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Mass spectrometry-based untargeted metabolomics study of polycystic ovary syndrome

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Abstract

Background Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder, and its diagnosis remains controversial due to heterogeneous phenotypes and varying diagnostic criteria. Insulin resistance and its metabolic consequences are central features of PCOS management. Metabolomics has increasingly been applied to elucidate the pathophysiology of complex disorders. In this study, we sought to determine which serum metabolites and metabolic pathways are differentially altered in women with PCOS compared with healthy controls, thereby addressing whether metabolomic profiling can reveal candidate biomarkers for early diagnosis and potential therapeutic targets.

Methods Fifty patients diagnosed with PCOS and 50 healthy controls matched for age and body mass index (BMI) were included in the study. Blood samples were collected for metabolomic analysis and routine biochemical parameters. Metabolomic analysis was performed by UPLC-HRMS and data were processed using MZmine, TidyMass and MetaboAnalyst. Metabolite annotation was performed using databases such as HMDB, MassBank and MoNA.

Results Metabolomic analysis revealed 49 compounds in the serum of PCOS patients, 39 of which were upregulated and 10 of which were downregulated. Compounds such as di(2-ethylhexyl) phthalate (DEHP), promethazine N-oxide, tetrahydromagnolol, 5-methyl-5-phenylhydantoin, valerenic acid, butylparaben, erucamide, DDAO, d-erythro-sphinganin-1-phosphate and 1-arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine were significantly higher in the PCOS group. Compounds such as L-methyladenosine, cystine, glu-gln and 2,2'-methylene-bis(6-tert-butyl-4-methylphenol) were significantly lower. In the pathway analysis performed using KEGG database, sphygolipid metabolism, sphygolipid signaling pathway, neuroactive ligand-receptor interaction and phenylalanine metabolism were found to be the most affected pathways.

Conclusion This study demonstrates distinct alterations in lipid and amino acid metabolism in PCOS and highlights the accumulation of exogenous molecules, including endocrine disruptors, in patient serum. By integrating metabolomic profiling with clinical phenotyping, our findings provide novel insights into PCOS pathophysiology and suggest potential serum biomarkers that may support early diagnosis and personalized therapeutic approaches.

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Keywords Polycystic ovary syndrome, Metabolomics analysis, UPLC-HRMS, Serum metabolites

"What does this study adds to the clinical work"

This study identified significant differences in lipid and amino acid metabolic pathways in women with PCOS, highlighting potential biomarkers for the disease. The research also found increased levels of exogenous molecules and pollutants in the serum of PCOS patients, which may contribute to the development of personalized treatment strategies and early diagnosis.

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine and metabolic disorder affecting approximately 5–10% of women of reproductive age worldwide [1]. The etiopathogenesis of PCOS remains multifactorial and incompletely understood, involving genetic, epigenetic, and environmental contributors. Clinical manifestations include cutaneous signs of hyperandrogenism such as acne, seborrhea, alopecia, and hirsutism, as well as menstrual irregularities, chronic anovulation, and metabolic disturbances including hyperinsulinemia and insulin resistance (IR). Polycystic ovarian morphology (PCOM) can be identified by ultrasonographic examination. Beyond reproductive implications, women with PCOS are at increased risk of central obesity, dyslipidemia, hypertension, atherosclerosis, and type 2 diabetes mellitus (T2DM), which collectively contribute to the development of cardiovascular disease (CVD) [2, 3].

The diagnosis of PCOS is often considered controversial due to the existence of multiple diagnostic criteria (NIH 1990, Rotterdam 2003 and AE-PCOS 2006) and the heterogeneity of clinical phenotypes. This lack of consensus frequently complicates early recognition and stratification of patients [4–7]. Consequently, treatment strategies must extend beyond the management of hyperandrogenic symptoms and infertility to include improving insulin resistance and mitigating long-term cardiometabolic sequelae.

Given the complex pathophysiology of PCOS, a comprehensive understanding of the underlying metabolic alterations is crucial for the identification of novel biomarkers that may facilitate timely diagnosis and personalized therapeutic interventions [8, 9]. In recent years, metabolomics has emerged as a powerful systems biology approach for characterizing the global metabolic state of biological systems.

