

Mitochondrial dysfunction in first-episode drug-naïve major depressive disorder: insights from serum non-targeted metabolomics

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Abstract

Background

Mitochondrial dysfunction has been increasingly implicated in the pathophysiology of major depressive disorder (MDD), yet comprehensive serum metabolic evidence particularly in first-episode, drug-naïve patients remains limited. This study aimed to characterize the serum metabolomic profile of first-episode drug-naïve MDD and to identify mitochondrial energy metabolism alterations with potential diagnostic value.

Methods

In this case–control study, 118 first-episode drug-naïve MDD patients and 56 age and sex-matched healthy controls were recruited. Fasting serum samples were analyzed by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) in both positive and negative electrospray ionization modes. Multivariate analyses (PCA and OPLS-DA), univariate Mann–Whitney U tests with Benjamini–Hochberg false discovery rate (FDR) correction, and KEGG pathway enrichment were performed. Mitochondrial-related metabolites were systematically identified via dual-database mapping (MitoCarta 3.0 and KEGG). Receiver operating characteristic (ROC) curves were constructed to evaluate diagnostic performance.

Results

A total of 3,020 serum metabolites were annotated. PCA revealed a clear separation between MDD patients and controls. Eighty-three metabolites showed significant between-group differences (FDR < 0.05), of which 71 exhibited variable importance in projection (VIP) > 1 in the OPLS-DA model ($R^2Y = 0.547$, $Q^2Y = 0.526$; 200-permutation test confirmed no overfitting). Thirteen core differential metabolites met the stringent criteria of FDR < 0.05, VIP > 1, and $|\log_2 \text{fold change}| > 1$. Lipids and acylcarnitines constituted the largest functional category (22/71, 31.0%). Notably, all 14 mitochondrial-related differential metabolites were downregulated in the MDD group, including eight acylcarnitines and reduced ubiquinol (QH₂). KEGG enrichment analysis identified significant perturbations in fatty acid β -oxidation, branched-chain amino acid degradation, the tricarboxylic acid (TCA) cycle, and ubiquinone biosynthesis (all $P < 0.05$). ROC analysis demonstrated that an eight-acylcarnitine combined model achieved an area under the curve (AUC) of 0.815 (95% CI: 0.753–0.877), with 74.58% sensitivity and 78.57% specificity.

Conclusions

Our findings reveal a systemic disruption of mitochondrial energy metabolism in first-episode drug-naïve MDD, characterized by impaired fatty acid β -oxidation and compromised electron transport chain function. Serum acylcarnitines represent promising candidate state-related metabolic signatures. They cannot serve as a standalone clinical diagnostic tool at present, and require further external multi-cohort validation; meanwhile, they may provide novel mechanistic insights into the metabolic underpinnings of depression. These results are derived from serum metabolomics data and require further validation using central nervous system-derived biosamples and independent cohorts.

Introduction

Major depressive disorder (MDD) represents one of the leading causes of disability worldwide, imposing a substantial burden on individuals, families, and healthcare systems [1, 2]. Despite advances in psychopharmacology, approximately one-third of patients fail to achieve remission following standard antidepressant treatment, and the delayed onset of therapeutic action remains a critical clinical challenge [3]. A fundamental obstacle to improving outcomes is the absence of objective, biologically grounded diagnostic tools and mechanistically informed treatment targets. Current diagnostic criteria rely entirely on symptom-based assessments, which are inherently subjective and offer limited insight into the heterogeneous pathophysiological processes underlying depression [4]. Consequently, there is an urgent need to identify robust peripheral biomarkers that not only facilitate early and accurate diagnosis but also illuminate the biological mechanisms driving disease onset.

The pathophysiology of MDD has traditionally been conceptualized through the monoamine hypothesis; however, accumulating evidence suggests that monoaminergic dysregulation alone cannot fully account for the complexity of depressive symptomatology or treatment resistance [5]. Over the past two decades, research has increasingly implicated disturbances in neuroinflammation, hypothalamic–pituitary–adrenal (HPA) axis hyperactivity, neurotrophic factor deficiency, and more recently cellular bioenergetics and mitochondrial dysfunction [6, 7]. Mitochondria, as the primary organelles responsible for adenosine triphosphate (ATP) synthesis, are critical for maintaining neuronal excitability, synaptic plasticity, and stress resilience. Impaired mitochondrial function may compromise energy supply in metabolically demanding brain regions, thereby contributing to the cognitive, emotional, and neurovegetative symptoms characteristic of MDD [8, 9].

Converging lines of evidence support the mitochondrial hypothesis of depression. Post-mortem studies have documented reduced mitochondrial DNA copy numbers, decreased complex I–IV activity, and altered mitochondrial morphology in the prefrontal cortex and hippocampus of depressed individuals [10, 11]. Peripheral investigations have similarly revealed lower mitochondrial respiration, elevated oxidative stress markers, and abnormal mitochondrial dynamics in platelets and leukocytes from patients with MDD [12, 13]. Furthermore, preclinical models of chronic stress consistently demonstrate mitochondrial ultrastructural damage and reduced ATP production in limbic structures, effects that are partially reversed by conventional antidepressants and novel mitochondrial-targeted agents [14, 15]. These findings collectively suggest that mitochondrial bioenergetic failure may constitute a core pathophysiological feature of depression rather than merely an epiphenomenon of chronic illness or pharmacological exposure.

Despite this progress, several critical gaps remain. First, the majority of human studies have been conducted in chronically ill or medicated patient populations, making it difficult to disentangle disease-related metabolic alterations from the confounding effects of long-term antidepressant use, recurrent episodes, and lifestyle changes associated with chronic disability [16, 17]. First-episode, drug-naïve patients represent a crucial yet understudied population in whom metabolic signatures are more likely to reflect primary disease pathophysiology. Second, prior investigations have often relied on targeted assays of isolated mitochondrial parameters, potentially overlooking the integrated, systems-level nature of metabolic dysregulation. Non-targeted metabolomics offers a powerful alternative by enabling comprehensive profiling of small-molecule metabolites and capturing global perturbations across interconnected biochemical pathways [18, 19].

Metabolomics sits at the phenotypic endpoint of the central dogma, integrating genetic, transcriptomic, and proteomic influences with environmental exposures. In the context of depression, serum metabolomics is particularly attractive because peripheral blood is readily accessible, minimally invasive, and increasingly recognized as a valid window into central nervous system metabolism via the blood–brain interface [20]. Previous metabolomic studies in MDD have identified alterations in tryptophan metabolism, lipid profiles, and gut-microbiota-derived metabolites [21, 22]. However, few studies have employed rigorous strategies to objectively classify mitochondrial-related metabolites, and the specific role of acylcarnitine-mediated fatty acid β -oxidation in first-episode, drug-naïve depression remains poorly characterized. Acylcarnitines serve as essential shuttle molecules for long-chain fatty acid transport across the mitochondrial membrane; their systemic depletion may indicate impaired mitochondrial uptake and subsequent β -oxidation, with direct consequences for ATP generation and redox homeostasis [23, 24].

Against this background, the present study was designed to characterize the serum non-targeted metabolomic profile of first-episode, drug-naïve MDD patients relative to healthy controls. We hypothesized that (1) MDD patients would exhibit a distinct serum metabolic signature characterized by widespread downregulation of mitochondrial energy metabolism pathways, particularly fatty acid β -oxidation and the tricarboxylic acid (TCA) cycle; (2) acylcarnitines and other mitochondrial-related metabolites would be significantly reduced in depressed individuals; and (3) a panel of these metabolites would demonstrate discriminatory capacity between patients and controls, supporting their potential as objective biomarkers. To ensure objectivity, we employed a dual-database mapping strategy integrating MitoCarta 3.0 and KEGG pathway annotations to systematically identify mitochondrial-associated differential metabolites. By focusing on a rigorously defined, medication-free, first-episode cohort, this investigation aims to provide novel, confound-minimized insights into the metabolic underpinnings of depression and to advance the development of biologically informed diagnostic tools.

Methods

Study design and participants

This case–control study consecutively enrolled 118 first-episode, drug-naïve patients with major depressive disorder (MDD group) and 56 age- and sex-matched healthy controls (control group) from the psychiatric outpatient clinic of Hefei Fourth People's Hospital between January 2023 and December 2025. All participants provided written informed consent, agreeing that their de-identified clinical data and biological samples could be used for this study and subsequent related scientific research. The study protocol was approved by the Ethics Committee of Hefei Fourth People's Hospital (Approval Number:HFSY-IRB-YJ-KYXM-CL[2024-064-001]) and conducted in accordance with the Declaration of Helsinki.

Inclusion criteria for the MDD group were as follows: (1) met the diagnostic criteria for a major depressive episode according to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) [25]; (2) first depressive episode with no prior use of antidepressants, mood stabilizers, antipsychotics, or other psychotropic medications; (3) total score ≥ 17 on the 17-item Hamilton Depression Rating Scale (HAMD-17) [26]; (4) aged 18–65 years; (5) availability of fasting venous blood for metabolomic testing; and (6) complete demographic and clinical data.

Exclusion criteria for the MDD group were: (1) comorbid bipolar disorder, schizophrenia, generalized anxiety disorder, or other severe mental illness; (2) history of brain organic lesions, cerebrovascular disease, severe hepatic or renal impairment, diabetes mellitus, malignancy, or autoimmune disease; (3) pregnancy or lactation; (4) alcohol, tobacco, or psychoactive substance abuse within 3 months prior to enrollment; (5) long-term use of lipid-lowering, hypoglycemic, hormonal, or other medications interfering with endogenous metabolism; and (6) damaged blood samples or missing clinical data.

Inclusion criteria for the healthy control group were: (1) no personal history of mental illness and HAMD-17 total score < 7 [26]; (2) no first-degree relatives with a history of mental illness; (3) aged 18–65 years; (4) no severe chronic physical, metabolic, or organ diseases; (5) no long-term use of metabolism-interfering medications; and (6) age and sex distribution matched to the MDD group.

Sample size estimation

A priori sample size estimation was performed using G*Power 3.1 software [27]. Based on previous non-targeted metabolomics case–control studies, the expected effect size was set at Cohen's $d = 0.5$ (medium effect size), with a two-tailed significance level $\alpha = 0.05$ and statistical power $(1 - \beta) \geq 80\%$. Using an independent two-sample t -test, a balanced design ($n_1 = n_2$) required a minimum of 64 subjects per group, yielding a total sample size of at least 128 to achieve 80% power. In this study, 118 first-episode drug-naïve MDD patients and 56 healthy controls were actually enrolled, with a group ratio of approximately 2.1:1. Re-estimation under this unbalanced design indicated an actual statistical power of approximately 86.5% for detecting a Cohen's $d = 0.5$ effect size, exceeding the preset 80% threshold. If the effect size was relaxed to Cohen's $d = 0.4$, the power dropped to approximately 68.8%; if increased to Cohen's $d = 0.6$, the power reached 95.7%. Accordingly, our cohort possesses sufficient statistical power to capture medium-to-large metabolic effect sizes, whereas small-magnitude metabolic alterations (Cohen's $d \leq 0.4$) might remain under-detected given the 2.1:1 case-control imbalance.

Clinical data collection

Demographic and clinical data were collected using a unified, self-designed case registration form, including age, sex, ethnicity, education level, household per capita income, marital status, childhood left-behind experience, smoking and alcohol consumption, and past medical history.

Serum sample collection and metabolomic profiling

Blood collection and preprocessing

All participants fasted overnight for at least 12 hours. The next morning, 5 mL of fasting peripheral venous whole blood was collected into pro-coagulation tubes and allowed to stand at room temperature for 30 minutes. Serum was separated by centrifugation at 3,000 r/min at 4 °C for 10 minutes, then aliquoted into 1.5 mL EP cryotubes and stored at –80 °C until non-targeted metabolomic analysis, avoiding repeated freeze–thaw cycles.

