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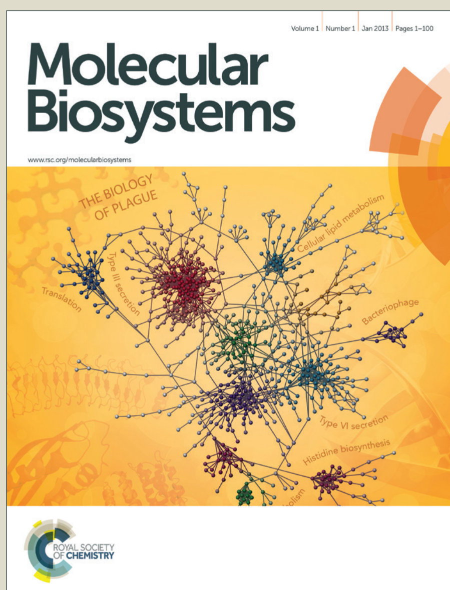

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Peripheral Blood Mononuclear Cell-based Metabolomic Profiling of a Chronic Unpredictable Mild Stress Rat Model of Depression

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Abstract

Major depressive disorder (MDD) is a debilitating mood disorder with various etiopathological hypotheses. However the pathogenesis and diagnosis are still unclear. Peripheral blood mononuclear cells (PBMCs) have been shown to be well-suited to biomarker investigation in major depressive disorder (MDD), as well as to unveil the underlying pathogenesis of MDD. In this study, PBMCs were obtained from a chronic unpredictable mild stress (CUMS) rodent model of depression. Gas chromatography-mass spectrometry (GC/MS) metabolomic approach coupled with principal components analysis (PCA) and open partial least-squares discriminant analysis (OPLS-DA) statistical analysis were conducted to detect differential metabolites in PBMC of depressed rats. A total number of 18 differential metabolites were screened out. Seven metabolites showed lower level in CUMS relative to healthy control rats, including aspartic acid, glutamic acid, dehydroascorbic acid, aminomalonic acid, glycine, β-alanine, and ethanolamine; while eleven metabolites increased in CUMS relative to healthy control rats, namely erythronic acid, fructose, β-tocopherol, adenosine-5-monophosphate, 5-hydroxytryptamine, 5-hydroxytryptamine, glycolic acid, α-tocopherol, tetradecanoic acid, creatinine, 4,5-dimethyl-2,6-dihydroxypyrimidine, and myo-inositol. These molecular changes were closely related to perturbations in neurotransmitter metabolism, energy metabolism and oxidative stress metabolism. Biochemical function analysis of these differential metabolites suggested altered neurotransmitter, energy and oxidative metabolism disorder might be evolved in the pathogenesis of MDD, which could be valuable assist in clinical diagnosis of MDD

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1 Introduction

Major depressive disorder (MDD) is a debilitating mood disorder with a lifetime prevalence of up to 15% [1] that adversely affects social interaction and general health. MDD contributes to 12.3% of the global burden of disease and is predicted to rise up to 15% by 2020[2]. Although there have been various etiopathological hypotheses concerning depression (e.g., the monoamine deficiency hypothesis, neurogenesis, hypothalamic-pituatary-adrenal axis dysfunction)[3], in terms of heterogeneity of the disease, none of them could independently explain the complex pathogenesis of the disease. In addition, the diagnosis of MDD is still largely unknown yet. The clinical diagnosis requires the occurrence of a set of symptoms which seldom present simultaneously, resulting in substandard diagnosis and undertreatment. Thus, there is an urgent need to develop a more comprehensive understanding of the molecular changes underlying depression in order to advance diagnostic biomarker development.

Peripheral blood mononuclear cells (PBMCs) have critical roles in immune response, metabolism, and communication with other cells and extracellular matrices almost everywhere in the human body. Previous studies have reported immune system dysfunction in MDD and post traumatic stress disorder (PTSD) patients [4-8], as well as showing an association between altered lymphocytic expression and depression [5, 9-11]. In particular, studies have shown that the brain and PBMCs have shown a number of parallel responses [12, 13], alterations of metabolism and cellular functions in the CNS, as well as disturbances in the main neurotransmitter and hormonal systems are concomitant with altered function and metabolism of blood lymphocytes [5]. Since Cerebrospinal Fluid (CSF) and brain biopsy samples can be clinically impractical to collect for routine screening or diagnostic purposes, PBMCs derived from convenient, low-cost blood sampling display practical advantages as a peripheral clinical sample in diagnostic biomarker development for MDD.

