinical symptoms in patients (N = 36) but increased significantly their body mass index (BMI, p = 0.001). These changes were accompanied by significantly reduced levels of C18:1 (p = 0.00003) and C18:2 (p = 0.0008) as well as increased level of C3 (p = 0.01). General linear model revealed the relation of LCACs (C16, C16:1, and C18:1) to the inflammatory markers (epidermal growth factor, IL-2, IL-4, IL-6), whereas SCAC C3 was linked to the metabolic markers (leptin, C-peptide) and BMI. FEP was associated with an imbalance of ACs in patients because the levels of several LCACs were significantly higher and the levels of several SCACs were significantly reduced compared with CSs. This imbalance was modified by 7 months of antipsychotic drug treatment, reversing the levels of both LCACs and SCACs to that established for CSs. This study supports the view that ACs have an impact on both inflammatory and metabolic alterations inherent for FEP.



KEYWORDS: *first-episode psychosis, antipsychotic drug treatment, body mass index, short-chain acylcarnitines, long-chain acylcarnitines, inflammatory biomarkers, metabolomic, metabonomic, metabolic biomarkers*

1. INTRODUCTION

Schizophrenia (SCH) is characterized by disturbances in multiple domains of brain functioning, including cognitive, emotional, and perceptual processes.[1] The conclusive identification of specific etiological factors or pathogenetic processes in SCH has remained elusive. Growing body of evidence demonstrates that patients with SCH differ from healthy controls regarding neuropathological, biochemical, and genetic parameters.[2,3]

Recent studies support the view that SCH is associated with an imbalance in redox or oxidative stress (OxS) status.[4−7] OxS is intimately linked to a variety of pathophysiological processes, such as mitochondrial dysfunction (MitoDys), inflammation, and hypoactive *N*-methyl-D-aspartate receptors among others.[8] MitoDys in SCH was first described in 1954 by Takahashi,[9] and recent studies have highlighted the impaired function of mitochondria in the pathology of SCH.[10,11] Mitochondria are the principal cellular compartments implicated in lipid metabolism, in particular, fatty acid oxidation (FAO) contributed by acylcarnitines (ACs). The studies on SCH and first episode of psychosis (FEP) indicate that psychotic disorders alter the composition of brain lipids (i.e., phospholipids and polyunsaturated fatty acids (PUFAs)), and lipid homeostasis may be affected by antipsychotics as well.[12−16] Despite these considerations, the alterations in whole ACs profile in FEP remain to be elucidated.

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ACs (from short-chain to long-chain ones, from C2 to C18) are produced via transfer of the acyl group of a fatty acyl-CoA to L-carnitine (CARN) by carnitine transferases. Assay of serum/plasma ACs is performed for the biochemical screening of inheritable disorders of FAO enzymes as they typically accumulate as a consequence of metabolic dysfunction resulting from poor coordination with β-oxidation and tricarboxylic acid cycle in mitochondria.[17] Problems with FAO manifest as altered plasma AC levels, mainly as increased levels of long-chain ACs (LCASs).[17,18] It is well known that CARN is obligatory for converting long-chain acyl-CoAs into LCACs by CARN palmitoyltransferase 1 (CPT1), followed by shuttling of LCACs from cytosol into the mitochondrial matrix, but in addition to using LCACs as biomarkers, recent evidence supports the hypothesis that LCACs have a potential to activate pro-inflammatory signaling pathways[19−21] and have implications in insulin resistance.[17,22]

Regarding FEP and inflammation, we have already studied the profile of cytokines of interleukin family (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), and vascular endothelial and epidermal growth factors (VEGF and EGF) in the serum before and 7 months after the start of antipsychotic medication.[23]

Drug-naïve FEP patients displayed significantly higher levels of EGF, IL-4, and IL-6 and significantly lower level of IL-1β compared with control subjects (CSs). Furthermore, regression analysis established significant correlations between disease symptoms and increased EGF/decreased IL-1β. Seven months of antipsychotic treatment resulted in significantly reduced levels of EGF, IL-2, VEGF, IL-6, IFN-γ, IL-4, IL-8, and IL-1α compared with premedication levels.[23] We also demonstrated that, in the same cohort, antipsychotic-naïve FEP patients had significantly higher levels of ferritin and resistin but lower level of leptin compared with CSs. Seven month antipsychotic drug treatment ameliorated clinical symptoms and caused a statistically significant increase in body mass index (BMI); these changes were accompanied by increased levels of C-peptide and leptin as well as decreased level of adiponectin.[24] Therefore, one can suggest that inflammatory processes are activated in FEP, whereas the antipsychotic drug-induced amelioration of disease symptoms is accompanied by a reduction of inflammatory markers and emerging signs of metabolic syndrome.[23,25−27]

On the basis of the evident impact of ACs on lipid metabolism, FAO, and insulin sensitivity as well as their associations with inflammatory responses, we decided to use this well-characterized cohort of antipsychotic-naïve FEP patients and CSs for profiling of CARN and ACs in their blood samples. Pretreatment levels of CARN and ACs in FEP patients were further compared with their corresponding levels after antipsychotic treatment for 7 months. It is apparent that the metabonomic approach is necessary to investigate the full list of ACs (from C2 to C18) in FEP patients. The currently applied approach evaluates the patterns and concentrations of low-molecular-weight metabolites over broad classes of compounds. The identities and concentrations of ACs represent the final products of cellular interactions reflecting the interplay between the gene regulation, enzymatic activity and metabolic reactions in a dynamic profile in terms of total cellular environment.[28] Metabolic profiling allows us to take a more detailed look at lipid metabolism and to study the levels and impact of numerous intermediates and products. Therefore, the application of metabolic profiling may have particular relevance in early detection, diagnosis, and prognosis of psychotic disorders. The comparison of changes in ACs profile with the established inflammatory and metabolic alterations may provide useful knowledge concerning the impact of CARN and ACs in the pathophysiology of FEP.