Metabolomic profiling has been successfully applied not only in PCOS but also in a wide spectrum of diseases, including diabetes, cardiovascular disorders, neurodegenerative diseases, iron deficiency anemia, and cancer, where it has provided valuable insights into disease mechanisms and biomarker discovery [10–15].

The untargeted metabolomics approach, also referred to as metabolic fingerprinting, aims to capture and quantify as many low-molecular-weight metabolites as possible in a biological sample, thereby providing a holistic snapshot of ongoing biochemical processes [16, 17]. Alterations in amino acid, lipid, and organic acid metabolism have previously been reported in PCOS, and these changes are closely linked to insulin resistance, inflammation, and oxidative stress. Importantly, metabolomic profiling offers the potential to identify clinically relevant biomarkers that can improve diagnosis, enable disease monitoring, and contribute to the development of individualized treatment strategies.

Based on this rationale, the present study aimed to comprehensively investigate metabolic alterations in PCOS by integrating clinical, hormonal, and untargeted metabolomic profiling. Using ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS), we sought to identify differentially expressed metabolites and elucidate dysregulated metabolic pathways. By characterizing these metabolic disturbances, we aimed to generate novel insights into the pathophysiology of PCOS and explore potential serum biomarkers that may support early diagnosis and personalized therapeutic approaches.

Material method

Study design and sample collection

This study included 50 patients diagnosed with Polycystic Ovary Syndrome (PCOS) and 50 age and BMI matched healthy controls. Patients were recruited from the Gynecology and Obstetrics outpatient clinic of Bezmialem Vakif University Faculty of Medicine Hospital. The study was approved by the Bezmialem Vakif University Ethics Committee. PCOS was diagnosed according to the 2003 Rotterdam criteria [18], requiring at least two of the following: (1) oligo/anovulation, (2) clinical or biochemical hyperandrogenism, or (3) polycystic ovary morphology on ultrasound, after exclusion of other related disorders. Controls were selected among women with regular cycles, no hyperandrogenism, normal ovarian ultrasound, and no history of endocrine, autoimmune, or surgical disease.

Sample size justification

There is no universally accepted standard for sample size determination in untargeted metabolomics studies. Therefore, we conducted a power analysis based on preliminary clinical data. The analysis indicated that a minimum of 45 participants per group would be required to detect medium effect sizes (Cohen's d = 0.5) with 80%

power at $\alpha = 0.05$. Accordingly, 50 participants were recruited for each group.

Clinical and biochemical assessments

Anthropometric measurements including height, weight, waist and hip circumference were taken, and BMI was calculated as weight (kg)/height $(m)^2$. Waist–hip ratio and Ferriman–Gallwey score were recorded, with FG ≥ 8 considered diagnostic of hirsutism. Hormones (LH, FSH, E2, PRL, testosterone, SHBG, DHEAS, androstenedione, fasting insulin) were analyzed using chemiluminescence (Siemens Atellica IM 1600). Lipid profile and fasting glucose were measured photometrically (Siemens Atellica CH 930). CRP was analyzed using an immunoturbidimetric method (Abbott Architect C4000). Free androgen index (FAI) was calculated as testosterone × 100/SHBG. HOMA-IR was calculated as fasting insulin × fasting glucose/405.

Sample preparation and quality control

Fasting venous blood samples were collected in clot activator tubes, left at room temperature for 30 min, and centrifuged at 2300×g for 10 min. Serum was aliquoted and stored at − 80 °C until analysis. For metabolomics, 250 µL serum was mixed with 500 µL methanol for protein precipitation, vortexed, and centrifuged at 10,000 g for 75 min. The supernatant was filtered through a 0.22 µm membrane, dried under vacuum, and reconstituted in 50 µL methanol. All samples were analyzed in two technical injections, and the mean intensity values were used in further analyses. Quality control (QC) samples were prepared by pooling 10 µL aliquots from each serum sample and injected after every 10 study samples to monitor instrument stability.