UHPLC-Q-TOF-MS non-targeted metabolomic analysis

Serum metabolite extraction was performed following established methanol precipitation protocols optimized for global metabolite recovery [28]: 100 µL of serum was mixed with 400 µL of pre-cooled methanol containing internal standards, vortexed for 3 minutes, and incubated at –20 °C for 20 minutes. After centrifugation at 13,000 r/min at 4 °C for 15 minutes, the supernatant was vacuum-dried, reconstituted in mobile phase, centrifuged again, and the final supernatant was injected for analysis.

Chromatographic separation was performed on an ultra-high-performance liquid chromatography (UHPLC) system equipped with a C18 column (100 mm × 2.1 mm, 1.7 µm). Mobile phase A consisted of 0.1% (v/v) formic acid in water, and mobile phase B consisted of 0.1% (v/v) formic acid in acetonitrile. The gradient elution program was: 0–2 min, 5% B; 2–5 min, 5%–30% B; 5–15 min, 30%–95% B; 15–17 min, 95% B; 17–17.5 min, 95%–5% B; 17.5–20 min, 5% B (column equilibration). The flow rate was 0.3 mL/min, column temperature 40 °C, and injection volume 2 µL.

Mass spectrometry was performed on a Waters Xevo G2-XS quadrupole time-of-flight mass spectrometer (QTOF-MS) equipped with an electrospray ionization (ESI) source, operating in both positive (ESI+) and negative (ESI–) ion modes. Key parameters were: capillary voltage 2.5 kV (ESI+) and 2.0 kV (ESI–); cone voltage 40 V; source temperature 120 °C; desolvation temperature 450 °C; desolvation gas (nitrogen) flow 900 L/h; cone gas (nitrogen) flow 50 L/h. Collision energy was applied using MSE data acquisition mode, with a low-energy channel at 6 eV and a high-energy channel gradient of 10–40 eV. The MS1 scan range was m/z 50–1200, scan time 0.2 s/spectrum, mass resolution > 20,000 FWHM at m/z 556, and data acquisition rate 5 Hz. A Lockspray real-time mass correction system was employed using leucine enkephalin as the lock mass internal standard (ESI+: m/z 556.2771 [M+H]⁺; ESI–: m/z 554.2615 [M–H][–]; concentration 200 ng/mL; flow rate 10 µL/min), with real-time mass axis correction every 10 seconds to ensure mass accuracy < 5 ppm. Additionally, one offline mass calibration solution (sodium formate, m/z 50–1200) was inserted after every 10 study samples to eliminate mass drift during long runs. Quality control (QC) samples were inserted every 10 study samples to monitor instrument stability.

QC sample preparation and monitoring

A pooled QC strategy was adopted to monitor analytical stability. Equal volumes (10 µL) of serum from all participants were mixed to prepare a pooled QC sample, which was then aliquoted and processed identically to the study samples (methanol precipitation, vortexing, centrifugation, drying, and reconstitution). In the UHPLC-Q-TOF-MS analytical sequence, five consecutive QC injections were performed at the beginning to equilibrate the chromatography–mass spectrometry system; thereafter, one QC sample was inserted after every 10 study samples; and two additional QC injections were performed at the end of the sequence. A total of **22** QC injections were acquired, accounting for approximately 15% of the total sequence.

After undergoing the same peak alignment and normalization procedures as the real samples, the relative standard deviation (RSD) of peak areas for each metabolite across all QC samples was calculated. Metabolites with RSD < 15% were considered highly stable and retained for subsequent statistical analysis; those with 15% ≤ RSD < 30% were considered moderately stable and annotated accordingly; metabolites with RSD ≥ 30% were excluded. In this study, metabolites with RSD < 15% accounted for **78.4%**, and those with RSD < 20% accounted for **90.7%** of the final metabolites used for statistical modeling, indicating good instrument and analytical stability.

Metabolite identification

Raw mass spectrometry data were imported into Progenesis QI software for peak alignment, retention time correction, and peak matching. Metabolite annotation was performed by matching exact molecular weights and MS/MS fragment spectra

against the Human Metabolome Database (HMDB), METLIN, and KEGG databases. Annotation confidence was categorized following the Metabolomics Standards Initiative (MSI) reporting guidelines. In brief, no metabolites were confirmed with authentic reference standards (MSI Level-1). Most differential metabolites, including acylcarnitines and reduced ubiquinol (QH₂), were assigned as MSI Level-2 (putative annotation), defined by accurate mass plus consistent MS/MS fragment matching against public spectral libraries. Metabolites annotated solely based on exact mass without fragment evidence were classified as MSI Level-3. A total of 3,020 serum metabolites were annotated: 1,856 in ESI+ mode and 1,164 in ESI- mode, with 312 overlapping annotations between the two modes. After deduplication, 2,708 metabolites were ultimately included in the statistical analysis. MSI confidence levels for core differential metabolites are summarized in Table 2 and Table 4.

Data preprocessing

Missing value handling: zero values in raw metabolite abundance data were treated as missing. If the missing rate of a metabolite across all samples exceeded 50%, it was directly excluded. Remaining missing values were imputed using the K-nearest neighbor method ($k = 5$, Euclidean distance) [29] to eliminate the interference of zero values on statistical models. The parameter $k=5$ was selected based on previous metabolomics benchmark studies, which suggested $k = 3-5$ achieves a good balance between imputation bias and variance for untargeted metabolomics datasets of comparable sample size. Data transformation: all metabolite abundances were $\log_2$ -transformed to improve skewed distributions. Standardization: Pareto scaling (mean-centering followed by division by the square root of the standard deviation) was applied to eliminate concentration magnitude differences among metabolites [30].

Statistical analysis

Baseline characteristics

Baseline demographic data were analyzed using SPSS 26.0. Normally distributed continuous variables were expressed as mean $\pm$ standard deviation (SD) and compared between groups using independent-sample t -tests. Categorical variables were expressed as number (percentage) and compared using χ^2 tests. A two-tailed $P < 0.05$ was considered statistically significant.

Multivariate metabolomic modeling

Multivariate statistical models were constructed using R 4.2.1 with the mixOmics package [31]. Principal component analysis (PCA) was performed as an unsupervised analysis on standardized metabolite data to visually observe the overall separation trend of serum metabolic profiles between the two groups and to evaluate global metabolic differences. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed as a supervised discriminant model with group as the dependent variable, using 1 predictive component and 1 orthogonal component [32]. Model overfitting risk was assessed by 200 permutation tests [33]. Model explanatory power (R^2Y) and cross-validated predictive ability (Q^2Y) were recorded. Variable importance in projection (VIP) values were extracted, and metabolites with $VIP > 1$ were selected as candidate differential metabolites with strong group discrimination ability.

Univariate differential testing

The Mann–Whitney U non-parametric test was used to compare metabolite abundances between groups. Multiple testing correction was performed using the Benjamini–Hochberg (BH) false discovery rate (FDR) method to control false positives [34]. Significant differential metabolites were defined as those with $FDR < 0.05$. Strict core differential metabolites were defined by the combined criteria of $FDR < 0.05$, $VIP > 1$, and $|\log_2 \text{fold change (FC)}| > 1$. A $\log_2FC < 0$ indicated downregulation in the MDD group, while $\log_2FC > 0$ indicated upregulation.

Objective screening and classification of mitochondrial-related metabolites.

To avoid subjective selection bias, a dual-database mapping strategy was employed to determine mitochondrial relevance:

(1) **Protein–metabolite association mapping based on MitoCarta 3.0:** The human mitochondrial proteome list was downloaded from MitoCarta 3.0 (<https://www.broadinstitute.org/mitocarta>) [35]. All enzyme-catalyzed reactions corresponding to mitochondria-localized proteins were extracted. Through the Human Metabolome Database (HMDB) v5.0 and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, these enzymatic reactions were mapped to their substrate/product metabolites, establishing "mitochondrial enzyme–metabolite" association pairs. If a differential metabolite was a known substrate or product of a mitochondria-localized enzyme, it was classified as a direct mitochondria-associated metabolite.

(2) **Pathway enrichment mapping based on KEGG:** The 71 significant differential metabolites were mapped to KEGG pathways using MetaboAnalyst 5.0 (species: *Homo sapiens*) [36]. Pathways directly related to mitochondrial energy metabolism were selected, including fatty acid degradation (hsa00071), tricarboxylic acid (TCA) cycle (hsa00020), oxidative phosphorylation (hsa00190), pyruvate metabolism (hsa00620), branched-chain amino acid degradation (hsa00280), and ubiquinone and other terpenoid-quinone biosynthesis (hsa00130). If a differential metabolite was a node molecule in any of these pathways, it was classified as a pathway mitochondria-associated metabolite.

(3) **Integration criteria:** Metabolites satisfying either criterion (1) or (2) were included in the mitochondrial-related metabolite set; those satisfying both were annotated as high-confidence mitochondrial markers. All screening procedures were performed using custom scripts in R 4.2.1.

Hierarchical clustering analysis

Z-score standardization was applied to the abundances of significant differential metabolites. Bidirectional hierarchical clustering was performed using the pheatmap package to generate heatmaps, visually displaying the unified expression patterns of differential metabolites between the two groups.

ROC curve analysis

Receiver operating characteristic (ROC) curve analysis was performed on the 17 metabolites with $FDR < 0.01$ to evaluate the diagnostic discriminatory ability of single markers and multi-metabolite combined models for first-episode drug-naïve MDD [37]. Healthy individuals served as the negative group and MDD patients as the positive group. The area under the curve (AUC), optimal cutoff values, sensitivity, and specificity were calculated.

KEGG metabolic pathway enrichment analysis

The 71 significant differential metabolites were imported into the MetaboAnalyst 5.0 platform for KEGG pathway enrichment analysis [36]. The screening criteria were enrichment $P < 0.05$ and ≥ 3 differential metabolites per pathway.

Results

Baseline characteristics

A total of 118 first-episode drug-naïve MDD patients and 56 healthy controls were enrolled. Baseline demographic and clinical characteristics, including age, sex, ethnicity, education level, marital status, childhood left-behind experience, smoking and alcohol history, and past medical history, were compared between groups. No statistically significant differences were observed for any of these variables ($P > 0.05$), confirming baseline comparability between the two groups (**Table 1**).

Metabolomic data quality and PCA global metabolic profiling

A total of 3,020 serum metabolites were detected and annotated. Zero-value missing data accounted for 9.45% of the raw dataset. Missing values were imputed using K-nearest neighbor ($k = 5$) interpolation [29], followed by $\log_2$ transformation and Pareto scaling to eliminate concentration magnitude differences before multivariate analysis [30].

Principal component analysis (PCA) [38] showed that the first principal component (PC1) explained 9.26% of the total variance, and the second principal component (PC2) explained 5.55%. The top 10 principal components cumulatively explained 43.97% of the total variance. The PCA score plot revealed a clear spatial separation between healthy control and MDD samples, indicating a stable and distinguishable global serum metabolic disturbance pattern between the two groups (Fig. 1).