2. MATERIALS AND METHODS

2.1. Participants

Thirty-eight FEP patients were recruited from the Psychiatric Clinic of Tartu University Hospital, Estonia. The patients fulfilled the following inclusion criteria: age between 18 and 45; had experienced FEP; the duration of their untreated psychosis had been <3 years; and no antipsychotic treatment received before the first contact with medical services for psychosis. Patients were excluded from the study if they had psychotic disorders owing to a general medical condition or had substance-induced psychosis. FEP diagnoses were based on clinical interviews according to the International Classification of Diseases, 10th ed. (ICD-10)[29] criteria. Thirty-six FEP patients underwent treatment using antipsychotic medication (two refused) and were included in the follow-up analysis. History of antipsychotic medication use was collected according to reviews of patients' medical charts. Patients were treated with various antipsychotic medications according to what was clinically indicated. During the follow-up period, patients received atypical (n = 24), typical (n = 1), or mixed (n = 11) antipsychotic medication; the mean theoretical chlorpromazine dose equivalent[30] was 396 ± 154 (range 80−640) mg. During the second blood collection, 13 patients received quetiapine (among them 7 cases as only antipsychotic treatment), 10 patients received aripiprazole (3 cases as only antipsychotic treatment), 12 were treated with olanzapine (9 cases as only antipsychotic treatment), 2 patients were assigned to risperidone (1 case as only treatment), 2 patients to sertindole (1 case as only antipsychotic treatment), 3 patients to ziprasidone, 2 patients to clozapine (in both cases clozapine was administered in the combination with other psychotropic drugs), and 2 patients to perfenazine (1 case as only treatment). Twenty-seven patients were treated only with antipsychotics, but 4 patients additionally needed mood stabilizers and 6 patients also received antidepressants or hypnotics.

Thirty-seven mentally healthy subjects participated in the study as CSs. The CSs sample was recruited by advertising in the same geographical area the FEP patients came from. CSs were interviewed by experienced psychiatric doctors to avoid the inclusion of CSs with mental disorders. Exclusion criteria for the control group also included psychotic disorders among close relatives. Because it was a naturalistic study, cigarette smoking and substance abuse were not exclusion criteria for either group. Eight patients (21.1%, all men) and 7 CSs (18.9%, three of them were men) were active cigarette smokers. In addition, 10 patients (eight of them were the same, who were current cigarette smokers) and 1 CS (also cigarette smoker) had used cannabis during lifetime. In addition, all participants were monitored for symptoms of infections or symptoms of severe systemic somatic illness throughout the investigation period. There was no need to exclude anyone for that reason. The study was approved by the Ethics Committee of the University of Tartu, Estonia. Written informed consent was also obtained from all participants.

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2.2. Procedure

Blood samples, clinical, and BMI data of the FEP patients were assessed at two time points: on admission and after the follow-up period (mean duration 7.18 ± 0.73 months). The period between the time points consisted of an initial stabilization of acute psychotic symptoms (took ∼1 month) and a further 6 month continuous treatment with antipsychotics. Symptom severity was measured using PANSS,[31] a rating instrument that evaluates the presence and severity of positive, negative, and general psychopathology using 30 items scored from 1 (absent) to 7 (severe).

2.3. Blood Collection and Clinical Laboratory Measurements

Fasting blood samples of participants were collected using standard venipuncture technique between 9:00 and 11:00 a.m. Blood (5 mL) was sampled in anticoagulant-free tubes and kept for 1 h at 4 °C (for platelet activation) before serum was isolated (centrifugation at 2000 rpm for 15 min at 4 °C). Serum was kept at −20 °C before testing. Blood samples and BMI data from CSs were collected cross-sectionally.

2.4. Measurement of Biomarkers

2.4.1. Measurement of Acylcarnitines. Serum level of ACs were determined with the AbsoluteIDQ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry ([FIA]−MS/MS) as well as liquid chromatography ([LC]−MS/MS) technique. The Biocrates AbsoluteIDQ p180 kit is a commercially available targeted metabolomics assay, and it is applied to many studies of human serum and plasma, including clinical ones. All measurements were performed as described in the manufacturer's manual UM-P180. Identification and quantification of the metabolites was achieved using multiple reactions monitoring along with internal standards. From all statistically important changes of ACs in our study, we used in ultimate discussion only those values that were at least 2.3 times higher than the level of detection (LOD) given in the manual of the Biocrates AbsoluteIDQ p180. Calculation of metabolite concentrations was automatically performed by MetIDQ software (BIOCRATES Life Sciences AG). To ensure data quality they were checked based on the LOD. Average values of all measured ACs are presented in Supplementary Table S-1.