LC–MS/MS analysis

Untargeted metabolomic profiling was performed at Bezmialem Vakif University Drug Research Center (İLMER) using a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher, Germany) coupled with a Fortis C18 column (3 µm, 150 × 2 mm). The mobile phases were water containing 0.1% formic acid (A) and methanol (B). The gradient program increased B from 5% to 95% over 15 min, followed by re-equilibration, for a total runtime of 17 min. The injection volume was 5 µL, and data were acquired in full scan mode (100–1100 m/z) under both positive and negative electrospray ionization.

Data treatment and metabolite annotation

Raw files were converted into mzXML and mgf formats using ProteoWizard [19] Data processing (peak detection, alignment, deconvolution) was performed with MZmine and TidyMass [20, 21]. Normalization was carried out using probabilistic quotient normalization (PQN), followed by log10 transformation and Pareto scaling. Missing values were imputed using the KNN algorithm. Metabolite annotation was performed against HMDB, MassBank, and MoNA databases using accurate mass and MS/MS matching. Annotation confidence was assigned according to Metabolomics Standards Initiative (MSI) levels.

Pathway analysis

Pathway enrichment and topology analyses were performed in MetaboAnalyst 5.0 [22] using the KEGG database. Over-representation analysis was combined with pathway topology analysis. Pathways with $p < 0.05$ and FDR < 0.05 were considered significantly altered.

Statistical analysis

Clinical and biochemical data were analyzed in SPSS v20. Normality was tested with Shapiro–Wilk, and normally distributed variables were compared using Student's t-test. A p-value < 0.05 was considered significant. For metabolomics, univariate analyses (t-test, fold-change < 0.5 or > 1.5, FDR adjustment) were performed. Principal Component Analysis (PCA) was conducted to visualize group separation and to assess data quality.

Results

The hormonal, metabolic, and demographic profiles of the study subjects are summarized in Table 1. There were no significant differences in age, BMI, and waist-hip ratio in the PCOS group compared with the control groups. Among the hormonal data, LH, LH/FSH ratio, total testosterone, calculated FAI, DHEA-S, and androstenedione were significantly higher in women with PCOS than in the control group ($p < 0.05$). At the same time, the FG score, which is a marker of hirsutism, was found to be significantly higher in the PCOS group. On the other hand, FSH and HDL were found to be significantly lower in patients with PCOS ($p < 0.05$). In addition, there was no significant difference between the groups in E2, PRL, SHBG, T-Ch, LDL-Ch, TG, FBG, FSIns, HOMA-IR and CRP.

To evaluate overall data structure and analytical performance, Principal Component Analysis (PCA) was conducted on both raw and normalized metabolomic datasets. In the raw data PCA, pooled QC samples clustered tightly, confirming instrumental stability and reproducibility throughout the analytical run. In contrast, the normalized and scaled PCA (PQN normalization, log10 transformation, Pareto scaling) revealed partial separation between PCOS patients and healthy controls, with the first two principal components (PC1: 28.22%, PC2: 11.12%) explaining a substantial proportion of the variance. These findings indicate that while inter-individual

Table 1 Baseline characteristics of PCOS and healthy individuals

Variable	PCOS group (*n* = 50)	Control group (*n* = 50)	*p*-value
Age (years)	22.37 ± 3.73	22.12 ± 4.11	0.758
BMI (kg/m2)	24.31 ± 5.47	23.63 ± 3.65	0.473
W/H	0.77 ± 0.07	0.78 ± 0.08	0.822
LH (U/L)	6.40 ± 4.16	4.76 ± 2.75	0.044*
FSH (U/L)	6.14 ± 1.29	6.87 ± 1.55	0.021*
LH/FSH ratio	1.02 ± 0.63	0.75 ± 0.55	0.047*
E2 (pg/mL)	40.77 ± 16.23	43.74 ± 19.70	0.465
PRL (ng/mL)	14.44 ± 8.70	12.81 ± 6.37	0.369
T (nmol/L)	1.07 ± 0.46	0.64 ± 0.47	0.001*
SHBG (nmol/L)	53.02 ± 26.96	53.85 ± 22.46	0.901
FAI	2.76 ± 1.76	1.90 ± 0.76	0.017*
DHEA-S (µg/dL)	254.12 ± 89.84	212.80 ± 78.32	0.036*
AS (ng/mL)	2.21 ± 1.24	1.48 ± 0.88	0.014*
T-Ch (mg/dL)	157.59 ± 22.26	164.32 ± 28.40	0.270
HDL-Ch (mg/dL)	54.95 ± 11.40	60.91 ± 12.74	0.034*
LDL-Ch (mg/dL)	91.06 ± 19.37	85.10 ± 19.05	0.225
TG (mg/dL)	73.23 ± 29.06	67.90 ± 21.48	0.401
FBG (mg/dL)	89.12 ± 6.45	86.18 ± 7.91	0.059
FSIns (mU/L)	10.54 ± 8.36	10.29 ± 10.23	0.905
HOMA-IR	2.10 ± 1.08	2.20 ± 2.51	0.819
CRP (mg/L)	1.65 ± 2.12	1.61 ± 1.83	0.925
FG Score	11.55 ± 7.49	2.49 ± 2.49	0.001*