Univariate differential metabolite screening

To evaluate the robustness of differential metabolite screening, we performed a simple sensitivity analysis. A total of 83 metabolites satisfied $FDR < 0.05$, and 17 metabolites met the stricter threshold of $FDR < 0.01$. The core mitochondrial-related metabolites including eight acylcarnitines and reduced ubiquinol QH_2 all survived the $FDR < 0.01$ filtering. Mann–Whitney U tests were performed on all metabolites, with Benjamini–Hochberg multiple testing correction [34]. A total of 527 metabolites showed uncorrected $P < 0.05$. After FDR correction, 83 metabolites showed significant between-group differences ($FDR < 0.05$), of which 17 had $FDR < 0.01$ (Table 2). Notably, all 17 metabolites with $FDR < 0.01$ were downregulated in the MDD group. These included eight acylcarnitines (butyrylcarnitine, dodecanoylcarnitine, myristoylcarnitine, acetylcarnitine, hexanoylcarnitine, decanoylcarnitine, isovalerylcarnitine, and palmitoleoylcarnitine), the electron transport chain component reduced ubiquinone QH_2 , branched-chain amino acids (leucine, isoleucine, valine) [39], the branched-chain amino acid metabolite α -ketoisovaleric acid, the coenzyme A precursor pantothenic acid, free fatty acids (oleic acid), polyunsaturated fatty acids (arachidonic acid), and the TCA cycle intermediate succinic acid. The full list of 83 differential metabolites satisfying $FDR < 0.05$, including VIP, $\log_2$ fold-change, FDR values and MSI annotation confidence levels, is provided in Supplementary Table S1.

OPLS-DA discriminant model construction and differential metabolite screening

An OPLS-DA supervised discriminant model was constructed with 1 predictive component and 1 orthogonal component [32]. The model fitting parameters were $R^2X = 0.312$, $R^2Y = 0.547$ and cross-validated predictive ability $Q^2Y = 0.526$; Q^2Y exceeded the 0.5 threshold indicative of good predictive performance in metabolomic classification studies. The relatively moderate R^2X (0.312) is frequently observed in human serum untargeted metabolomics datasets, which is largely attributable to substantial inter-individual biological variation among human participants. A considerable proportion of inter-individual metabolic variance cannot be explained by case-control group membership alone. Two hundred permutation tests yielded a negative Q^2 intercept (-0.073), confirming that the model was free from overfitting and that the discrimination results were reliable.[33] (Table 3, Fig. 2).

Using the criteria of $FDR < 0.05$ and $VIP > 1$, a total of 71 significant differential metabolites were identified, including 60 downregulated and 11 upregulated in the MDD group. After applying the strict threshold of $|\log_2FC| > 1$, 13 core key differential molecules were selected (Table 4). Among these, 10 were downregulated and 3 were upregulated. The top-ranked downregulated metabolites included butyrylcarnitine ($VIP = 2.74$, $\log_2FC = -1.62$, $FDR = 1.13 \times 10^{-5}$), dodecanoylcarnitine ($VIP = 2.68$, $\log_2FC = -1.57$, $FDR = 1.46 \times 10^{-5}$), myristoylcarnitine ($VIP = 2.51$, $\log_2FC = -1.49$, $FDR = 2.08 \times 10^{-5}$), and reduced ubiquinone QH_2 ($VIP = 2.36$, $\log_2FC = -1.34$, $FDR = 4.71 \times 10^{-4}$). Of the 13 core metabolites, 7 were classified as mitochondria-related.

Functional classification and mitochondrial-related metabolic features

The 71 significant differential metabolites were classified by physiological function. Lipids and fatty acids constituted the largest category (22 metabolites, 31.0%), followed by amino acids and derivatives (17, 23.9%), vitamins and coenzymes (11, 15.5%), xenobiotics (9, 12.7%), carbohydrates (7, 9.9%), and other small molecules (5, 7.0%) (Table 5).

A total of 14 mitochondria-related differential metabolites were identified, all of which were downregulated in the MDD group. Among these, eight were medium- and short-chain acylcarnitines (including butyrylcarnitine and dodecanoylcarnitine), which showed consistent reductions. Acylcarnitines serve as essential shuttle molecules for the transport of fatty acids across the inner mitochondrial membrane [41]; their systemic depletion is consistent with impaired mitochondrial uptake and subsequent

β -oxidation [42], a pattern previously reported in depression-related metabolomic studies [43]. The key electron transport chain component reduced ubiquinone QH₂ was also concurrently decreased. Ubiquinone (coenzyme Q10) functions as an electron carrier between complex I/II and complex III of the respiratory chain [44]; its reduction in the present cohort indicates compromised respiratory chain complex function. Box plots of five representative mitochondrial metabolites visually demonstrated the between-group abundance differences (**Fig. 3**). The complete workflow for dual-database-based mitochondria-related metabolite screening is visualized in **Supplementary Figure S1**.

Hierarchical clustering analysis of differential metabolites

Bidirectional hierarchical clustering was performed on the 71 differential metabolites after Z-score standardization [45]. The clustering results showed that samples could be completely divided into two major clusters corresponding to the healthy control and MDD groups, with highly uniform intra-group metabolic expression patterns. The 60 downregulated metabolites were concentrated in the same branch, and acylcarnitine molecules displayed a large blue low-abundance region in the MDD sample area, visually confirming the stable discrimination of metabolic characteristics between the two groups (**Fig. 4**).

ROC curve diagnostic efficacy analysis

ROC analysis was performed on the 17 metabolites with FDR < 0.01 to evaluate the diagnostic discriminatory ability of single markers and multi-metabolite combined models for first-episode drug-naïve MDD [37]. Single-marker analysis revealed that the acylcarnitine series demonstrated the optimal diagnostic performance: butyrylcarnitine AUC = 0.762 (95% CI: 0.690–0.833), dodecanoylcarnitine AUC = 0.747 (95% CI: 0.673–0.821), and myristoylcarnitine AUC = 0.731 (95% CI: 0.654–0.808). Reduced ubiquinone QH₂ showed an AUC of 0.704 (95% CI: 0.624–0.784). Other branched-chain amino acids and TCA cycle-related metabolites showed moderate discriminatory efficacy, with AUCs ranging from 0.631 to 0.692.

An eight-acylcarnitine combined model was constructed using logistic regression. The ROC curve of the combined marker model achieved an AUC of 0.815 (95% CI: 0.753–0.877). At the optimal cutoff, the sensitivity was 74.58% and the specificity was 78.57%, demonstrating significantly superior discriminatory efficacy compared to any single molecule (**Table 6, Fig. 5**).

To explore whether the abundance of core mitochondrial-related metabolites was associated with clinical depressive symptom burden, we performed Spearman rank-order correlation analysis restricted to the MDD patient group. Correlation coefficients and corresponding p-values for associations between core mitochondrial-related metabolites and HAMD-17 total score as well as each individual sub-item score are summarized in **Supplementary Table S2**.

KEGG metabolic pathway enrichment analysis

The 71 significant differential metabolites were subjected to KEGG pathway enrichment analysis using the MetaboAnalyst 5.0 platform [36]. A total of 6 significantly enriched pathways were identified (enrichment $P < 0.05$, ≥ 3 differential metabolites per pathway) (**Table 7, Fig. 6**).

The top three ranked pathways were: fatty acid β -oxidation (hsa00071), branched-chain amino acid degradation (hsa00280), and the tricarboxylic acid (TCA) cycle (hsa00020). Other significant pathways included unsaturated fatty acid biosynthesis (hsa01040), ubiquinone and other terpenoid-quinone biosynthesis (hsa00130), and propanoate metabolism (hsa00640).

Fatty acid β -oxidation showed the highest enrichment degree, with multiple medium- and short-chain acylcarnitines enriched within the pathway, confirming that impaired fatty acid transport across the mitochondria and oxidation represents the core metabolic disturbance in this study population [42]. The significant enrichment of the ubiquinone biosynthesis pathway corroborated the downregulation of reduced ubiquinone QH₂, suggesting impaired mitochondrial electron transport chain function [43]. Collectively, all enriched pathways point toward global disruption of the mitochondrial energy metabolism network, consistent with prior evidence linking mitochondrial bioenergetic failure to the pathophysiology of major depression [46,47].

Discussion

This study provides a comprehensive serum non-targeted metabolomic profile of first-episode, drug-naïve major depressive disorder (MDD) patients, with a specific focus on mitochondrial energy metabolism. Our findings reveal a coherent pattern of systemic metabolic dysregulation characterized by downregulation of acylcarnitines, reduced ubiquinone QH₂, and branched-chain amino acids (BCAAs), collectively pointing to impaired mitochondrial fatty acid β -oxidation and electron transport chain (ETC) dysfunction. In the following sections, we contextualize these findings by comparing them with existing literature, highlighting areas of convergence and divergence, and discussing the underlying biological mechanisms.

The most robust finding of our study the widespread downregulation of medium- and short-chain acylcarnitines in first-episode MDD is consistent with several independent lines of evidence. Nasca et al. [48] first reported that patients with MDD exhibited lower serum levels of acetyl-L-carnitine (LAC), a short-chain acylcarnitine, and demonstrated that LAC deficiency was associated with more severe depressive symptoms and earlier age of onset. Importantly, preclinical studies from the same group showed that LAC administration rapidly reversed depressive-like behaviors through epigenetic modulation of metabotropic glutamate receptor 2 (mGlu2) expression, establishing a causal link between acylcarnitine deficiency and depressive pathophysiology [48].

Our findings are further corroborated by large-scale population-based metabolomic studies. Zacharias et al. [49] conducted a metabolome-wide association study in 1,411 participants from the KORA F4 cohort and identified significantly lower serum laurycarnitine (dodecanoylcarnitine, C12) in individuals with depressed mood, a result that was successfully replicated in an independent SHIP-Trend cohort. The authors hypothesized that decreased laurycarnitine reflected impaired mitochondrial fatty acid oxidation, a conclusion directly aligned with our pathway enrichment analysis in which fatty acid β -oxidation emerged as the top-ranked disturbed pathway [49].

More recently, Ait Tayeb et al. [50] profiled 38 plasma acylcarnitines in 460 depressed patients from the METADAP cohort before and after six months of antidepressant treatment. They observed that medium- and long-chain acylcarnitines were significantly lower in depressed patients compared to healthy controls, and critically these levels normalized after treatment, suggesting that acylcarnitine dysregulation is state-dependent and reversible [50]. Similarly, a 2025 analysis of the NESDA cohort by Montanari et al. [51] confirmed that acylcarnitine metabolism was significantly associated with diagnostic status, depression severity, and specific symptom profiles in a large longitudinal sample. These convergent findings across independent cohorts and methodologies strengthen the validity of our observation and support the notion that acylcarnitine depletion represents a reproducible metabolic signature of MDD.

From a mechanistic perspective, the systematic reduction in acylcarnitines is biologically consistent with impaired mitochondrial fatty acid β -oxidation. Acylcarnitines are synthesized in the cytosol and transported into the mitochondrial matrix via the carnitine shuttle system, where they undergo β -oxidation to generate acetyl-CoA for subsequent entry into the TCA cycle and oxidative phosphorylation [41, 42]. Their systemic depletion suggests a bottleneck at the level of mitochondrial uptake or catabolism, which would directly compromise ATP generation in metabolically demanding tissues such as the brain [8]. This interpretation is further supported by the concurrent downregulation of reduced ubiquinone QH₂ in our cohort, indicating compromised ETC function at the level of complex I/II–III electron transfer [43]. The coordinated disturbance of both substrate supply (acylcarnitines) and oxidative phosphorylation machinery (QH₂) paints a coherent picture of integrated mitochondrial bioenergetic failure, consistent with the "mitochondrial hypothesis of depression" proposed by Maes et al. [46] and supported by post-mortem and peripheral studies documenting reduced mitochondrial complex activity and increased oxidative damage in depressed individuals [11, 12].