2.4.2. Measurement of Inflammatory Markers and Growth Factors. We used a high-sensitive biochip array technology (Randox Biochip, RANDOX Laboratories, Crumlin, U.K., Cytokine & Growth Array for Evidence Investigator) to measure the profile of cytokines and growth factors. The following cytokines and growth factors were measured according to the manufacturer's protocol: TNF-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, MCP-1, VEGF, and EGF. A more detailed description of these measured markers in relation to the same cohort has been provided elsewhere.[23]

2.4.3. Measurement of Metabolic Biomarkers. The following inflammatory and metabolic biomarkers were measured by a biochip array technology (Randox Biochip, RANDOX Laboratories, Metabolic Syndrome Array I for Evidence Investigator): C-peptide, insulin, leptin, resistin, ferritin, and PAI-1. Relevant information about the analyte detection and quality-control method used has been previously described.[24]

2.5. Statistical Analyses

Group differences with regards to demographic measurements were analyzed using the t test or Chi-square test. The application of Shapiro-Wilk tests indicated that age and BMI followed normal distributions, but the ACs values had non-normal distributions. Therefore, nonparametric statistical methods were used to establish biomarker levels between and within group differences. The Mann−Whitney U-test was applied to compare the raw data of two independent samples (FEP patients' pretreatment condition versus CSs) and the Wilcoxon matched pairs test to compare the two dependent samples (FEP patients' pre- versus post-treatment condition). For within-subjects' analyses, patients were paired one by one. The Bonferroni correction was applied for the number of biomarkers within the particular analysis.[32] Differences between FEP patients and CSs (based on the Mann−Whitney U-test) and differences between the pre- and post-treatment values within the patients group (based on the Wilcoxon matched pairs test) were considered to be significant at $p \leq 0.001$. To demonstrate effect size (eta^2) estimates for the nonparametric tests, the values of squared standardized test statistics (Z) were divided by the total number of observations on which Z was based. Effect sizes were interpreted as small, medium, and large, with corresponding eta^2 ranging from 0.01 to 0.05, 0.06 to 0.13, and ≥0.14, respectively.[33]

General linear models (GLMs) were used to demonstrate biomarkers levels' differences between the groups (i.e., drug-naïve FEP patient versus CSs and FEP patients after treatment versus CSs) and within the group (i.e., drug-naïve FEP patient versus FEP patients after treatment). Because GLM analyses required normally distributed data, biomarker values were $\log_{10}$-transformed to approximate normality. Categorical (disease, gender, smoking status) and continuous (age) covariates were used in the GLM to compare biomarkers levels (dependent variables) between groups. In the next step, we used backward variable elimination until the best model fit was reached. Each subsequent step removed the least significant variable in the model until all remaining variables had individual p values smaller than 0.05. F-tests were used to further compare the fits of linear models and analyze significant (disease or treatment) main effects in the final models. Furthermore, partial eta^2 values (the proportion of the effect in addition to error variance that is attributable to the effect) were established for the final models. Partial eta^2 values of 0.02, 0.13, and 0.26 were defined as small, medium, and large effects, respectively.[34] The statistical analyses were performed using Statistica software (StatSoft, 13th ed.) for Windows.[35]

3. RESULTS

3.1. General Description of the Study Groups

There were no statistically significant differences between antipsychotic-naïve FEP patients and CSs in terms of age ($t_{(73)} = 0.49$, $p = 0.62$), gender ($\chi^2_{(1)} = 1.08$, $p = 0.30$), or mean (±s.d.) scores of BMI (22.55 ± 2.94 and 23.02 ± 3.05, respectively; $t_{(73)} = -0.69$, $p = 0.49$). During the 7 month antipsychotic treatment, positive symptom ($Z = 5.16$, $p < 0.000001$), negative symptom ($Z = 5.23$, $p < 0.000001$), general symptom ($Z = 5.18$, $p < 0.000001$), and total symptom ($Z = 5.23$, $p < 0.000001$) scores decreased significantly. The 7 month treatment caused a significant change in BMI ($t_{(35)} = -8.07$, $p < 0.000001$). Mean BMI gain at 7 months follow-up was 2.97 kg/m^2 (±2.21). In addition, the difference in tobacco use (8

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Table 1. Comparison of Acylcarnitine Serum Levels between FEP Patients at Baseline (Before Treatment with Antipsychotics) and Control Subjects[a]