Recently, metabolomics, which is a quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification, has emerged as a tool in the discovery of molecular biomarkers for diagnosing or prognosing disease, exploring potential pathophysiological mechanisms, and assessing therapeutic effects of drugs [14, 15]. There is mounting evidence showing metabolic irregularities in the brain tissue, urine, and plasma of animal depression models [16-20]. However, no reports regarding PBMC-based metabolic changes in animal depression models have been published thus far. In humans, PBMC-based metabolic patterns are often influenced by medications, causing difficulty in distinguishing the effects of the disease from those of the drugs.

Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and

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nuclear magnetic resonance (NMR) coupled with multivariate statistical methods have been extensively applied in metabolomic research. Compared with other methods, GC-MS has been widely applied on account of its high sensitivity, peak resolution, and reproducibility. Here, through a GC-MS metabolomic approach coupled with principal components analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) statistical analysis, we constructed the well-validated chronic unpredictable mild stress (CUMS) rat model of depression [16, 21, 22] in order to comparatively analyze PBMC-based metabolic patterns in CUMS and healthy control rats. This work should provide valuable insight into PBMC-based metabolic changes that may aid in diagnostic biomarker discovery and possibly paved way to revealing the underlying mechanism(s) of depression.

2 Materials and Methods

2.1 Ethic Statement

This study was performed strictly according to the recommendations of *Guide for the Care and Use of Laboratory Animals* [23]. This study was approved by the Ethics Committee of Chongqing Medical University (Permit number: 20120126). Male Sprague-Dawley rats were obtained from Chongqing Medical University (CQMU)'s animal center. Throughout the experiment, all rats were maintained under a standard laboratory condition (24 ± 1°C, 45 ± 15 relative humidity, and a 12h/12h light/dark cycle) with free access to a standard rat diet and tap water.

2.2 Chronic Unexpected Mild Stress Procedure

Only rats with stable sucrose consumption performances and sucrose preferences of greater than 90% were selected for further study. On this basis, rats weighing approximately 250g were randomly divided into a chronic unpredictable mild stress (CUMS, n=50) group and a healthy control (CON, n=30) group. Previous studies have shown that approximately 20% of rats do not reduce their sucrose intake after CUMS treatment [24-26]; therefore, more rats were placed into the CUMS group.

The CUMS procedure was similar to our previous work [21, 22]. Herein, we made some appropriate adjustments to strengthen the stimulation and increase the unpredictability. Stressors were randomly paired (except for restriction, which is relatively stronger stimulation). The entire CUMS procedure lasted for four weeks. CON rats were left undisturbed in their home cages besides regular cage cleaning and body weight measurement, which were performed simultaneously with the CUMS rats.

CUMS rats could be measured as decreased sucrose consumption by means of the sucrose preference test (SPT) [24, 27, 28]. The ratio of 24 h sucrose solution preference was calculated as follows: sucrose preference % = sucrose consumption / (sucrose consumption + water consumption) * 100% [29]. SPT was conducted in triplicate

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before and after the CUMS model construction and after 24 h deprivation of food and water. One day of recovery was needed before conducting another SPT. The mean value was used in the data analysis. Prior to experimentation, all rats were trained to consume a palatable 2% sucrose solution. Each rat was placed in their own cage with free access to one bottle of the 2% sucrose solution and another bottle of tap water. Following exposure to CUMS, rats were characterized as depressed if their SPT was less than 70% [28]. The rats' weights were measured each week during the CUMS period.