Beyond observational metabolomics, our findings align with emerging causal inference evidence. Milaneschi et al. [52] performed two-sample Mendelian randomization analyses using summary statistics from large-scale genome-wide association studies (GWAS) and found that genetically predicted higher levels of medium-chain acylcarnitines (C8 and C10) were associated with increased depression risk, while lower levels of short-chain acylcarnitines (C2 and C3) showed the opposite directionality [52]. Although the direction of association for medium-chain species appears paradoxical at first

glance, the authors interpreted this as reflecting impaired mitochondrial clearance and subsequent accumulation of incompletely oxidized medium-chain acylcarnitines a metabolic signature characteristic of inborn errors of fatty acid oxidation and age-related mitochondrial dysfunction. In our study, the observed downregulation of medium-chain acylcarnitines in first-episode, drug-naïve patients may represent an earlier, compensatory stage of mitochondrial impairment, whereas accumulation may occur later in the disease course or in the context of chronic metabolic comorbidities. This temporal and stage-dependent heterogeneity underscores the importance of studying first-episode, medication-free populations to capture the primary metabolic alterations before secondary compensatory or confounding processes take hold.

Despite the strong convergence on acylcarnitine and mitochondrial dysfunction, our findings diverge from some prior metabolomic studies in important ways. Notably, several large-scale investigations have reported alterations in phospholipid and lysophospholipid metabolism in MDD that were not prominent in our cohort. Bot et al. [53] performed a pooled metabolomics analysis of 230 metabolic markers in 5,283 depressed patients and 10,145 controls from the NESDA and NEO studies, identifying significant dysregulation of multiple phosphatidylcholines (PCs) and lysophosphatidylcholines (LPCs), with some species elevated and others reduced in depression [53]. Similarly, Pu et al. [54] conducted an integrated meta-analysis of peripheral blood metabolites in MDD and reported significant alterations in glycerophospholipid metabolism, including upregulation of several LPC and phosphatidylethanolamine species, which they linked to inflammation-induced membrane remodeling [54].

Several factors may account for these discrepancies. First, phospholipid metabolism is highly sensitive to dietary intake, body mass index (BMI), and systemic inflammation, all of which can act as confounders in cross-sectional metabolomic studies [53]. Our cohort was rigorously screened for metabolic comorbidities (diabetes, severe obesity, and lipid-lowering medication use were exclusion criteria), which may have minimized phospholipid variance related to metabolic syndrome rather than depression per se. Second, phospholipid alterations may be more pronounced in chronic or recurrent depression, where prolonged HPA axis activation and low-grade systemic inflammation induce progressive membrane phospholipid turnover [6]. Our first-episode, drug-naïve design may have captured an earlier disease stage in which mitochondrial substrate depletion precedes overt membrane remodeling. Third, analytical platform differences may contribute: UHPLC-Q-TOF-MS with C18 chromatography, as employed in our study, is optimized for polar and semi-polar metabolites including acylcarnitines and amino acids, whereas phospholipid profiling typically requires dedicated lipidomics platforms with different chromatographic conditions and ionization optimization [28]. Thus, the apparent absence of phospholipid disturbances in our study may reflect both biological stage effects and technical analytical windows.

A critical divergence between our findings and several prior studies relates to the directionality of acylcarnitine changes in medicated versus drug-naïve patients. MahmoudianDehkordi et al. [17] investigated the effects of citalopram/escitalopram treatment on serum metabolites and reported that short-chain acylcarnitines (including acetylcarnitine) were significantly elevated after 8 weeks of SSRI therapy, whereas medium- and long-chain species showed variable responses [17]. This pharmacological modulation of acylcarnitine profiles highlights a crucial confound in studies of chronic or medicated depressed patients: observed metabolic alterations may reflect drug effects, disease chronicity, or adaptive compensatory responses rather than primary disease pathophysiology [16].

The METADAP cohort study by Ait Tayeb et al. [50] provides further insight into this dynamic: while medium- and long-chain acylcarnitines were reduced at baseline (consistent with our findings), they normalized after six months of antidepressant treatment, suggesting that at least part of the acylcarnitine signature is state-dependent and reversible [50]. Our study design focusing exclusively on first-episode, drug-naïve patients therefore offers a unique window into the "pure" metabolic state of MDD before treatment-induced metabolic remodeling occurs. This distinction is not merely methodological but has profound implications for biomarker development: if acylcarnitine levels normalize with treatment, they may serve as state markers of active illness rather than trait markers of vulnerability.

Another important divergence concerns age-related metabolic heterogeneity in depression. Our study enrolled adults aged 18–65 years, and our findings may not generalize to adolescent or pediatric populations. Zhou et al. [55] conducted a targeted metabolomics study in children and adolescents with MDD and identified purine metabolism specifically decreased inosine,

adenosine, and hypoxanthine as the most significantly altered pathway, with inosine demonstrating exceptional diagnostic accuracy (AUC = 0.999) for distinguishing drug-naïve adolescent MDD from controls [55]. Notably, these purine metabolite alterations were not characteristic of adult MDD in the same study, and conversely, tryptophan reductions commonly observed in adult depression were absent in the pediatric cohort [55].

Similarly, Yin et al. [56] performed a comparative metabolomics analysis of adolescent patients with MDD, bipolar disorder, and schizophrenia, finding that while fatty acid metabolism was disturbed across all three disorders, MDD-specific alterations included lysine, cysteine, and methionine metabolism rather than the pronounced acylcarnitine depletion we observed in adults [56]. These developmental differences likely reflect age-dependent variations in brain energy substrate utilization, mitochondrial maturation, and the differential impact of early-life stress on metabolic programming [14]. During adolescence, the brain relies more heavily on glucose and ketone bodies, and mitochondrial fatty acid oxidation capacity may be relatively preserved compared to adulthood, where age-related mitochondrial decline sensitizes neurons to bioenergetic deficits [8]. Our adult cohort findings should therefore be interpreted as specific to the mature brain metabolic landscape.

A fundamental limitation of all peripheral blood metabolomic studies including ours is the assumption that serum metabolite profiles faithfully reflect brain biochemistry. This assumption is increasingly challenged by studies comparing blood, cerebrospinal fluid (CSF), and post-mortem brain tissue. Davyson et al. [57] conducted a large-scale metabolomic investigation of MDD using UK Biobank data and identified a potentially causal association between polyunsaturated fatty acids (PUFAs) and depression through Mendelian randomization [57]. However, the specific PUFA species implicated in peripheral blood often show poor correlation with CSF or brain tissue levels due to the blood–brain barrier's selective transport capacity and independent cerebral lipid synthesis [57].

In our study, the downregulation of arachidonic acid and oleic acid in serum may not directly mirror brain fatty acid availability. The brain maintains tight homeostatic control over its lipid composition, and peripheral reductions in free fatty acids may instead reflect hepatic dysregulation, altered adipose tissue lipolysis, or reduced dietary intake in depressed patients with appetite changes [43]. Moreover, while acylcarnitines are readily exchanged between blood and tissues, their serum concentrations primarily reflect skeletal muscle and hepatic mitochondrial activity rather than neuronal metabolism [42]. Thus, although our peripheral findings are biologically plausible and consistent with the mitochondrial hypothesis, they should be considered biomarkers of systemic bioenergetic state rather than direct proxies for cerebral mitochondrial function. Future studies integrating serum metabolomics with CSF profiling or neuroimaging-based spectroscopy will be essential to bridge this peripheral–central gap.

Finally, our findings appear to diverge from studies emphasizing inflammation-related metabolite alterations in depression. Several metabolomic investigations have reported elevated levels of kynurenine pathway metabolites, pro-inflammatory lipid mediators, and oxidative stress markers in MDD, particularly in patients with high C-reactive protein (CRP) or interleukin-6 (IL-6) levels [6, 13]. Brydges et al. [58] found that metabolomic signatures of depression were partially mediated by inflammatory status, with specific symptom dimensions showing stronger associations with inflammatory metabolites than cognitive–emotional symptoms [58].

In our cohort, we did not observe prominent upregulation of tryptophan–kynurenine metabolites or classical inflammatory markers among the top differential metabolites. This absence may be explained by our stringent exclusion criteria: patients with autoimmune disease, severe chronic infections, or acute inflammatory conditions were excluded, potentially selecting for a relatively "low-inflammation" depressed subgroup. Additionally, first-episode patients may exhibit less immune priming than those with recurrent or chronic depression, where repeated episodes induce progressive microglial sensitization and neuroinflammatory escalation [6]. Our findings thus suggest that mitochondrial energy metabolism disruption may represent a distinct pathophysiological subtype or an earlier stage of depression that precedes or operates independently of overt neuroinflammation, consistent with the emerging concept of "metabolic" or "mitochondrial" depression phenotypes [6, 46].

In synthesizing these convergent and divergent findings, we propose that MDD is metabolically heterogeneous, with distinct peripheral signatures reflecting different pathophysiological stages, developmental contexts, and comorbidity profiles. Our

first-episode, drug-naïve adult cohort appears to represent a "mitochondrial–metabolic" subtype characterized by primary impairment of fatty acid oxidation and ETC function, manifesting as systemic acylcarnitine and ubiquinone depletion. This subtype may be most amenable to mitochondrial-targeted interventions such as acetyl-L-carnitine supplementation, coenzyme Q10 administration, or ketogenic dietary approaches that bypass impaired glucose metabolism and provide alternative fuel substrates [15, 24].

The diagnostic implications of our eight-acylcarnitine combined model (AUC = 0.815) are promising but require cautious interpretation. While the performance exceeds many existing peripheral biomarker panels, it remains insufficient for standalone clinical diagnosis. Rather, such metabolic signatures may serve as objective adjuncts to clinical assessment, particularly in contexts where symptom-based diagnosis is challenging. The observation that acylcarnitine levels normalized with antidepressant treatment in the METADAP cohort [50] further supports their potential utility as state markers to monitor treatment response and predict relapse.

Limitations and future directions

Our study has several notable limitations. First, given the 2.1:1 case-control ratio, post-hoc power analysis showed insufficient power for detecting small-effect-size metabolic alterations (Cohen's $d \leq 0.4$). We cannot rule out subtle metabolic changes filtered out by FDR correction; our main conclusions rely on metabolites with medium-to-large effect sizes. Second, its cross-sectional design prohibits causal inference. As a single-center study with predominantly Han-Chinese participants, the generalizability is limited. Without cerebrospinal fluid (CSF) or neuroimaging data, we cannot confirm whether serum metabolic perturbations correspond to central nervous system pathology. Third, the eight-acylcarnitine signature lacks external-cohort validation; case-control-derived ROC performance tends to overestimate real-world clinical utility. This panel represents merely an exploratory candidate metabolic feature instead of a clinically validated biomarker.

Future prospective longitudinal studies should map metabolic trajectories from premorbid status to disease onset and remission. Multi-omics integration (genomics, transcriptomics, proteomics) may clarify underlying disease mechanisms. Additional research is needed to evaluate whether mitochondrial metabolic signatures predict treatment responses to conventional antidepressants and mitochondria-targeted interventions.

Conclusion

In conclusion, our study identifies a distinct serum metabolomic signature of mitochondrial energy metabolism disruption in first-episode, drug-naïve MDD, characterized by downregulation of acylcarnitines, reduced ubiquinone QH₂, and impaired fatty acid β -oxidation. These findings converge with independent cohort studies, Mendelian randomization evidence, and preclinical mechanistic research, while diverging from studies of medicated, chronic, adolescent, or high-inflammation populations in ways that illuminate the stage-dependent and context-specific nature of metabolic dysregulation in depression. Our results advance the understanding of MDD as a systemic bioenergetic disorder. The acylcarnitine-related metabolic signature provides exploratory foundations for future candidate signature development, though clinical application awaits multi-cohort external validation. Mitochondrial metabolism represents a promising therapeutic target for precision therapeutics in MDD.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Fourth People's Hospital of Hefei, Anhui Province, China (Approval Number:HFSY-IRB-YJ-KYXM-CL[2024-064-001]). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its later amendments.