biomarkers	ß	ß (95% CI)	*t* value	*p* value
C0_Carnitine	−0.06	−0.30, 0.17	−0.54	0.59
C10_Decanoylcarnitine	0.32	0.09, 0.54	2.82	**0.006**
C10:1_Decenoylcarnitine	0.15	−0.08, 0.39	1.28	0.21
C10:2_Decadienylcarnitine	−0.09	−0.32, 0.14	−0.80	0.43
C12_Dodecanoylcarnitine	0.28	0.06, 0.51	2.48	**0.02**
C12-DC_Dodecanedioylcarnitine	−0.04	−0.26, 0.19	−0.31	0.76
C12:1_Dodecenoylcarnitine	0.25	0.03, 0.48	2.23	**0.03**
C14_Tetradecanoylcarnitine	0.30	0.08, 0.52	2.73	**0.008**
C14:1_Tetradecenoylcarnitine	0.43	0.22, 0.64	4.08	**0.0001**
C14:1-OH_Hydroxytetradecenoylcarnitine	0.29	0.06, 0.52	2.55	**0.01**
C14:2_Tetradecadienylcarnitine	0.39	0.17, 0.61	3.56	**0.0007**
C14:2-OH_Hydroxytetradecadienylcarnitine	0.004	−0.23, 0.24	0.03	0.98
C16_Hexadecanoylcarnitine	0.48	0.28, 0.69	4.63	**0.00002**
C16-OH_Hydroxyhexadecanoylcarnitine	0.25	0.02, 0.48	2.19	**0.03**
C16:1_Hexadecenoylcarnitine	0.55	0.36, 0.74	5.81	**0.000001**
C16:1-OH_Hydroxyhexadecenoylcarnitine	0.41	0.20, 0.62	3.93	**0.0002**
C16:2_Hexadecadienylcarnitine	0.18	−0.06, 0.41	1.52	0.13
C16:2-OH_Hydroxyhexadecadienylcarnitine	−0.12	0.36, 0.12	−1.02	0.31
C18_Octadecanoylcarnitine	0.33	0.11, 0.56	2.93	**0.005**
C18:1_Octadecenoylcarnitine	0.57	0.37, 0.76	5.77	**0.000001**
C18:1-OH_Hydroxyoctadecenoylcarnitine	0.20	−0.04, 0.43	1.70	0.09
C18:2_Octadecadienylcarnitine	0.42	0.21, 0.63	3.93	**0.0002**
C2_Acetylcarnitine	0.18	−0.04, 0.40	1.65	0.10
C3_Propionylcarnitine	−0.46	−0.67, −0.26	−4.51	**0.00003**
C3-DC(C4-OH)_Malonylcarnitine (Hydroxybutyrylcarnitine)	0.35	0.13, 0.57	3.14	**0.003**
C5-OH(C3-DC-M)_Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	0.08	−0.15, 0.31	0.70	0.49
C3-OH_Hydroxypropionylcarnitine	−0.02	−0.26, 0.22	−0.19	0.86
C3:1_Propenoylcarnitine	0.07	−0.17, 0.30	0.54	0.59
C4_Butyrylcarnitine	−0.36	−0.58, −0.15	−3.33	**0.001**
C4:1_Butenylcarnitine	0.21	−0.02, 0.44	1.80	0.08
C6(C4:1-DC)_Hexanoylcarnitine (Fumarylcarnitine)	0.42	0.20, 0.64	3.83	**0.0003**
C5_Valerylcarnitine	−0.30	−0.52, −0.08	−2.69	**0.009**
C5-DC(C6-OH)_Glutarylcarnitine (Hydroxyhexanoylcarnitine)	0.23	0.004, 0.45	2.03	**0.046**
C5-M-DC_Methylglutarylcarnitine	0.05	−0.18, 0.29	0.45	0.66
C5:1_Tiglylcarnitine	0.01	−0.23, 0.25	0.09	0.93
C5:1-DC_Glutaconylcarnitine	0.18	−0.05, 0.40	1.53	0.13
C6:1_Hexenoylcarnitine	0.14	−0.09, 0.38	1.20	0.24
C7-DC_Pimelylcarnitine	0.22	−0.01, 0.45	1.91	0.06
C8_Octanoylcarnitine	0.25	0.02, 0.48	2.19	**0.03**
C9_Nonaylcarnitine	−0.37	−0.59, −0.15	−3.32	**0.001**
H1_Hexose	0.28	0.06, 0.50	2.49	**0.02**

[a]ß, regression coefficients; CI, confidence interval; p values (derived from GLM analysis), significance values of $\log_{10}$-transformed acylcarnitine levels with disease, adjusted for gender, age and smoking status. Significant *t* values ($p < 0.05$) are marked in bold.

patients [21.1%] versus 7 controls [18.9%]) was not statistically significant ($\chi^2_{(1)} = 0.05$, $p = 0.82$).

3.2. Serum Acylcarnitine Profiles

3.2.1. AC Profile Alterations in FEP Patients before and after 7 Month Antipsychotic Treatment. To the best of our knowledge, this is the first study that demonstrates an overview of the composite panel of ACs profile results and their significance in FEP patients before and after antipsychotic treatment. In consequence, preliminary analyses (Mann−Whitney *U*-test and Wilcoxon matched pairs test) were first run to report base-level differences in AC levels between the groups (FEP patients and CSs) and within the FEP group.

Twenty-four out of 39 ACs exhibited shifts in FEP patients compared with CSs, but only eight of them (C14:1, C16, C16:1, C16:1-OH, C18:1, C18:2, C3, C6[C4:1-DC]) survived the Bonferroni correction for multiple comparisons ($p \leq 0.001$), and demonstrated large effect sizes. Thereafter, the impact of treatment to ACs levels was established (see Supplementary Tables S-1 and S-2). In particular, statistically significant trends were observed for 17 of the measured markers, and 4 of them (C16, C18:1, C18:2, C3) survived the Bonferroni correction for multiple comparisons and reflected large effect sizes.

3.2.2. AC Differences Specific to FEP and 7 Month Antipsychotic Treatment. Therefore, to confirm the existence of significant main effects of disease and treatment condition on the levels of ACs, an alternative methodological approach (GLM) was utilized, and important covariates (gender, smoking status, age) were included for the further analyses. After applying GLM, levels of 22 ACs (and hexoses)