2.3 PBMC Collection and Pretreatment

Rats were sacrificed under anesthesia by an overdose of chloral hydrate and blood samples (approximately 8 ml) were quickly collected via heart puncture in EDTA-coated tubes. Plasma was separated by blood centrifugation at 1500 g for 15 min within 1 hr after blood collection. The remainder was diluted with phosphate buffer saline (PBS) to the original blood sample volume. Subsequently, plasma was overlaid onto lymphoprep (Lymphoprep-density, 1.077 ± 0.001 g/ml; GE Healthcare Bio-Sciences AB, Sweden) and centrifuged at 2000 rpm for 20 min at room temperature. The supernatant was carefully removed. After washing three times with PBS, PBMC pellets were stored at −80°C until later GC-MS analysis.

2.4 GC-MS Sample Preparation and Derivatization

For GC/MS analysis, each frozen PBMC sample was added to 1 ml chromatographic grade methanol and 10 μl leucine-13C6 (0.5 mg/ml) as an internal standard and stored at 0°C overnight. The mixture was sonicated for 30 min and subsequently centrifuged at 14000 g at 4°C for 15 min. An 800 μl volume of supernatant was transferred into a new 1.5 ml EP tube, mixed with 400 μl extracting solution, and then evaporated to dryness under a stream of nitrogen gas. The dried residue was mixed with 30 μl methoxamine hydrochloride (20 mg/ml pyridine) and incubated at 37°C for 90 min with continuous shaking. Subsequently, the solution was derivatized with 30 μl BSTFA (1% TMCS) at 70°C for 60 min and then placed at room temperature for 30 min before GC/MS analysis.

2.5 GC-MS Analysis

GC-MS analysis was performed using the Agilent 7890A/5975C GC-MS System (Agilent Technologies Inc., USA). Chromatography was performed on a HP-5 MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA). The temperatures of the injector, EI iron source, and quadrupole rods were set at 280°C, 230°C, and 150°C, respectively. High purity helium carrier gas flowed at a rate of 1 ml/min. A total of 1 μl for each sample was applied for metabolite separation. The column temperature was initially maintained at 80°C for 2 min and then raised to 320°C by 10°C/min, maintained for 6 min. MS detection was conducted with electron impact ionization mode in the full scan mode (m/z, 50-600). To avoid the influence induced by instrument signal

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fluctuations, a random order of continuous sample analysis was adopted.

2.6 Metabolomic Data Analysis and Identification of Metabolic Signatures

Nine CUMS PBMC samples were comparatively analyzed against nine CON PBMC samples. Visual inspections of total ion current chromatogram (TIC) chromatograms were applied to all samples. All displayed strong signals for analysis as well as large peak capacity and good reproducibility in retention time (Fig. 1). A total of 292 individual peaks were detected, which existed in over 80% percent of the samples in each group. In this study, compound annotation was performed by comparing the accurate mass (m/z) and retention time (Rt) of reference standards in our in-house library and the accurate mass of compounds obtained from the web-based resources (NIST online databases). All the GC/MS metabolic profiles were processed after conversion into a NetCDF format using TagFinder [30]. This processing enabled deconvolution, alignment, and data reduction to produce a list of mass and retention time pairs with corresponding intensities for all detected peaks from each data file in the data set. The resulting table was exported into Microsoft Excel where normalization was performed prior to multivariate analysis. The resulting two-dimensional matrix, involving peak index (RT-m/z pair), sample names (observations), and normalized peak area square, were introduced into SIMCA-P 11.0 software (Umetrics, Umeå, Sweden).

PCA was applied to the unit variance (UV)-scaled spectral data to reveal intrinsic CUMS-related patterns. Then, OPLS-DA was performed to discriminate CUMS samples from CON samples, yielding two parameters: R^2Y being the cumulative model variation in Y and Q^2 - being the cumulative predicted variation. As these parameters approach 1.0, a stable model with predictive reliability is indicated. The discriminating metabolites were obtained using a statistically significant threshold of variable influence on projection (VIP) values obtained from the OPLS-DA model and two-tailed Student's *t*-test on the normalized raw data at a univariate analysis level. Metabolites with VIP values greater than 1.0 and *p*-values of less than 0.05 were deemed to be statistically significant. Fold change was calculated as the logarithm of the average mass response (area) ratio between two arbitrary classes.