The comparison was performed using the Student's t test method. Data was shown as the mean ± SD

BMI body mass index, *W/H* Waist–hip ratio, *LH* luteinizing hormone, *FSH* follicle stimulating hormone, *E2* estradiol, *PRL* prolactin, *T* testosterone, *SHBG* sex hormone-binding globulin, *FAI* the free androgen index (FAI = T × 100/SHBG), *DHEAS* dehydroepiandrosterone sulfate, *AS* androstenedione, *T-Ch* total cholesterol, *HDL-Ch* high-density lipoprotein cholesterol, *LDL-Ch* low-density lipoprotein cholesterol, *TG* triglyceride, *FBG* fasting blood glucose, *FSIns* fasting serum insulin, *HOMA-IR* the homeostasis model assessment of insulin resistance (HOMA-IR = FBG × FSIns/405), *CRP* C-reactive protein, *FG score* Ferriman-Gallwey score

variability is present, distinct metabolic alterations contribute to the differentiation of PCOS and control groups (Fig. 1).

We have been identified in the serum of PCOS women as compared to healthy control 49 compounds, 39 of them were up regulated and 10 of them were down regulated (Table 2). 14 significant compounds were shown in volcano plot about 10 of them were up regulated and 4 down regulated (Fig. 2). In individuals with PCOS patient, it was determined that the compounds Di(2-ethylhexyl) phthalate (DEHP), Promethazine N-oxide, Tetrahydromagnolol, 5-Methyl-5-phenylhydantoin, Valerenic acid, Butylparaben, Erucamide, DDAO, D-erythro-Sphinganine-1-phosphate, 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine were significantly higher compared to the healthy control group. On the other hand, it was found that the compounds L-Methyladenosine, Cystine, Glu-Gln, and 2,2'-Methylene-bis (6-tert-butyl-4 methylphenol) were significantly lower (Fig. 2).

KEGG database was utilized to annotate pathways for differential metabolites in the PCOS and healthy control group. Some of the compounds found were substances that did not participate in metabolic pathways in the body.

Metabolites showing significant differences between the groups were found to be involved in Sphingolipid metabolism, Sphingolipid signaling pathway, Neuroactive ligand-receptor interaction, Phenylalanine metabolism, Apoptosis, Fc gamma R mediated phagocytosis, Apelin signaling pathway, Adrenergic signaling in cardiomyocytes, Necroptosis and Phospholipase D signaling pathway (Fig. 3).

Discussion

Multiple metabolomics studies on the serum, urine, and follicular fluids of PCOS patients have identified common changes in carbohydrate, amino acid, and fatty acid metabolism [23–25]. Additionally, specific dysfunctions have been recorded in various subgroups of patients [26].

Our untargeted UPLC-HRMS analysis revealed significant perturbations in lipid and amino acid metabolism in women with PCOS (Fig. 3). The changes in lipid molecules were noted in sphingolipid and phospholipid metabolism, alongside impaired fatty acid metabolism. Furthermore, alterations were identified in several molecules related to amino acid metabolism, including phenylalanine, cysteine, L-methyladenosine, and the dipeptide Glu-Gln (Table 2). We also observed decreased bile acid levels and increased concentrations of environmental pollutants, including endocrine-disrupting chemicals, in patient serum. Pathway analysis identified the five most affected pathways as sphingolipid metabolism, the sphingolipid signaling pathway, neuroactive ligand-receptor interaction, phenylalanine metabolism, and apoptosis (Fig. 3).