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Table 2. Effects of 7 Month Treatment with Antipsychotics on Metabolite Levels Among FEP Patient Group[a]

biomarkers	ß	ß (95% CI)	*t* value	*p* value
C0_Carnitine	0.08	−0.24, 0.40	0.52	0.61
C10_Decanoylcarnitine	0.21	−0.09, 0.50	1.40	0.17
C10:1_Decenoylcarnitine	0.06	−0.24, 0.36	0.40	0.69
C10:2_Decadienylcarnitine	0.09	−0.21, 0.39	0.61	0.54
C12_Dodecanoylcarnitine	0.27	−0.02, 0.56	1.87	0.07
C12-DC_Dodecanedioylcarnitine	−0.16	−0.47, 0.15	−1.03	0.31
C12:1_Dodecenoylcarnitine	0.17	−0.13, 0.47	1.11	0.27
C14_Tetradecanoylcarnitine	0.38	0.09, 0.67	2.64	**0.01**
C14:1_Tetradecenoylcarnitine	0.41	0.14, 0.68	3.02	**0.004**
C14:1-OH_Hydroxytetradecenoylcarnitine	0.34	0.05, 0.64	2.32	**0.02**
C14:2_Tetradecadienylcarnitine	0.41	0.13, 0.69	2.94	**0.005**
C14:2-OH_Hydroxytetradecadienylcarnitine	0.23	−0.08, 0.53	1.48	0.15
C16_Hexadecanoylcarnitine	0.47	0.20, 0.74	3.44	**0.001**
C16-OH_Hydroxyhexadecanoylcarnitine	0.29	−0.01, 0.59	1.91	0.06
C16:1_Hexadecenoylcarnitine	0.44	0.19, 0.70	3.42	**0.001**
C16:1-OH_Hydroxyhexadecenoylcarnitine	0.43	0.15, 0.71	3.06	**0.003**
C16:2_Hexadecadienylcarnitine	0.31	0.02, 0.61	2.10	**0.04**
C16:2-OH_Hydroxyhexadecadienylcarnitine	−0.16	−0.47, 0.16	−0.99	0.33
C18_Octadecanoylcarnitine	0.38	0.08, 0.68	2.51	**0.02**
C18:1_Octadecenoylcarnitine	0.55	0.31, 0.80	4.47	**0.00003**
C18:1-OH_Hydroxyoctadecenoylcarnitine	0.33	0.04, 0.63	2.25	**0.03**
C18:2_Octadecadienylcarnitine	0.47	0.20, 0.74	3.51	**0.0008**
C2_Acetylcarnitine	0.03	−0.27, 0.32	0.17	0.87
C3_Propionylcarnitine	−0.39	−0.68, −0.10	−2.66	**0.01**
C3-DC(C4-OH)_Malonylcarnitine (Hydroxybutyrylcarnitine)	0.38	0.10, 0.66	2.71	**0.009**
C5-OH(C3-DC-M)_Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	0.07	−0.24, 0.38	0.48	0.64
C3-OH_Hydroxypropionylcarnitine	0.14	−0.17, 0.45	0.90	0.37
C3:1_Propenoylcarnitine	0.16	−0.16, 0.47	1.00	0.32
C4_Butyrylcarnitine	−0.17	−0.49, 0.14	−1.12	0.27
C4:1_Butenylcarnitine	0.23	−0.08, 0.55	1.50	0.14
C6(C4:1-DC)_Hexanoylcarnitine (Fumarylcarnitine)	0.29	−0.01, 0.59	1.99	0.05
C5_Valerylcarnitine	−0.27	−0.57, 0.03	−1.79	0.08
C5-DC(C6-OH)_Glutarylcarnitine (Hydroxyhexanoylcarnitine)	0.27	−0.02, 0.56	1.88	0.07
C5-M-DC_Methylglutarylcarnitine	0.17	−0.14, 0.48	1.10	0.28
C5:1_Tiglylcarnitine	0.06	−0.25, 0.37	0.42	0.68
C5:1-DC_Glutaconylcarnitine	0.12	−0.18, 0.42	0.80	0.43
C6:1_Hexenoylcarnitine	0.03	−0.27, 0.33	0.20	0.84
C7-DC_Pimelylcarnitine	0.28	−0.02, 0.58	1.89	0.06
C8_Octanoylcarnitine	0.16	−0.14, 0.46	1.06	0.29
C9_Nonaylcarnitine	−0.19	−0.49, 0.11	−1.26	0.21
H1_Hexose	0.17	−0.13, 0.48	1.13	0.26

[a]ß, regression coefficient; CI, confidence interval; *p* values (derived from GLM repeated measure), significance values of $\log_{10}$-transformed acylcarnitines levels in patients group before treatment compared with biomarkers values measured after 7 month treatment with antipsychotics, adjusted for gender, and smoking status. Significant *t* values ($p < 0.05$) are marked in bold.

demonstrated statistically significant differences, and the most prominent difference emerged in five ACs levels (C14:1, C16, C16:1, C18:1, and C3) when comparing drug-naïve FEP patients and CSs (Table 1).

The main effect of the treatment occurred in relation to 14 ACs levels, and the most salient among others was C18:1 after comparing FEP patients before and after treatment (Table 2). Interestingly, no differences in AC levels were detected by means of GLM between CSs and post-treatment FEP patients (Supplementary Table S-3).

3.3. Effects of Disease and Antipsychotic Drugs on the Levels of ACs, Inflammatory, and Metabolic Biomarkers

Furthermore, to provide a more comprehensive view on the essence of ACs profile differences between and within the groups, we expanded our data analysis. On the basis of the metabolic and inflammatory biomarkers results of our previous studies[23,24] we additionally included the values of the following markers in the GLM analysis: ferritin, PAI-1, C-peptide, insulin, leptin, adiponectin, resistin, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8 and IL-10, IFN-γ, MCP-1, TNF-α, as well as VEGF and EGF. We used the backward elimination technique to demonstrate disease and treatment effects on biomarker value differences.