3 Results

3.1 Successful Construction of the CUMS Rat Model

We successfully constructed the CUMS rat model of depression. Data were presented as means ± SD and analyzed using SPSS software. To evaluate the quality of the CUMS model, two parameters were used: changes in body weight and sucrose preference. Body weight was analyzed by means of a repeated measurement ANOVA

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with the CUMS or CON group as an independent factor and time as a repeated measure. A Student's *t*-test was used to analyze significant differences between the two groups. All tests were two-tailed. The significance threshold was set at $p<0.05$ (Fig. 2a, b).

With respect to body weight, ANOVA yielded a main effect of time [F=2419.936, $p<0.001$], a main effect of group [F=15.724, $p<0.001$], and significant group by time interaction [F=385.382, $p<0.001$]. No significant differences were found in the baseline body weight of the two groups, but the body weight of the CUMS group was significantly lower than that of the CON group during the last three weeks of the CUMS period (t=4.204, $p<0.001$; t=8.404, $p<0.001$; t=6.506, $p<0.001$). Regarding sucrose preferences, CUMS rats showed a dramatic reduction in relative sucrose intake compared with CON rats (t=6.462, $p<0.01$).

3.2 Multi-dimensional Statistic Analysis and Identification of Differential Metabolites

PCA ($R^2X=0.421$; Fig. 3a) was used to identify differences in the PBMC metabolic profiles between the CUMS and CON groups. OPLS-DA analysis indicated that this model was efficient and showed clear separation between the two groups ($R^2Y=0.99$, $Q^2=0.671$; Fig. 3b, c).

According to the OPLS-DA analysis, 18 significant differential metabolites were screened out (Table 1). Seven of abovementioned metabolites showed lower levels in CUMS than CON rats, including aspartic acid, glutamic acid, dehydroascorbic acid, aminomalonic acid, glycine, β-alanine, and ethanolamine; while eleven metabolites increased in CUMS relative to CON rats, namely erythronic acid, fructose, β-tocopherol, adenosine-5-monophosphate, 5-hydroxytryptamine, 5-hydroxytryptamine, glycolic acid, α-tocopherol, tetradecanoic acid, creatinine, 4,5-dimethyl-2,6-dihydroxypyrimidine, and myo-inositol.

4 Discussion

MDD is a debilitating mood disorder that has been associated with changes in peripheral lymphocytes. Through a GC-MS metabolomic approach coupled with PCA and OPLS-DA statistical analysis, this study revealed a set of 18 differential metabolites that clearly distinguishes CUMS from CON rats. To better understand the underlying pathogenesis of depression, the differential metabolites were assigned to the following four dominating categories according to their biochemical functions (KEGG http://www.genome.jp/kegg/; HMDB http://www.hmdb.ca/; METLIN http://metlin.scripps.edu/index.php): 1) amino acid metabolism disorder, including aspartic acid, glutamic acid, glycine and 5-hydroxytryptamine; 2) Energy metabolism disorder, which includes creatinine and AMP; 3) antioxidants metabolism disorder, which includes elevated β-tocopherol and α-tocopherol, decreased dehydroascorbic acid and erythronic acid; 4) others, including myo-inositol and alaine. This is the first

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study to report PBMC-based metabolic changes in a CUMS rat model of depression.

4.1 Disturbed Neurotransmitter Metabolism

In this study, decreased levels of aspartic acid, glutamic acid and glycine were observed in CUMS relative to CON rats. Aspartic acid is the precursor of N-acetylaspartate (NAA), the second most abundant amino acid in the CNS that is a marker of the formation and/or maintenance of myelin, dendritic, and synaptic proliferation [31, 32]. Elevated hippocampal NAA levels have been related to better working memory performance in humans [33]. Brain imaging studies of MDD patients had reported lower level of NAA in caudate, the dorsolateral prefrontal cortex and hippocampus, which is in consistent to our study [34-36]. Significant reduction of NAA was also observed in plasma of MDD patients [37]. The decreased aspartic acid in this study might suggest decreased NAA biosynthesis in PBMC. However, in animal studies, NAA showed either elevated or decreased level in different brain regions [38], whilst increased NAA in plasma CUMS rat model of depression [39]. Our previous study also found augmented NAA in prefrontal cortex of rat model of depression [40]. Of note, we might raise questions on inconsistent changes between animal models and MDD patients. Furthermore, provoking the thoughts that animal models could simulate the disease to some extent, it still could not reveal the intricate process of MDD.