Sphingolipid metabolism

Lipids are the largest group of molecules whose metabolism is notably altered in PCOS, and they represent one of the most common disruptions revealed by metabolic approaches [9]. In the study, the primary changes observed in the serum lipid profile included higher levels of D-erythro-Sphinganine-1-phosphate, D-erythro-Sphingosine-1-phosphate, phytosphingosine 1-phosphate, palmitoyl sphingomyelin, and D-sphingosine (Table 2). In accordance with these findings, the two most significant metabolic pathways identified in the serum of patients were sphingolipid metabolism and sphingolipid signaling pathways. This result aligns with another untargeted LC-MS study, which indicated that sphingolipid and glycerophospholipid metabolisms were the most impacted in the follicular fluids of PCOS [27].

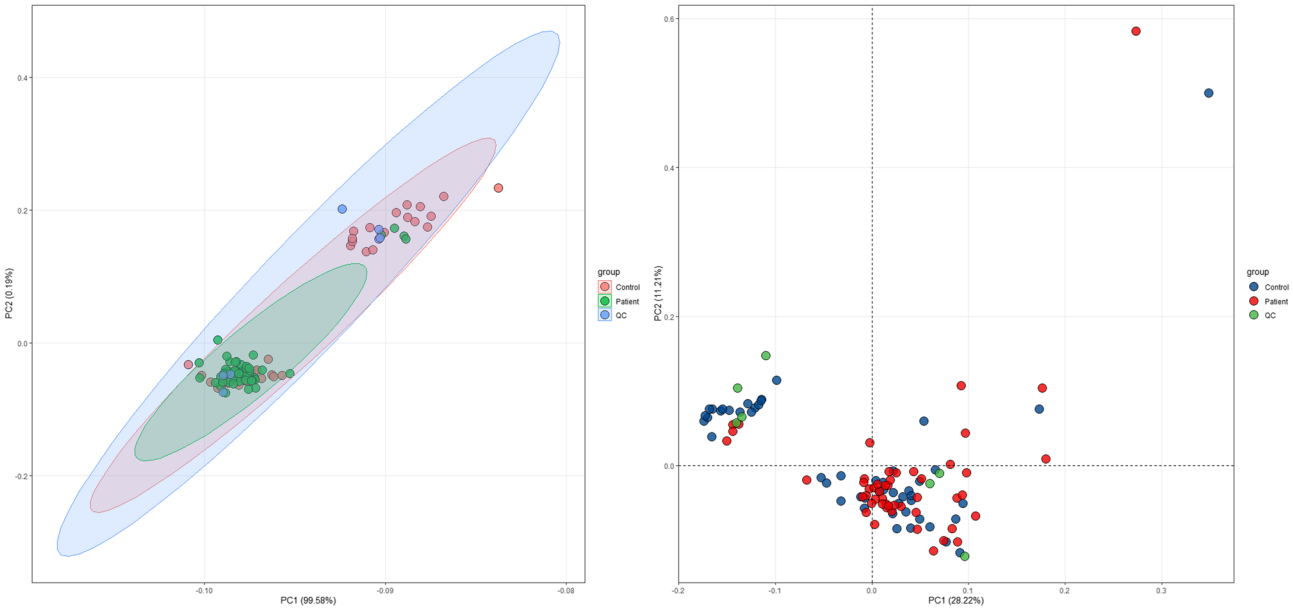

Fig. 1 PCA of serum metabolomic profiles in PCOS patients and healthy controls. ((Left) Raw data PCA showing serum metabolomic features from PCOS patients (red), healthy controls (blue), and pooled QC samples (green). QC samples cluster tightly, demonstrating analytical reproducibility and instrumental stability. (Right) Normalized and scaled PCA after probabilistic quotient normalization (PQN), log10 transformation, and Pareto scaling)