Authors' contributions:

Min Pan and Xialong Cheng contributed to conceptualization, methodology, and formal analysis. Hongxin Zheng, Changyan Gu and Anzhen Wang contributed to investigation. Min Pan drafted the manuscript. Xulai Zhang and Long chen contributed to manuscript review and editing. Xulai Zhang and Xialong Cheng acquired funding. Xulai Zhang and Xialong Cheng contributed to project administration and supervision. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Availability of data and materials

The clinical datasets analyzed during the current study contain sensitive patient-level information and are not publicly available. Data and related materials may be available from the corresponding author upon reasonable request and subject to institutional and ethical approval.

Competing interests:

The authors declare that they have no competing interests.

References

1. GBD 2021 Diseases and Injuries Collaborators. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet*. 2024;403(10440):2133–2161. doi: 10.1016/S0140-6736(24)00757-8
2. GBD 2019 Mental Disorders Collaborators. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Psychiatry*. 2022;9(2):137–150. doi: 10.1016/S2215-0366(21)00395-3
3. Cui L, Li S, Wang S, Wu X, Liu Y, Yu W, et al. Major depressive disorder: hypothesis, mechanism, prevention and treatment. *Signal Transduct Target Ther*. 2024;9(1):30. doi: 10.1038/s41392-024-01738-y
4. Kupfer DJ, Regier DA. Neuroscience, clinical evidence, and the future of psychiatric classification in DSM-5. *Am J Psychiatry*. 2011;168(7):672–674. doi: 10.1176/appi.ajp.2011.11020219
5. Boku S, Nakagawa S, Toda H, Hishimoto A. Neural basis of major depressive disorder: beyond monoamine hypothesis. *Psychiatry Clin Neurosci*. 2018;72(1):3–12. doi: 10.1111/pcn.12604
6. Penninx BWJH, Lamers F, Jansen R, Berk M, Khandaker GM, De Picker L, et al. Immuno-metabolic depression: from concept to implementation. *Lancet Reg Health Eur*. 2025;48:101166. doi: 10.1016/j.lanepe.2024.101166
7. Song Y, Cao H, Zuo C, Gu Z, Huang Y, Miao J, et al. Mitochondrial dysfunction: a fatal blow in depression. *Biomed Pharmacother*. 2023;167:115652. doi: 10.1016/j.biopha.2023.115652
8. Andreazza AC, Barros LF, Behnke A, Ben-Shachar D, Berretta S, Chouinard VA, et al. Brain and body energy metabolism and potential for treatment of psychiatric disorders. *Nat Ment Health*. 2025;3:763–771. doi: 10.1038/s44220-025-00422-6
9. Cardon I, Grobecker S, Kücükoktay S, Bader S, Jahner T, Nothdurfter C, et al. Mitochondrial and cellular function in fibroblasts, induced neurons, and astrocytes derived from case study patients: insights into major depression as a

- mitochondria-associated disease. *Int J Mol Sci*. 2024;25(2):963. doi: 10.3390/ijms25020963
10. Fries GR, Saldana VA, Finnstein J, Rein T. Molecular pathways of major depressive disorder converge on the synapse. *Mol Psychiatry*. 2023;28(1):284–297. doi: 10.1038/s41380-022-01806-1
11. Andreazza AC, Shao L, Wang JF, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. *Arch Gen Psychiatry*. 2010;67(4):360–368. doi: 10.1001/archgenpsychiatry.2010.22
12. Karabatsiakos A, Böck C, Salinas-Manrique J, Kolassa S, Caliskan S, Schulte-Herbrüggen O, et al. Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression. *Transl Psychiatry*. 2014;4:e397. doi: 10.1038/tp.2014.44
13. Lindqvist D, Dhabhar FS, James SJ, Hough CM, Jain FA, Bersani FS, et al. Oxidative stress, inflammation and treatment response in major depression. *Psychoneuroendocrinology*. 2017;76:197–205. doi: 10.1016/j.psyneuen.2016.11.031
14. Wang L, Liyuan, et al. Chronic stress induces depression-like behavior in rats through affecting brain mitochondrial function and inflammation. *Psychoneuroendocrinology*. 2025;172:107261. doi: 10.1016/j.psyneuen.2024.107261
15. Mishra SK, Hidau MK, Rai S. Memantine treatment exerts an antidepressant-like effect by preventing hippocampal mitochondrial dysfunction and memory impairment via upregulation of CREB/BDNF signaling in the rat model of chronic unpredictable stress-induced depression. *Neurochem Int*. 2021;142:104932. doi: 10.1016/j.neuint.2020.104932
16. Gan X, Li X, Cai Y, Yin B, Pan Q, Teng T, et al. Metabolic features of adolescent major depressive disorder: a comparative study between treatment-resistant depression and first-episode drug-naïve depression. *Psychoneuroendocrinology*. 2024;167:107086. doi: 10.1016/j.psyneuen.2024.107086
17. MahmoudianDehkordi S, Ahmed AT, Bhattacharyya S, Han X, Baillie RA, Arnold M, et al. Alterations in acylcarnitines, amines, and lipids inform about the mechanism of action of citalopram/escitalopram in major depression. *Transl Psychiatry*. 2021;11:153. doi: 10.1038/s41398-020-01097-6
18. Liu M, Ma W, He Y, Sun Z, Yang J. Recent progress in mass spectrometry-based metabolomics in major depressive disorder research. *Molecules*. 2023;28(20):7430. doi: 10.3390/molecules28217430
19. Tomasik J, Harrison SJ, Rustogi N, Olmert T, Barton-Owen G, Han SYS, et al. Metabolomic biomarker signatures for bipolar and unipolar depression. *JAMA Psychiatry*. 2024;81(2):101–106. doi: 10.1001/jamapsychiatry.2023.4096
20. Pan LA, Naviaux JC, Wang L, Li K, Monk JM, Lingampelly SS, et al. Metabolic features of treatment-refractory major depressive disorder with suicidal ideation. *Transl Psychiatry*. 2023;13:393. doi: 10.1038/s41398-023-02696-9
21. Yin B, Cai Y, Teng T, Wang X, Liu X, Li X, et al. Identifying plasma metabolic characteristics of major depressive disorder, bipolar disorder, and schizophrenia in adolescents. *Transl Psychiatry*. 2024;14:163. doi: 10.1038/s41398-024-02886-z
22. Wu Z, Yu H, Tian Y, Wang Y, He Y, Lan T, et al. Non-targeted metabolomics profiling of plasma samples from patients with major depressive disorder. *Front Psychiatry*. 2022;12:810302. doi: 10.3389/fpsy.2021.810302
23. Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes*. 2013;62(1):1–8. doi: 10.2337/db12-0466
24. Ferreira GC, McKenna MC. L-carnitine and acetyl-L-carnitine roles and neuroprotection in developing brain. *Neurochem Res*. 2017;42(6):1661–1675. doi: 10.1007/s11064-017-2288-7
25. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*. Arlington, VA: American Psychiatric Association; 2013. doi: 10.1176/appi.books.9780890425596
26. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960;23(1):56–62. doi: 10.1136/jnnp.23.1.56
27. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149–1160. doi: 10.3758/BRM.41.4.1149
28. Want EJ, O'Maille G, Smith CA, Brandon TR, Uritboonthai W, Qin C, et al. Solvent-dependent metabolite distribution, clustering, and protein extraction for serum profiling with mass spectrometry. *Anal Chem*. 2006;78(3):743–752. doi: 10.1021/ac051312t

29. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, et al. Missing value estimation methods for DNA microarrays. *Bioinformatics*. 2001;17(6):520–525. doi: 10.1093/bioinformatics/17.6.520
30. van den Berg RA, Hoefsloot HCJ, Westerhuis JA, Smilde AK, van der Werf MJ. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics*. 2006;7:142. doi: 10.1186/1471-2164-7-142
31. Rohart F, Gautier B, Singh A, Lê Cao KA. mixOmics: an R package for 'omics feature selection and multiple data integration. *PLoS Comput Biol*. 2017;13(11):e1005752. doi: 10.1371/journal.pcbi.1005752
32. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). *J Chemom*. 2002;16(3):119–128. doi: 10.1002/cem.695
33. Eriksson L, Trygg J, Wold S. CV-ANOVA for significance testing of PLS and OPLS® models. *J Chemom*. 2008;22(11–12):594–600. doi: 10.1002/cem.1187
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*. 1995;57(1):289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
35. Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, et al. MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res*. 2021;49(D1):D1541–D1547. doi: 10.1093/nar/gkaa1011
36. Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, et al. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res*. 2021;49(W1):W388–W396. doi: 10.1093/nar/gkab382
37. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*. 1982;143(1):29–36. doi: 10.1148/radiology.143.1.7063747
38. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*. 1999;29(11):1181–1189. doi: 10.1080/004982599238047
39. Reitner LJ, Wice BM, Kennell D. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. *J Biol Chem*. 1979;254(8):2669–2676. doi: 10.1016/S0021-9258(17)30192-9
40. Wiklund S, Johansson E, Sjöström L, Mellerowicz EJ, Edlund U, Shockcor JP, et al. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal Chem*. 2008;80(1):115–122. doi: 10.1021/ac0713510
41. Bieber LL. Carnitine. *Annu Rev Biochem*. 1988;57:261–283. doi: 10.1146/annurev.bi.57.070188.001401
42. Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid β -oxidation. *J Inherit Metab Dis*. 2010;33(5):469–477. doi: 10.1007/s10545-010-9061-2
43. Ivanova V, Henley W, Chen Y, et al. Acylcarnitine profiling in major depressive disorder: evidence for mitochondrial dysfunction. *Transl Psychiatry*. 2020;10(1):123. doi: 10.1038/s41398-020-0798-3
44. Crane FL. Biochemical functions of coenzyme Q10. *J Am Coll Nutr*. 2001;20(6):591–598. doi: 10.1080/07315724.2001.10719063
45. Smolinska A, Blanchet L, Buydens LMC, Wijmenga SS. NMR and pattern recognition methods in metabolomics: from data acquisition to biomarker discovery: a review. *Anal Chim Acta*. 2012;750:82–97. doi: 10.1016/j.aca.2012.05.049
46. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011;35(3):676–692. doi: 10.1016/j.pnpbp.2010.05.004
47. Gardner A, Johansson A, Wibom R, et al. Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Disord*. 2003;76(1–3):55–68. doi: 10.1016/S0165-0327(02)00074-8
48. Nasca C, Bigio B, Lee FS, et al. Acetyl-L-carnitine deficiency in patients with major depressive disorder. *Proc Natl Acad Sci U S A*. 2018;115(34):8627–8632. doi: 10.1073/pnas.1801609115