Glutamic acid (glutamate, GLU) is accepted as the major excitatory neurotransmitter in the nervous system. GLU play a key role in the long-term potentiation and are important in learning and memory [41]. The glutamatergic system is a critical mediator of stress responses through regulating the hypothalamic–pituitary–adrenal axis function [42-44]. In animal models of depression, alterations in glutamatergic neurotransmission proteins induce depressive-like behaviors [45, 46]. Changes in glutamate levels have been noted in plasma, serum, CSF and brain tissue of individuals with mood and psychotic disorders, as well as in suicide victims [47]. However the interpretations of glutamate levels in different samples is challenging, given confounds such as medication exposure, post-mortem metabolic effects, and the inability to distinguish the source of glutamate (central vs peripheral). The present finding of decreased glutamic acid in the PBMC of depressed rats may reinforce previous reports that indirectly suggest the involvement of glutamate in the pathophysiology.

Glycine is an inhibitory amino acid neurotransmitter that participates in many essential cellular processes. Glycine, which showed significant lower level in plasma of MDD patients [48], however was augmented in urine of MDD patients [49]. The metabolomic profiling of different brain regions in CUMS rats also revealed significant higher levels of glycine [38]. The discrepant distribution of glycine might suggest different signal regulating pathway in CNS and peripheral blood stream.

5-hydroxytryptamine (5-HT, serotonin), a monoamine neurotransmitter derived from tryptophan, was found

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augmented in CUMS relative to CON rats. The monoamine hypothesis of depression states that depressive illness arises from decreased brain 5-HT function [50]. As (1) decreased tryptophan levels have been observed in the postmortem brains of suicidal MDD patients [51] as well as in the plasma and urine of CUMS rats [16, 52]; (2) altered 5-HT transporter expression has been observed in disinhibited infant rhesus macaques exposed to early life [53]; (3) the influence of life stress on human depression development is moderated by a polymorphism in the 5-HT transporter gene [54]; and (4) we found increased 5-HT levels in the PBMCs of CUMS rats here, these combined facts suggest increased tryptophan or 5-HT importation into PBMCs from the extracellular environment. In order to test this hypothesis, further research should investigate tryptophan and 5-HT transporter expression and activity in the PBMCs of CUMS rats and MDD patients.

In determination of the aforementioned set of neurotransmitters, may be the conductive to the clinical diagnosis of depression disease.

4.2 Disturbed Energy Metabolism

Creatinine is synthesized via a metabolic pathway consisting of creatine, phosphocreatine (alternatively termed creatine phosphate), and adenosine triphosphate (ATP). The creatine- phosphocreatine-ATP pathway plays a major role in cellular energy transport. In this study, there were significant increases in creatinine and adenosine-5-monophosphate (AMP) in the PBMCs of CUMS relative to CON rats. Zheng et al. reported significant decreases in creatinine in both the plasma and urine of MDD patients [48, 49]. The increased PBMC creatinine levels observed here suggest an altered energy metabolism in the PBMCs of CUMS rats, while the decreased plasma and urine creatinine levels observed by Zheng et al. suggest deficiencies in creatinine transport. Although we did not detect any significant differences in creatine levels here, Gruber et al. reported that absolute creatine levels in the prefrontal cortex (PFC) of MDD patients was higher than that of healthy individuals [36], and Milne et al. reported increased hippocampal creatine levels associated with recurrent episodes of MDD [55].

4.3 Disturbed Oxidative Stress Metabolism

MDD has been previously associated with an increase in oxidative stress and a decrease in antioxidant status that damages neurons [56] and has an important role in the pathophysiology of depressive disorders. In this study, elevated levels of α-tocopherol and β-tocopherol – antioxidant analogs of vitamin E [57] – were observed in CUMS relative to CON rats. α-tocopherol has been shown to exhibit antidepressant properties in preclinical studies [58], and clinical studies have demonstrated that depressed patients possess lower serum and plasma vitamin E levels [59, 60]. Low plasma α-tocopherol levels have been associated with poor memory performance in elderly individuals and with major depression states. Increased PBMC levels of α-tocopherol and β-tocopherol suggest enhanced oxidative

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stress activity in PBMCs, supporting the role of oxidative stress in the pathogenesis of MDD.