D-erythro-Sphinganine-1-phosphate emerged as a prominent candidate biomarker, as highlighted by the volcano plot analysis (Fig. 2), supported by a previous study [11]. It is a derivative of the serum molecule D-erythro-sphingosine-1-phosphate, which plays a key role in various signaling pathways, as well as in cell growth, proliferation, and apoptosis [28]. Sphingosine 1-Phosphate (S1P) is the active form of sphingolipids, and its roles in various diseases, from infections to tumor immunology, are well established [29]. Recent research has shown increased serum levels of S1P species, specifically D-erythro-Sphinganine-1-phosphate, D-erythro-Sphingosine-1-phosphate, and phytosphingosine 1-phosphate, highlighting significant alterations in S1P metabolism in PCOS. This observation is consistent with previous serum LC–MS studies reporting elevated levels of S1P along with changes in nine sphingosine and three sphinganine molecules [30]. In the same study, all sphingomyelin species, including palmitoyl sphingomyelin and D-sphingosine, were elevated in patient serum, correlating with the current results.

As shown in a mechanistic study, S1P reduced the viability of mouse ovarian granulosa cells and inhibited estrogen secretion through a specific pathway [31]. Excessive levels of S1P may contribute to the development of PCOS, as shown in previous literature and supported by serum UPLC-HRMS findings. While most of the literature supports the established findings, one study presents evidence of a significant decrease in serum levels of sphinganine [32].

In the study participants, elevated serum D-sphingosine levels may contribute to the development of PCOS as it serves as a precursor for ceramide. Elevated levels of serum ceramides are directly associated with insulin resistance, as they disrupt insulin signaling [33]. This disruption leads to increased insulin production and subsequently higher androgen levels, triggering the typical symptoms of PCOS [30].

Phospholipid metabolism

All detected glycerophospholipid species were elevated in the serum of PCOS patients compared to those in healthy individuals. These molecules included 1-heptadecanoyl-sn-glycero-3-phosphocholine (LysoPC 17), 1-(1Z-octadecenyl)-sn-glycero-3-phosphocholine (LysoPC 18), 1-arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine (LysoPC (20:0/0:0)), 1-O-hexadecyl-2-O-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-sn-glycero-3-phosphocholine (DHA-PC (16:0/22:6)), and phosphocholine (PC (16:0/0:0)). Previous studies have validated similar disruptions in glycerophospholipid metabolism, showing higher levels of these molecules [8, 27].

LysoPC (20:0/0:0) showed the highest impact among bioactive LysoPCs in the serums of women with PCOS, with a fold change value over 2 (Table 2). LysoPCs are generated in plasma during the deacylation/reacylation cycle and primarily associate with albumin. Inflammatory processes can increase the ratio of free to bound LysoPC in circulation, potentially due to heightened formation of LysoPC or decreased albumin levels [34]. In women

Table 2 Statistical distribution of metabolite alterations between PCOS and control groups based on fold-change, *p*-value, and adjusted *p*-value