We found significant differences across the groups (i.e., drug-naïve FEP patients versus CSs) in 32 analytes (Supplementary Table S-4). The most notable among these were increases in the levels of EGF ($p < 0.0000001$), C16:1 ($p = 0.0000003$), C18:1 ($p = 0.000001$), C16 ($p = 0.00002$), C14:1 ($p = 0.00005$), C16:1-OH ($p = 0.00007$), C18:2 ($p = 0.0001$), C14:2 ($p = 0.0002$), C6(C4:1-DC) ($p = 0.0002$), and IL-6 ($p = 0.0008$) in the patient group. In addition, the level of C3 was

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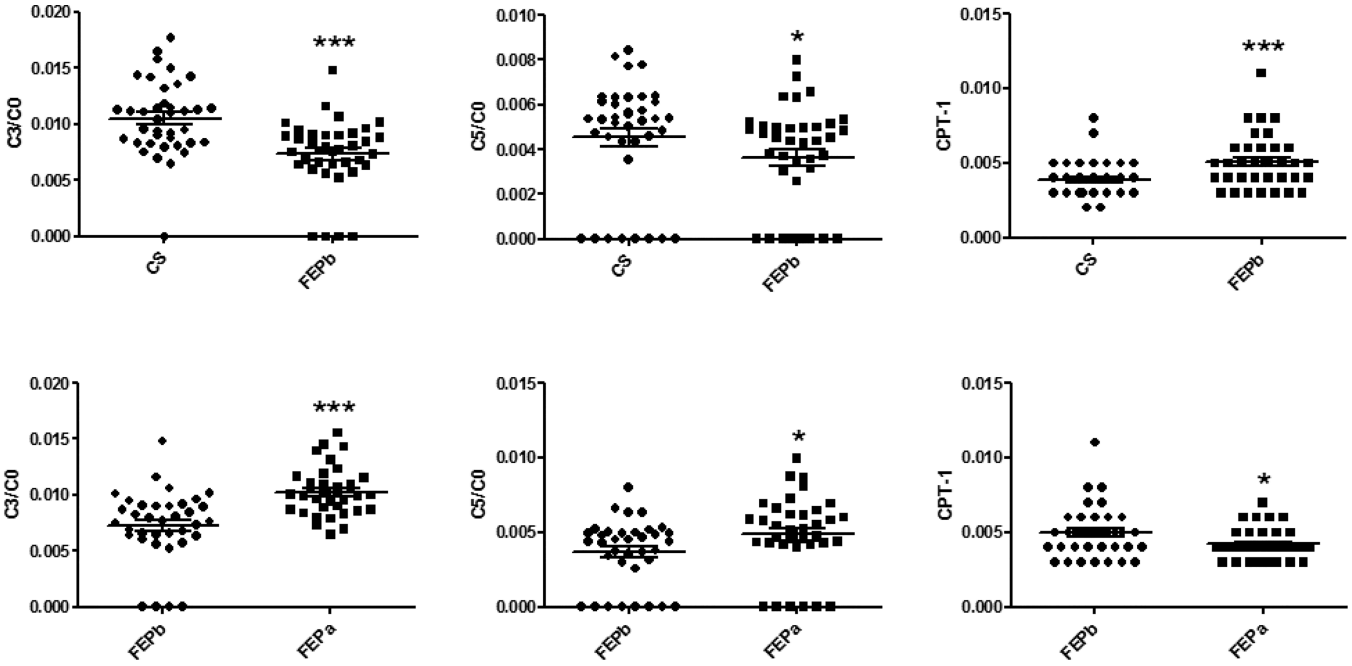

Figure 1. Ratio between SCACs and LCACs and L-carnitine (CARN) in FEP patients before and after antipsychotic treatment. CSs, control subjects; FEPb, first episode psychosis (FEP) patients before the treatment; FEPa, FEP patients after treatment with antipsychotic drugs; CPT-1, the ratio between long-chain ACs and CARN; CSs versus FEPb, Mann–Whitney *U*-test: * – $p < 0.05$, *** – $p < 0.001$; FEPb versus FEPa, Wilcoxon matched pairs test: *, $p < 0.05$; ***, $p < 0.001$.

significantly ($p = 0.0002$) reduced in the FEP patients group as compared with CSs. The final model demonstrated a strong main effect of the disease ($F_{(32,34)} = 6.22$, $p = 0.0000004$, partial eta^2 = 0.85). Thus our results are indicating that there is a disease-dependent interplay between ACs and metabolic and inflammatory markers.

Statistically significant main effects of the 7 month antipsychotic treatment included 13 ACs, IL-2, IL-4, INF-γ, EGF, C-peptide, leptin, and BMI (Supplementary Table S-5). The most prominent changes emerged for levels of EGF ($p < 0.00000001$), IL-2 ($p = 0.000003$), and C18:1 ($p = 0.0002$). The size of the treatment effect (partial eta^2) was 0.83 ($F_{(20.44)} = 10.49$, $p = 0.0000000001$).