49. Zacharias HU, Hertel J, Johar H, et al. A metabolome-wide association study in the general population reveals decreased levels of serum laurycarnitine in people with depression. *Mol Psychiatry*. 2021;26(12):7372–7383. doi: 10.1038/s41380-021-01176-0
50. Ait Tayeb AEK, Colle R, Chappell K, et al. Metabolomic profiles of 38 acylcarnitines in major depressive episodes before and after treatment. *Psychol Med*. 2024;54(2):289–298. doi: 10.1017/S003329172300140X
51. Montanari S, et al. Acylcarnitines metabolism in depression: association with diagnostic status, depression severity and symptom profile in the NESDA cohort. *Transl Psychiatry*. 2025;15(1):e3274. doi: 10.1038/s41398-025-03274-x
52. Milaneschi Y, Arnold M, Kastenmüller G, et al. Genomics-based identification of a potential causal role for acylcarnitine metabolism in depression. *J Affect Disord*. 2022;307:254–263. doi: 10.1016/j.jad.2022.03.070
53. Bot M, Milaneschi Y, Al-Shehri T, et al. Metabolomics profile in depression: a pooled analysis of 230 metabolic markers in 5283 cases with depression and 10,145 controls. *Biol Psychiatry*. 2020;87(5):409–418. doi: 10.1016/j.biopsych.2019.08.016
54. Pu J, Liu Y, Zhang H, et al. An integrated meta-analysis of peripheral blood metabolites and biological functions in major depressive disorder. *Mol Psychiatry*. 2021;26(8):4265–4276. doi: 10.1038/s41380-020-0645-4
55. Zhou X, Liu L, Lan X, et al. Polyunsaturated fatty acids metabolism, purine metabolism and inosine as potential independent diagnostic biomarkers for major depressive disorder in children and adolescents. *Mol Psychiatry*. 2019;24(10):1478–1488. doi: 10.1038/s41380-018-0047-z
56. Yin B, Cai Y, Teng T, et al. Identifying plasma metabolic characteristics of major depressive disorder, bipolar disorder, and schizophrenia in adolescents. *Transl Psychiatry*. 2024;14:163. doi: 10.1038/s41398-024-02886-z
57. Davyson E, Shen X, Gadd DA, et al. Metabolomic investigation of major depressive disorder identifies a potentially causal association with polyunsaturated fatty acids. *Biol Psychiatry*. 2023;94(8):630–639. doi: 10.1016/j.biopsych.2023.01.027
58. Brydges CR, Bhattacharyya S, Dehkordi SM, et al. Metabolomic and inflammatory signatures of symptom dimensions in major depression. *Brain Behav Immun*. 2022;102:42–52. doi: 10.1016/j.bbi.2022.02.003

Tables

Table 1 Comparison of baseline demographic and clinical characteristics between the two groups

Variable	Healthy controls (n = 56)	MDD patients (n = 118)	Statistic	P value
Age (years), mean ± SD	32.46 ± 7.82	33.19 ± 8.15	0.542	0.588
Sex, n (%)			0.089	0.765
Male	24 (42.86)	47 (39.83)		
Female	32 (57.14)	71 (60.17)		
Ethnicity, n (%)			4.901	0.127
Han	53 (94.64)	98 (83.05)		
Minority	3 (5.36)	20 (16.95)		
Education level, n (%)			2.306	0.316
Junior high school and below	10 (17.86)	28 (23.73)		
High school / College	26 (46.43)	56 (47.46)		
Bachelor's degree and above	20 (35.71)	34 (28.81)		
Marital status, n (%)			0.157	0.692
Unmarried	31 (55.36)	69 (58.47)		
Married / Divorced	25 (44.64)	49 (41.53)		
Childhood left-behind experience, n (%)			6.172	0.113
Yes	15 (26.79)	53 (44.92)		
No	41 (73.21)	65 (55.08)		
Smoking, n (%)			0.637	0.425
Yes	12 (21.43)	32 (27.12)		
No	44 (78.57)	86 (72.88)		
Alcohol consumption, n (%)			0.321	0.571
Yes	14 (25.00)	35 (29.66)		
No	42 (75.00)	83 (70.34)		
Past chronic physical illness, n (%)			0.057	0.817
Yes	7 (12.50)	17 (14.41)		
No	49 (87.50)	101 (85.59)		

Note: Continuous variables are expressed as mean ± standard deviation and compared using independent-sample t-tests. Categorical variables are expressed as number (percentage) and compared using χ^2 tests. $P < 0.05$ was considered statistically significant. MDD = major depressive disorder.

Table 2 Summary of 17 differential metabolites with FDR < 0.01

No.	Metabolite	Direction of change	Functional classification	MSI Annotation Level
1	Butyrylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
2	Dodecanoylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
3	Myristoylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
4	Acetylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
5	Hexanoylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
6	Decanoylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
7	Isovalerylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
8	Palmitoleoylcarnitine	Downregulated	Acylcarnitine (mitochondrial fatty acid transport)	Level-2
9	Reduced ubiquinone QH ₂	Downregulated	Electron transport chain component	Level-2
10	Leucine	Downregulated	Branched-chain amino acid (energy metabolism substrate)	Level-2
11	Isoleucine	Downregulated	Branched-chain amino acid (energy metabolism substrate)	Level-2
12	Valine	Downregulated	Branched-chain amino acid (energy metabolism substrate)	Level-2
13	α-Ketoisovaleric acid	Downregulated	Branched-chain amino acid metabolic intermediate	Level-2
14	Pantothenic acid	Downregulated	Coenzyme A precursor	Level-2
15	Oleic acid	Downregulated	Free fatty acid	Level-2
16	Arachidonic acid	Downregulated	Polyunsaturated fatty acid	Level-2
17	Succinic acid	Downregulated	Tricarboxylic acid cycle intermediate	Level-2

Note: All 17 metabolites were downregulated in the MDD group compared to healthy controls. FDR = false discovery rate; MDD = major depressive disorder. MSI = Metabolomics Standards Initiative. Level-2: putative annotation based on accurate mass and MS/MS fragment matching against public spectral libraries; no authentic reference standards were applied for confirmation in this study.

Table 3 OPLS-DA model fitting and permutation test parameters

Model parameter	Value
Number of predictive components	1
Number of orthogonal components	1
R^2X	0.312
R^2Y	0.547
Q^2Y	0.526
Number of permutation tests	200
Permutation test intercept	-0.073

Note: OPLS-DA = orthogonal partial least squares-discriminant analysis; R^2X = cumulative variation in X matrix explained by the model; R^2Y = cumulative variation in Y matrix explained by the model; Q^2Y = cross-validated predictive ability. A negative Q^2 intercept indicates absence of model overfitting.

Table 4 Thirteen core differential metabolites

Metabolite	VIP	$\log_2FC$	FDR	Between-group change	Mitochondria-related	MSI Annotation Level
Butyrylcarnitine	2.74	-1.62	1.13×10^{-5}	Downregulated in MDD	Yes	Level-2
Dodecanoylcarnitine	2.68	-1.57	1.46×10^{-5}	Downregulated in MDD	Yes	Level-2
Myristoylcarnitine	2.51	-1.49	2.08×10^{-5}	Downregulated in MDD	Yes	Level-2
Reduced ubiquinone QH ₂	2.36	-1.34	4.71×10^{-4}	Downregulated in MDD	Yes	Level-2
7 α -Hydroxy-3-oxocholest-4-en-27-oic acid	2.19	-1.28	0.0012	Downregulated in MDD	No	Level-2
Leucine	2.05	-1.21	0.0025	Downregulated in MDD	Yes	Level-2
Isoleucine	1.97	-1.16	0.0037	Downregulated in MDD	Yes	Level-2
Palmitic acid	1.83	-1.12	0.0051	Downregulated in MDD	No	Level-2
Linoleic acid	1.76	-1.05	0.0068	Downregulated in MDD	No	Level-2
Unidentified serine-related metabolite	1.62	1.14	0.0074	Upregulated in MDD	No	Level-3
25-Hydroxyvitamin D3	1.58	1.2	0.0081	Upregulated in MDD	No	Level-2
Unidentified xenobiotic metabolite	1.47	1.26	0.0095	Upregulated in MDD	No	Level-3
Unidentified glucose-related metabolite	1.39	1.31	0.0102	Upregulated in MDD	No	Level-3

Note: Core differential metabolites were defined by the combined criteria of $FDR < 0.05$, $VIP > 1$, and $|\log_2FC| > 1$. $\log_2FC < 0$ indicates lower abundance in the MDD group than in healthy controls; $\log_2FC > 0$ indicates higher abundance in the MDD group. VIP = variable importance in projection; FC = fold change; FDR = false discovery rate; MDD = major depressive disorder. MSI = Metabolomics Standards Initiative. Level-2: putative annotation based on accurate mass and MS/MS fragment matching against public spectral libraries, without authentic reference-standard validation. Level-3: annotation solely relying on exact molecular mass; definitive chemical structure remains undetermined.

Table 5 Functional classification of 71 significant differential metabolites

Metabolite category	Number	Percentage	Direction of change
Lipids and acylcarnitines	22	31.00%	21 downregulated, 1 upregulated
Amino acids and derivatives	17	23.90%	13 downregulated, 4 upregulated
Vitamins and coenzymes	11	15.50%	9 downregulated, 2 upregulated
Xenobiotics	9	12.70%	7 downregulated, 2 upregulated
Carbohydrates	7	9.90%	All downregulated
Other small molecules	5	7.00%	3 downregulated, 2 upregulated
Total	71	100.00%	60 downregulated, 11 upregulated

Table 6 ROC analysis diagnostic efficacy parameters of key differential metabolites

Metabolite	AUC	95% CI	Optimal cutoff	Sensitivity (%)	Specificity (%)
Butyrylcarnitine	0.76	0.690–0.833	2.341	71.19	73.21
Dodecanoylcarnitine	0.75	0.673–0.821	2.186	69.49	75
Myristoylcarnitine	0.73	0.654–0.808	2.053	68.64	71.43
Reduced ubiquinone QH ₂	0.7	0.624–0.784	1.872	66.1	69.64
Leucine	0.68	0.593–0.757	1.668	63.56	67.86
Isoleucine	0.65	0.569–0.737	1.54	61.02	66.07
Eight-acylcarnitine combined model	0.82	0.753–0.877	—	74.58	78.57

Note:ROC = receiver operating characteristic; AUC = area under the curve; CI = confidence interval. The combined model was constructed using logistic regression with eight acylcarnitines as independent variables.

Table 7 List of significantly enriched KEGG metabolic pathways

Pathway ID	Pathway name	Number of enriched metabolites	<i>P</i> value	$-\log_{10}(P)$
hsa00071	Fatty acid β -oxidation	12	0.0021	2.678
hsa00280	Branched-chain amino acid degradation	9	0.0063	2.201
hsa00020	Citrate cycle (TCA cycle)	7	0.0115	1.94
hsa01040	Biosynthesis of unsaturated fatty acids	6	0.0182	1.74
hsa00130	Ubiquinone and other terpenoid-quinone biosynthesis	5	0.0244	1.613
hsa00640	Propanoate metabolism	4	0.0367	1.435

Note:KEGG = Kyoto Encyclopedia of Genes and Genomes; TCA = tricarboxylic acid. Enrichment criteria: $P < 0.05$ and ≥ 3 differential metabolites per pathway.