Compound name	Adduct	Fold-change	*p*-value	Adjusted *p*-value
Di(2-ethylhexyl) phthalate (DEHP)	(M + H)+	5.22	0.001075	0.011082
Promethazine N-oxide	(M + H)+	4.17	0.000876	0.010187
Tetrahydromagnolol	(M + H)+	4.16	0.002709	0.016894
5-Methyl-5-phenylhydantoin	(M + H)+	2.42	0.008844	0.042778
Valerenic acid	(M − H)−	2.41	0.000008	0.000476
Butylparaben	(M − H)−	2.39	0.000000	0.000034
Erucamide	(M + H)+	2.15	0.008217	0.040569
DDAO	(M + H)+	2.12	0.000847	0.010187
D-erythro-Sphinganine-1-phosphate	(M − H)−	2.09	0.000000	0.000038
LPC (20:0/0:0)	(M + H)+	2.05	0.000248	0.005333
Muscone	$(M + H - H_2O)+$	1.94	0.000659	0.009261
cis, cis-9,12-Octadecadien-1-ol	$(M + H - H_2O)+$	1.92	0.002606	0.016894
Pristanic acid	(M + H)+	1.91	0.001498	0.013153
2-Linoleoylglycerol	$(M + NH_4)+$	1.89	0.000530	0.008374
Palmitoyl-L-carnitine	(M + H)+	1.86	0.000228	0.005333
D-erythro-Sphingosine-1-phosphate	(M + H)+	1.78	0.000058	0.002302
Phytosphingosine-1-phosphate	$(M - H_2O - H)-$	1.75	0.000000	0.000034
Stearoyl-L-carnitine	(M + H)+	1.71	0.000317	0.005981
Monoelaidin	(M + H)+	1.70	0.002098	0.015940
Palmitoyl sphingomyelin	$(M + CH_3CN + H)+$	1.69	0.006299	0.031763
Oleoyl-L-carnitine	(M + H)+	1.69	0.000090	0.003035
DHA-PC (16:0/22:6)	(M + H)+	1.68	0.004497	0.025379
2-Hexyldecanoic acid	$(M + H - H_2O)+$	1.65	0.005740	0.029573
LPC (17:0/0:0)	(M + H)+	1.64	0.001196	0.011452
LPC (18:1/0:0)	(M + H)+	1.60	0.001321	0.012038
Hexaethylene glycol	(M + H)+	1.56	0.000664	0.009261
Leupeptin	(M + H)+	1.56	0.002586	0.016894
SFP-AMP-(5FPe)	(M + H)+	1.56	0.001900	0.015528
PC (16:0/0:0)	(M + H)+	1.52	0.002216	0.015940
MMV687762	(M + H)+	1.51	0.000194	0.005096
D-Sphingosine	(M + H)+	1.47	0.002680	0.016894
8,9-EPETE	(M + H)+	1.46	0.002127	0.015940
Poldine	(M + H)+	1.45	0.000461	0.007798
Glu-Leu-Asp-Lys-Trp-Ala	(M + H)+	1.45	0.001629	0.013791
Spectinomycin	(M + H)+	1.44	0.000158	0.004672
Palmitamide	(M + H)+	1.44	0.001208	0.011452
Isoproterenol	$(M + H - H_2O)+$	1.41	0.004852	0.026364
L-Phenylalanine	(M + H)+	1.40	0.004895	0.026364
Phosphocholine	(M + H)+	1.33	0.005358	0.028219
Triptophenolide	(M − H)−	0.90	0.002287	0.015940
C10-LAS (tentative)	(M − H)−	0.89	0.000903	0.010187
Dodecylbenzenesulfonic acid	(M − H)−	0.84	0.003913	0.022617
7α-OH-3-oxo-4-cholestenoic acid	(M + H)+	0.80	0.003433	0.020339
Hippuric acid	(M + H)+	0.68	0.002277	0.015940
Glycocholic acid	(M + H)+	0.51	0.003205	0.019478
L-Methyladenosine	(M + H)+	0.49	0.000949	0.010227
Cystine	(M + H)+	0.48	0.000746	0.009827
Glu-Gln	(M + H)+	0.46	0.000328	0.005981
2,2′-Methylene-bis (6-tert-butyl-4 methylphenol)	(M − H)−	0.21	0.000053	0.002302