3.4. Ratio of CARN with LCACs and SCACs

For calculation of ratio of CARN with LSACs and SCACs we used the raw data. The ratio between SCACs and CARN in drug-naïve FEP patients revealed a significant decrease in C3 (Mann–Whitney *U*-test, $Z = 4.24$, $p = 0.00002$) and C5 ($Z = 2.38$, $p = 0.018$) (Figure 1). By contrast, CPT-1, reflecting the ratio between LCACs and CARN, demonstrated increased accumulation of former ones (C14, C16, C18) ($Z = 3.42$, $p = 0.0006$). Treatment with antipsychotic drugs reversed all established shifts. The levels of C3 (Wilcoxon matched paired tests, $Z = 4.10$, $p = 0.00004$) and C5 ($Z = 2.38$, $p = 0.017$) were significantly increased compared with CARN (Figure 1). By contrast, CPT-1 showed that the levels of LCACs ($Z = 2.19$, $p = 0.029$) tended to be reduced compared with CARN.

4. DISCUSSION

SCH and related disorders have been shown to induce a shift in the composition of brain lipids (i.e., phospholipids, PUFAs), and chronic treatment with antipsychotic drugs may also affect lipid metabolism.[13−16] Consequently, one may suggest that such alterations in brain lipid levels due to SCH and antipsychotic treatment may have an impact on the ACs profile. However, to our best knowledge these kinds of studies involving whole ACs profile (involving CARN as well as ACs from C2 to C18) have not been performed in FEP patients compared with CSs, before and after treatment with antipsychotic drugs. Therefore, the necessity of such studies is beyond any doubt because there is ample evidence of the impact of ACs on FAO, insulin sensitivity, inflammatory processes, as well as the metabolism of amino acids and ketone bodies.[17,18] Moreover, ACs cannot simply be regarded as the byproducts of CARN transfer system but rather as indirect measures for altered mitochondrial homeostasis because their accumulation may signify MitoDys.[36,37] Consequently, examination of the contribution of CARN and AC profile is critical for adequate characterization of the status of metabolism in psychiatric patients.[38]

Therefore, considering the above-described prerequisites and having access to a diversely characterized cohort of antipsychotic-naïve FEP patients,[7,23,24] we examined the peculiarities of the profile of CARN and ACs (from short- to long-chain representatives) in patients with FEP in comparison with CSs. A profound profiling was performed in FEP patients both before and after 7 month treatment with antipsychotic drugs. We established that 24 out of 39 ACs demonstrated shifts in FEP patients compared with CSs. However, only eight (C14:1, C16, C16:1, C16:1-OH, C18:1, C18:2, C3, C6(C4:1-OH)) of them survived Bonferroni correction for multiple comparisons ($p \leq 0.001$) (Supplementary Table S-1). After applying GLM and stringent criteria that the measured sample concentrations have to be above the limit of detection (LOD; BIOCRATES Life Sciences AG), only four ACs remained in the list. ACs (C14:1, C16, and C18:1) were significantly elevated and C3 was substantially decreased in FEP patients compared with CSs (Table 1). Treatment with antipsychotic drugs significantly affected 17 metabolites in FEP patients. Only

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four of them (C16, C18:1, C18:2, C3) survived Bonferroni correction for multiple comparisons (Supplementary Table S-2 and Table 2). After applying both GLM and stringent LOD criteria, only C18:1 remained significantly different between FEP patients before and after treatment. None of the ACs was significantly different between CSs and post-treatment FEP patients (Supplementary Table S-3), showing that the altered values of ACs returned to the CSs level after treatment with antipsychotic drugs.

The AC profile in FEP reveals some significant changes in LCACs and SCACs. It may have several reasons. First, there is the activation of inflammatory processes in FEP, whereas antipsychotic drug ameliorated the disease symptoms accompanied by a reduction of low-grade inflammation and emerging signs of metabolic syndrome.[23,25,26] In addition, among various biological pathways receiving a significant amount of attention in the search for biomarkers of SCH are those involved in inflammatory processes, as cytokines regulate immune/inflammatory reactions and brain development and influence the dopaminergic, serotonergic, noradrenergic, and glutamatergic neurotransmission.[25,39,40] The results indicate that the levels of peripheral inflammatory markers may be changed in different disease stages, but taken together, these results suggest that the peripheral immune system is overactivated in both individuals undergoing their FEP and in people suffering from chronic SCH.[41]

Second, several articles have studied associations between FEP and OxS.[7,42,43] Although the relative importance of the nature (lipid-related, protein-related) and grade (low-grade, high-grade) of OxS in FEP remains to be elucidated, the present data demonstrate that antipsychotic treatment causes a decrease in OxS status with an improvement of inflammatory status.[7,23] One may suggest that the shift in inflammatory and OxS status in FEP is associated with modifications in LCAC profile and these molecules may reflect the severity of inflammation. Indeed, 7 month treatment with antipsychotic drugs normalizes inflammatory and OxS responses but also restores the profile of LCACs.

Comparison of AC levels with previously established inflammatory and metabolic markers showed a strong positive relation of several LCACs (C14:1, C16.1, C18:1) and negative relation of SCAC C3 with EGF, IL-4, and IL-6 (Supplementary Table S-4). EGF was the strongest marker of psychosis among the studied cytokines in our FEP patient group,[23] IL-4 was also a significant marker of FEP, whereas IL-6 was linked to both the FEP and chronic SCH.[23,27] According to this outcome, one could expect that LCACs may reflect low-grade inflammation in FEP. This statement receives further support by the fact that the ratio between LCACs and CARN (CPT-1) was significantly shifted toward the formation of LCACs (Figure 1). By contrast, the ratio of SCACs with CARN demonstrated reduced formation of C3 and C5. The SCACs profile resembled to a certain extent the response profile of leptin in our previous studies, probably reflecting the role of SCACs in the regulation of energy metabolism. After treatment with antipsychotic drugs we established a strong decrease in LCAC C18:1, and it reached the levels measured in CSs. The change of C18:1 was in line with the decrease in EGF and IL-2 after antipsychotic medication of FEP patients.[23] Recent evidence suggests that LCAC C18:1 inhibits glycine transport via glycine transporter GlyT2 (related to the nociceptive pathways) at high nanomolar concentrations.[44] The close relation of nociceptive and inflammatory processes is a well-established phenomenon. By contrast, the ratio of C3 and C5 with CARN (Figure 1) was elevated after repeated treatment with antipsychotic drugs. This response followed the same trend established for the metabolic markers (leptin, C-peptide) and BMI. This finding seems to underline the role of SCACs in the regulation of energy metabolism.