4.4 Other Metabolites

As myo-inositol is a significant intracellular osmolyte, changes in myo-inositol levels suggest alterations in tissue osmolarity. Consistent with our findings, a post-mortem study on bipolar disordered patients showed elevated brain myo-inositol levels [61]. The higher PBMC myo-inositol levels in CUMS rats relative to CON rats observed here may be a result of higher myo-inositol uptake or retention, changes in the cellular or extracellular matrix, or perturbation of the coupling mechanism in the receptor-secondary messenger system complex [62].

Metabolic changes of PBMC found herein to that reported in other parts of the body may not be reflexed as a counterpart perfectly, whereas, to large extent, studies have shown that the brain and PBMCs could express substantial parallel responses so far. Additionally, though differential metabolites have shed some light on understanding the pathogenesis of MDD, as well as pushing clinical diagnostic more convincible with biomarker detection and identification, there are still limitations which need more supplements in further studies. Firstly, the total sample size of this study was limited, as only nine CUMS PBMC samples were comparatively analyzed against nine CON PBMC samples. Secondly, stress-related mental disorders, such as MDD and PTSD, are gender-related [63]; however, all rats used in this study were male. Therefore, further studies with both male and female subjects are necessary to rule out sex-based effects. Thirdly, although this study has identified several candidate PBMC biomarkers from a CUMS rat model of depression, human PBMC-based metabolomic analysis including diagnostic specificities and sensitivities are required to advance biomarker development.

5 Conclusion

In conclusion, this is the first study on PBMCs metabolomics in CMUS rat model of depression. A set of differential metabolites associated with disturbed neurotransmitter, energy and oxidative stress metabolism in the PBMCs of CUMS rats, suggesting altered neurotransmitter, energy and oxidative stress metabolism disorder might be evolved in the pathogenesis of MDD. Aspartic acid, glutamic acid, glycine and 5-HT, which were key neurotransmitters may be useful for the clinical diagnosis of depression. Future metabolomic research should focus on identifying and validating human PBMC-based biomarkers for MDD.

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Conflict of Interest

The authors have declared no conflict of interest in the submission of this manuscript.

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Figure Legends

Figure 1. GC/MS Total Chromatogram of (a) Chronic Unpredictable Mild Stress (CUMS) and (b) Healthy Control (CON) Rats. Peaks: (1) glycolic acid, (2) ethanolamine, (3) glycine, (4) 4,5-dimethyl-2,6dihydroxypyrimidine, (5) β-alanine, (6) aminomalonic acid, (7) aspartic acid, (8) erythronic acid, (9) creatinine, (10) glutamic acid, (11) tetradecanoic acid, (12) dehydroascorbic acid, (13) fructose, (14) myo-inositol, (15) 5-hydroxytryptamine, (16) β-tocopherol, (17) adenosine-5-monophosphate, and (18) α-tocopherol.

Figure 2. Body Weight Changes During the Chronic Unpredictable Mild Stress (CUMS) Procedure. (a) At the second, third, and fourth week of the CUMS procedure, significant decreases in body weight were found in CUMS relative to CON rats ($p<0.001$). (b) Comparisons by sucrose preference testing (SPT) before and after the CUMS procedure. CUMS rats showed a significant decrease in sucrose preference as compared to CON rats ($p<0.05$). *$p<0.05$, ***$p<0.001$

Figure 3. Metabolomic Analysis of Peripheral Blood Mononuclear Cell (PBMC) Samples from Chronic Unpredictable Mild Stress (CUMS) and Healthy Control (CON) Rats. (a) Principal components analysis (PCA) scores plot derived from gas chromatography-mass spectrometry (GC/MS) spectra of PBMCs from CUMS and CON rats. (b) Open partial least-squares discriminant analysis (OPLS-DA) model showing a clear separation between CUMS and CON rats. (c) Statistical validation of the OPLS-DA model by permutation testing.