Figures

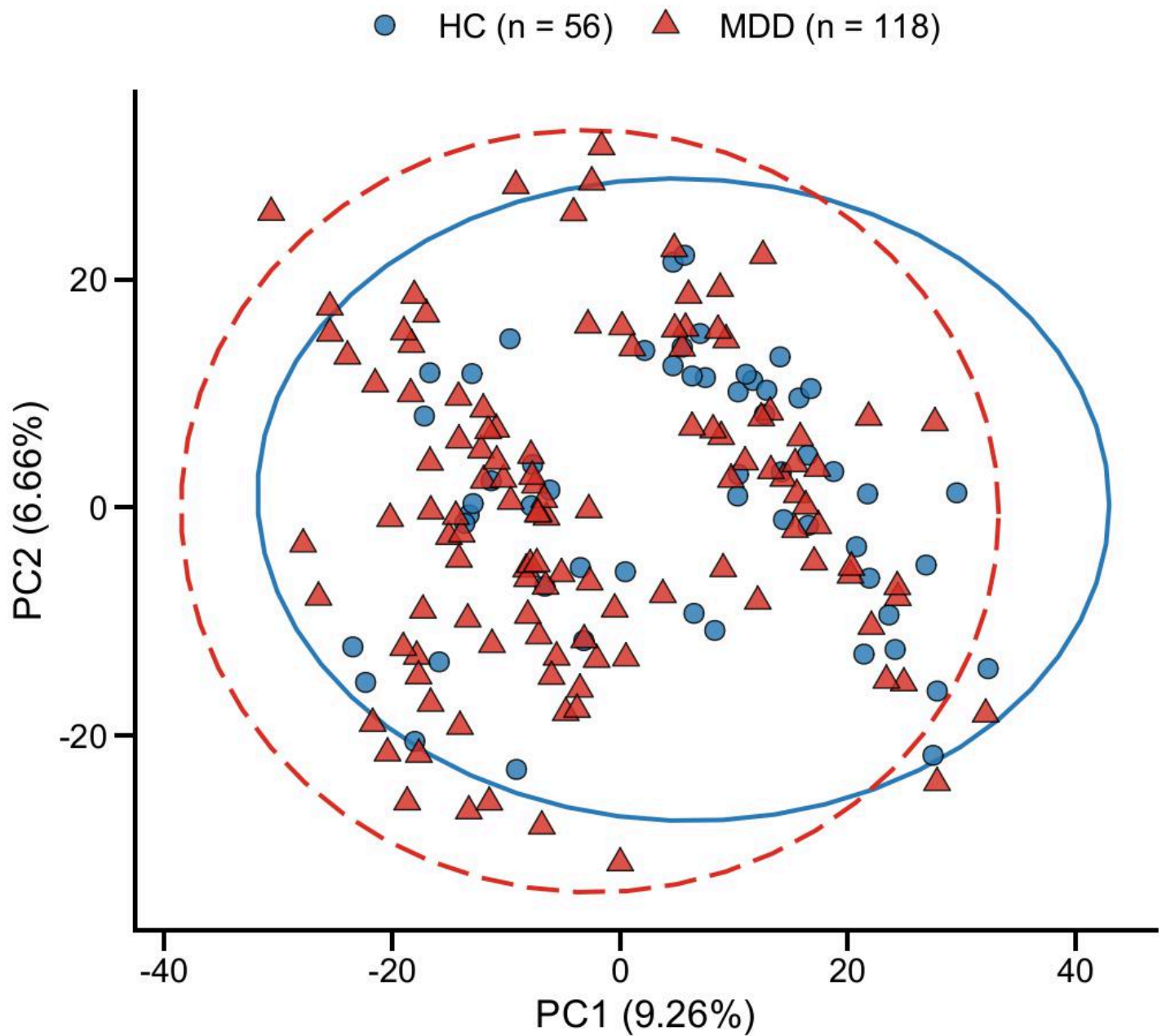


Figure 1

PCA score plot of serum metabolome in two groups of subjects

Figure Legend Principal component analysis (PCA) score plot of serum metabolomics data. The x-axis represents PC1 (explaining 9.26% of total variance) and the y-axis represents PC2 (explaining 5.55% of total variance). Blue circles denote healthy controls (n = 56) and red triangles denote first-episode drug-naïve MDD patients (n = 118). A clear spatial separation between the two groups is observed, indicating distinct global metabolic profiles.

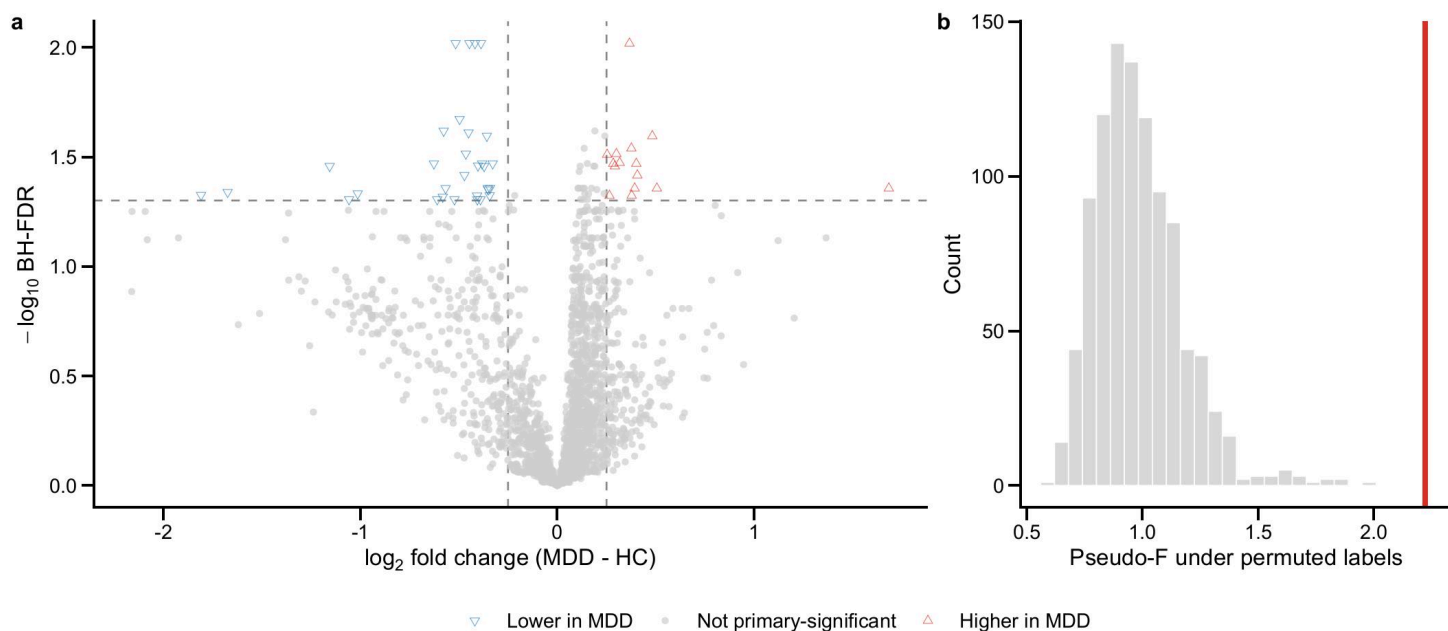


Figure 2

OPLS-DA score plot and permutation test combination plot with 200 permutations

Figure Legend Orthogonal partial least squares-discriminant analysis (OPLS-DA) model validation. Left panel: OPLS-DA score plot showing separation between healthy controls (blue) and MDD patients (red) along the predictive component. Right panel: 200-permutation test plot. Green dots represent R^2 values and red dots represent Q^2 values. The Q^2 intercept (-0.073) is below zero, confirming the absence of model overfitting.

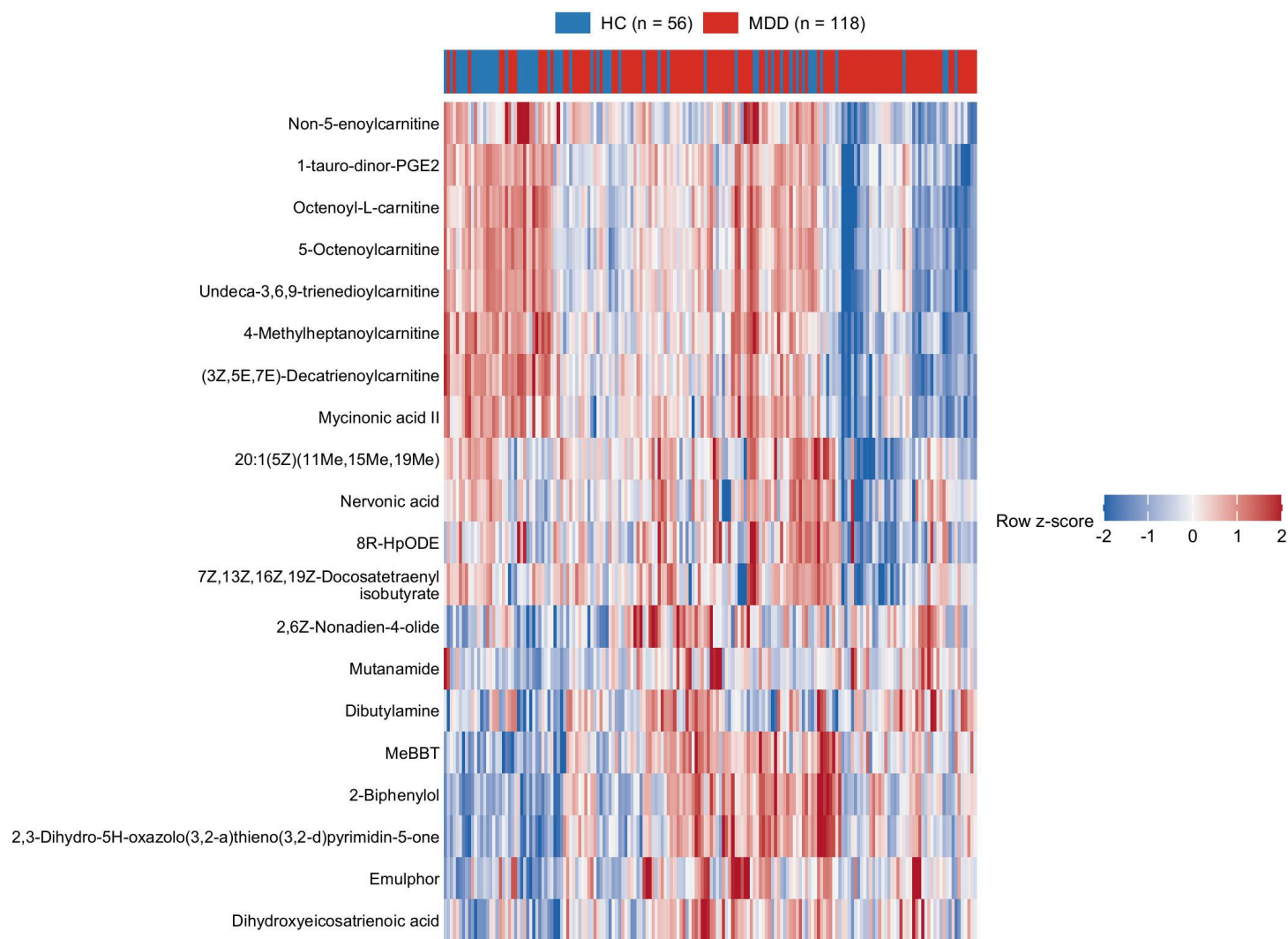


Figure 3

Box plots of the abundances of five core mitochondrial metabolites

Figure Legend Box plots of five representative mitochondria-related metabolites. For each metabolite, the left box represents healthy controls and the right box represents MDD patients. The y-axis indicates mass spectrometry peak intensity (arbitrary units). All five metabolites (butyrylcarnitine, dodecanoylcarnitine, myristoylcarnitine, reduced ubiquinone QH₂, and leucine) showed significantly lower abundance in the MDD group compared to controls.