Third, FEP patients have been shown to display some signs of prediabetes and glucose dysregulation.[45−48] The shift toward domination of LCACs fits with mitochondrial overload, and incomplete FAO refers to a decline in insulin sensitivity. LCACs (C16, C18) can reside in cell membranes due to amphipathic nature and thus can interfere with insulin signaling directly within the membrane. In addition, the alterations of glucose metabolism are especially relevant in the context of increased risk of metabolic syndrome or type 2 diabetes in SCH. Although this can often be linked with antipsychotics side effects, recent meta-analysis by Pillinger et al.[49] confirmed that altered glucose homeostasis is intrinsic to the disease and present from the illness onset. Evidence suggests that FEP is accompanied by disturbances in glucose utilization and energy production.[50] Interestingly, we established an increase in hexoses levels in FEP patients compared with CSs (Table 1 and Supplementary Table S-1), which refers to possible shifts in carbohydrate metabolism. All of the above-mentioned shifts (LCACs, hexoses) may indicate a certain MitoDys in FEP as mitochondria are a fundamental-functional "cross-road" for metabolism of lipids, glucose, and amino acids. It should be noted that regarding amino acid metabolism we established decreased C3 and C5 levels in FEP patients compared with CSs. It is known that SCACs are tightly linked to amino acid and ketone bodies' metabolism.

Some limitations of this study need consideration: First, the limited sample size may create generalization problems. Small cohort size in our study arose from the rarity of first episode, antipsychotic-naïve patients. We suggest that further studies including more patients with a longer follow-up period are necessary to draw firm conclusions regarding the association between treatment with psychotropic medications and the levels of ACs and CARN. In our study, we were not able to control the possible effects of any specific pharmacological agent on the measured biomarkers because of our small sample size and that different kinds of treatment arms were used during the follow-up period. For that reason we analyzed the main effects of treatment at the group level.

Second, we collected data from CSs at one point in time and did not control their health condition or biomarker levels after the same follow-up period, as was done for the FEP patient group. Furthermore, we did not evaluate the participants' dietary or physical activity habits. Diet could be one of the factors that contributes to diversity among study population metabolomes, and the changes may result in metabolic profiles after acute or chronic dietary interventions. This is worth mentioning because disease chronicity and continuous antipsychotic treatment may adversely affect lifestyle factors and cause environmental variation in the group of patients with psychotic disease. However, according to our naturalistic and longitudinal study design as well as because of the peculiarity of this group of patients it was infeasible to follow dietary standardization or specific dietary restriction procedures before the blood collections.

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5. CONCLUSIONS

Our findings indicate that antipsychotic-naïve FEP patients are characterized by different changes in the profile of SCACs and LCACs. The levels of LCACs (C14:1, C16, C18:1) are elevated in FEP patients compared with that of CSs. The comparison of LCACs with CARN demonstrates a shift for increased formation of former ones. Association with the inflammatory and metabolic markers reveals a similar behavior of the mentioned LCACs to the strongest inflammatory markers EGF, IL-4, and IL-6 in FEP patients. This probably reflects the role of LCACs in low-grade inflammation. The levels of SCACs (C3, C5) and their ratio with CARN show reduced formation of these molecules in drug-naïve FEP patients. This change is similar to alteration of leptin in FEP patients and probably underlines the role of these molecules in energy metabolism. Treatment with antipsychotic drugs reverses the levels of LCACs and SCACs to that measured in CSs. By taking into account the changes in low-grade inflammation, OxS, and metabolic modifiers,[7,23,24] it is likely that LCACs contribute more to the signs of inflammation and OxS, whereas SCACs are more implicated in the development of metabolic syndrome. One LCAC (C18:1) is of particular interest because of its significant pharmacological activity. The major target of this molecule, glycine transporter Gly2T, plays a role in the nociceptive pathways.[44] In summary, the present study underlines the distinct role of SCACs and LCACs in the mechanisms of FEP by modulating inflammatory processes and energy metabolism.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.7b00279.

Table S-1. Identification of differences in serum acylcarnitine levels between first-episode psychosis patients at baseline and control subjects. Table S-2. Identification of differences in serum acylcarnitine levels between the first-episode psychosis patients at baseline and FEP patients after 7 month treatment with antipsychotics. Table S-3. Metabolite levels in FEP patients after 7 month treatment with antipsychotics compared with control subjects. Table S-4. Acylcarnitine, metabolic, and inflammatory biomarker serum levels in first-episode psychosis patients at baseline (before treatment with antipsychotics) compared with control subjects. Table S-5. Effects of 7 month treatment with antipsychotics on biomarkers levels in FEP patients. (PDF)

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Notes

The authors declare no competing financial interest.

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