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Tables

Table 1. Differential Peripheral Blood Mononuclear Cell (PBMC) Metabolites Detected by Gas Chromatography-Mass Spectrometry (GC/MS)

No.	Metabolite	RT/min	M/Z	VIP value (OPLS-DA)*	*P*-value (Student's *t*-test)*	Fold change†
1	Aspartic acid	11.64	232	1.94	6.69E-03	-0.66
2	Glutamic acid	12.81	246	2.04	1.17E-03	-0.60
3	Dehydroascorbic acid‡	15.4	173	1.76	9.60E-03	-0.45
4	Aminomalonic acid	11.02	218	1.85	9.31E-03	-0.35
5	Glycine	8.88	174	2.23	8.51E-04	-0.35
6	β-alanine	10.44	248	1.81	1.96E-02	-0.28
7	Ethanolamine	8.31	174	1.67	3.52E-02	-0.24
8	Erythronic acid	12.01	292	1.75	2.01E-02	0.17
9	Fructose	15.84	103	1.52	3.44E-02	0.25
10	β-tocopherol	24.61	222	1.56	3.39E-02	0.25
11	Adenosine-5-monophosphate	25.29	315	1.77	1.97E-02	0.26
12	5-hydroxytryptamine	20.88	174	1.53	3.11E-02	0.27
13	Glycolic acid	5.66	177	1.72	3.06E-02	0.28
14	α-tocopherol	25.73	502	1.50	4.21E-02	0.30
15	Tetradecanoic acid	15.18	285	1.75	2.20E-02	0.35
16	Creatinine	12.09	115	1.50	7.31E-03	0.36
17	4,5-dimethyl-2,6-dihydroxypyrimidine	9.84	269	1.61	1.09E-02	0.38
18	Myo-inositol	16.57	305	1.59	2.73E-02	0.40

† Only metabolites with variable influence on projection (VIP) values of greater than 1.0 and p-values of less than 0.05 were deemed to be statistically significant.

†Fold change was calculated as the logarithm of the average mass response (area) ratio between the two classes (i.e., fold change = $\log_2$[CUMS/CON]). Thus, positive fold-change values indicate significantly higher levels in CUMS relative to CON rats, and negative fold-change values indicate significantly lower levels in CUMS relative to CON rats.

‡May be a combination of dehydroascorbic and ascorbic acids, since extraction was performed under oxidative conditions.

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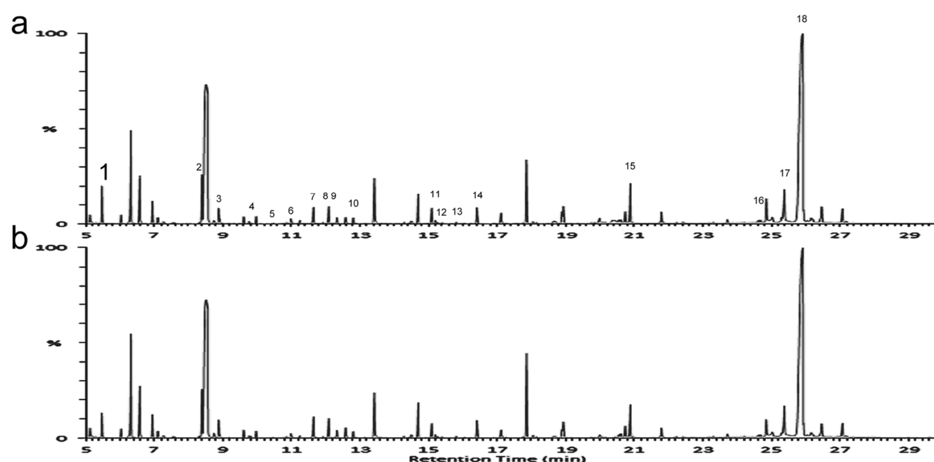