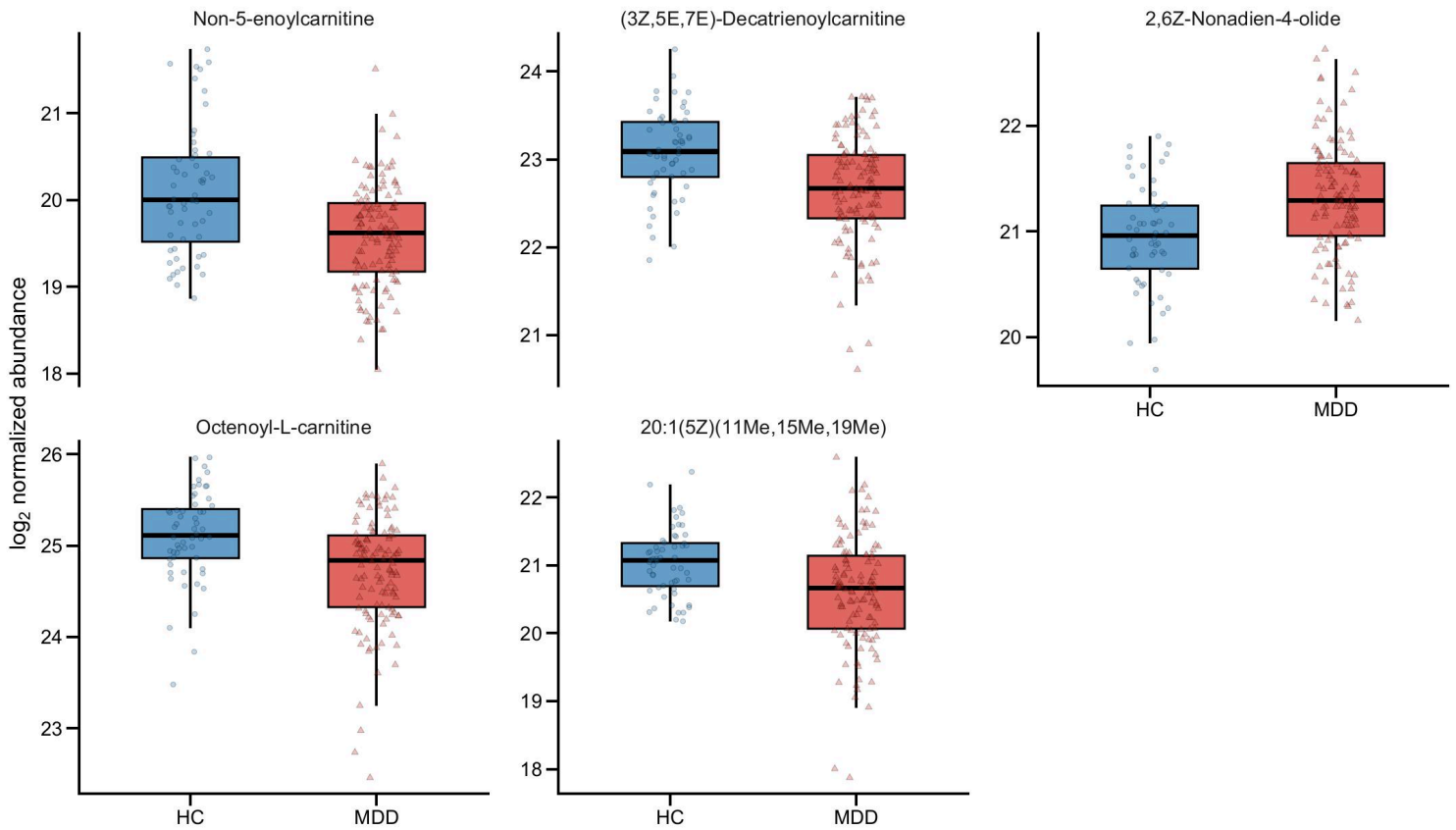


Figure 4

Hierarchical clustering heatmap of 71 significantly different metabolites

Figure Legend—Bidirectional hierarchical clustering heatmap of 71 significant differential metabolites. Columns represent all individual subjects; rows represent metabolites. The top color bar indicates group assignment: blue = healthy controls, red = MDD patients. The color scale from blue to white to red represents Z-score standardized relative abundance from low to high. The dendrogram shows clear separation into two major branches corresponding to healthy controls and MDD patients. Acylcarnitines exhibit concentrated low expression (blue region) in the MDD cluster.

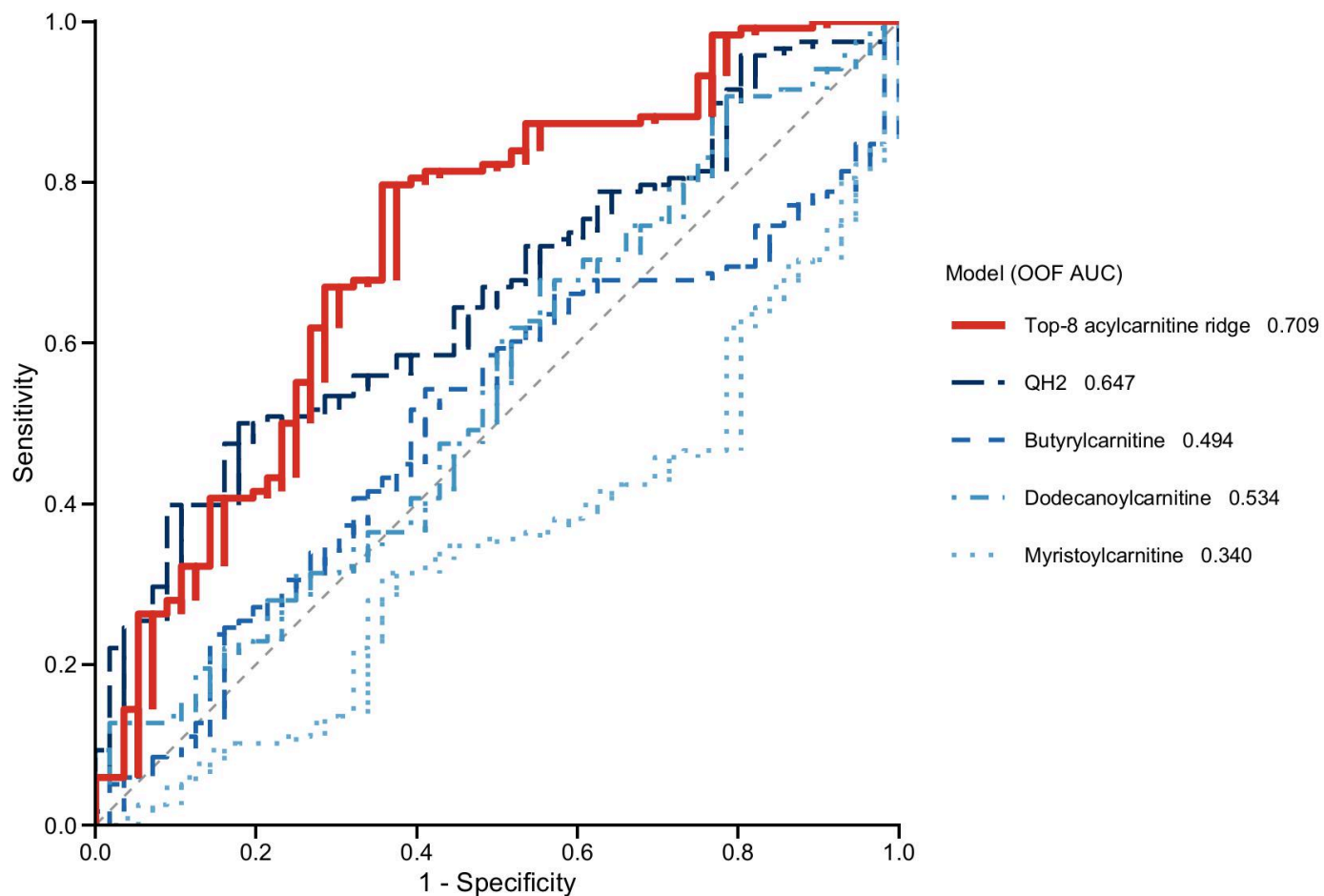


Figure 5

ROC Curve of the Combined Model of Core Metabolites and Acylcarnitines

Figure Legend The abscissa represents 1-Specificity, and the ordinate represents Sensitivity. The gray dotted line is the reference diagonal line ($y=x$). The black solid line indicates the combined diagnostic model of 8 acylcarnitines, and the colored dotted lines represent individual core metabolites. The legend shows the AUC value of each indicator.

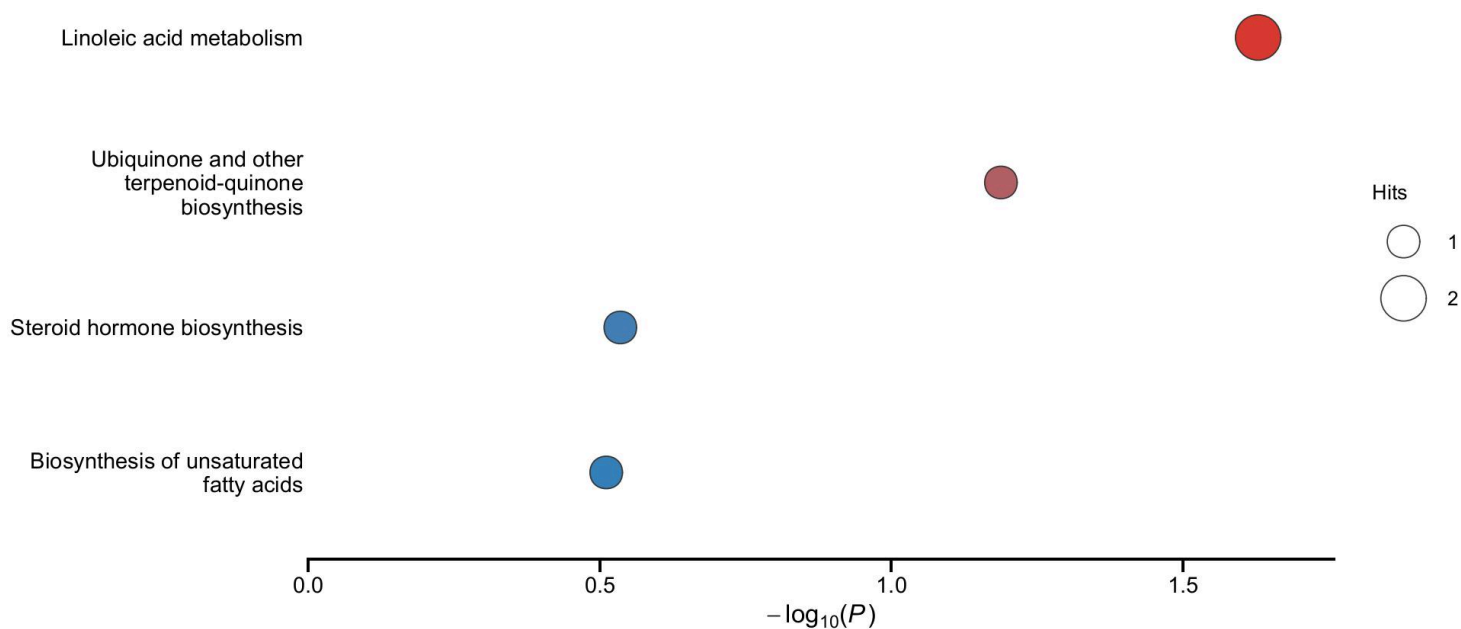


Figure 6

Bubble Chart for KEGG Metabolic Pathway Enrichment

Figure Legend Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment bubble plot. The x-axis represents pathway impact, and the y-axis represents $-\log_{10}(P)$. Bubble size indicates the number of enriched differential metabolites in each pathway. Bubble color gradient from yellow to red indicates increasing enrichment significance (decreasing P value). The top three ranked pathways are fatty acid β -oxidation (hsa00071), branched-chain amino acid degradation (hsa00280), and the citrate cycle (hsa00020).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1Acompletelistof83differentiallyexpressedmetaboliteswithaFDRof0.05.xlsx](#)
- [SupplementaryTableS2CoremitochondrialmetabolitesandcorrelationanalysiswithHAMD17.xlsx](#)
- [SupplementaryFigureS1CompleteFlowchartforScreeningofMitochondrialrelatedMetabolites.png](#)