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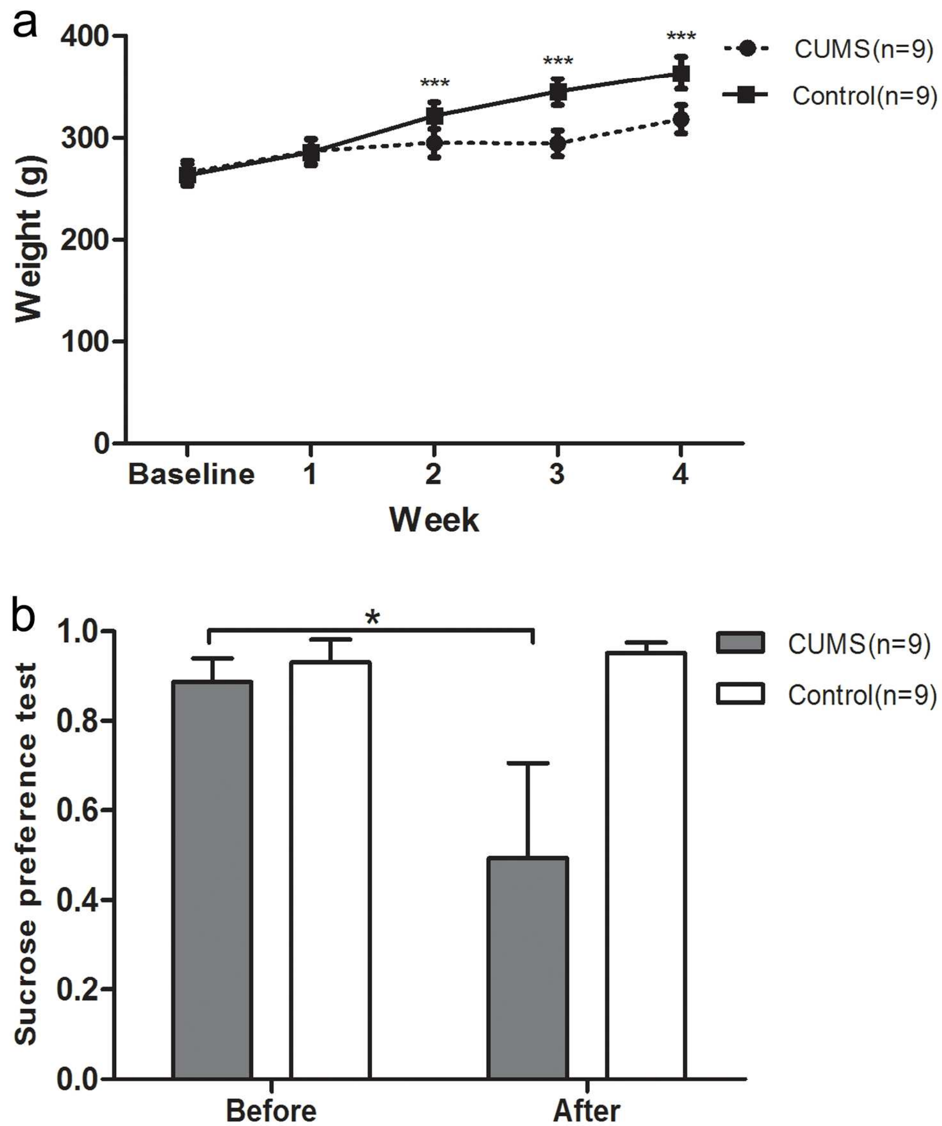

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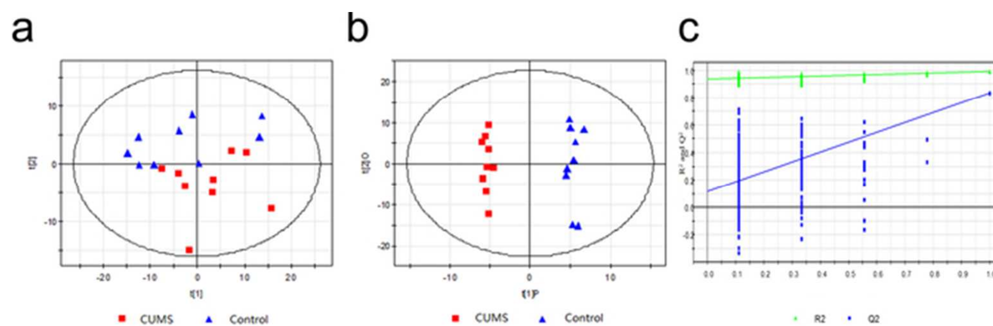

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57x19mm (300 x 300 DPI)

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