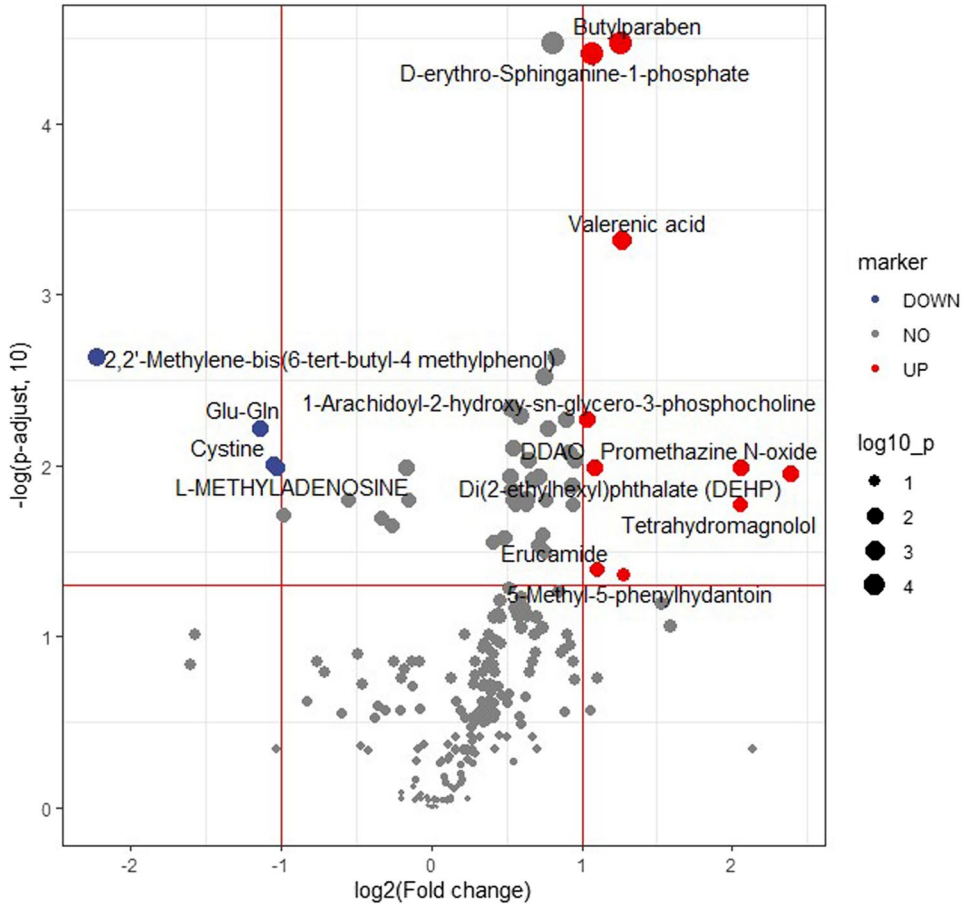

Fig. 2 Volcano plot of differential serum metabolites between PCOS patients and healthy controls. (Each dot represents a detected metabolite. The x-axis shows the log2 fold change (PCOS vs. control), and the y-axis shows the − log10 adjusted *p*-value. Red dots indicate significantly upregulated metabolites in PCOS, blue dots indicate significantly downregulated metabolites, and grey dots represent metabolites without significant changes. The size of each dot reflects the level of statistical significance (log10 p))

with PCOS, the production of LysoPC was found to be up-regulated in follicular fluid [27] along with higher urinary levels of LysoPC (18:1) [25]. In alignment with the current research findings, the LysoPC (18:2) molecule has been recognized as one of the most notable up-regulated metabolites found in the serum of individuals diagnosed with PCOS [11]. The authors highlighted that the strength of their study was the careful timing of sample collection during the follicular phase, which may account for the conflicting results found in other studies.

In contrast to both earlier reports and our current findings, various studies have also shown lower LysoPC levels in body fluids of PCOS patients [9]. A recent study observed a reduction in the levels of LysoPC (16:0), phytosphingosine, LysoPC (14:0), and LysoPC (18:0) in the composition of follicular fluid [35]. A study using shotgun lipidomics revealed increased total serum levels of ceramide and phosphatidylcholine (PC), along with decreased LysoPC [36]. Several other studies have also reported lower levels of serum or plasma LysoPCs [26, 37, 38].

LysoPCs have demonstrated several anti-inflammatory effects, including vasoprotective properties [34] and a role in insulin signaling [39]. Thus, the lower serum levels [32] were linked to an increased risk of type 2 diabetes mellitus in individuals with PCOS [40]. However, increasing evidence suggests that higher serum levels of LysoPCs are associated with induced inflammation and oxidative stress [8], which may increase the risk of developing type 2 diabetes [41]. Given these conflicting results, further mechanistic studies are crucial to elucidate the role of LysoPCs in PCOS due to their strong potential as a therapeutic target.

Fatty acids and derivatives

The UPLC-HRMS analysis of patient serum revealed significant alterations, particularly within lipid-class metabolites. Notably, carnitine-associated fatty acids showed pronounced changes.

Serum carnitine is crucial for transporting long-chain fatty acids to mitochondria and for beta-oxidation, serving as a key marker of metabolic dysfunction in disease

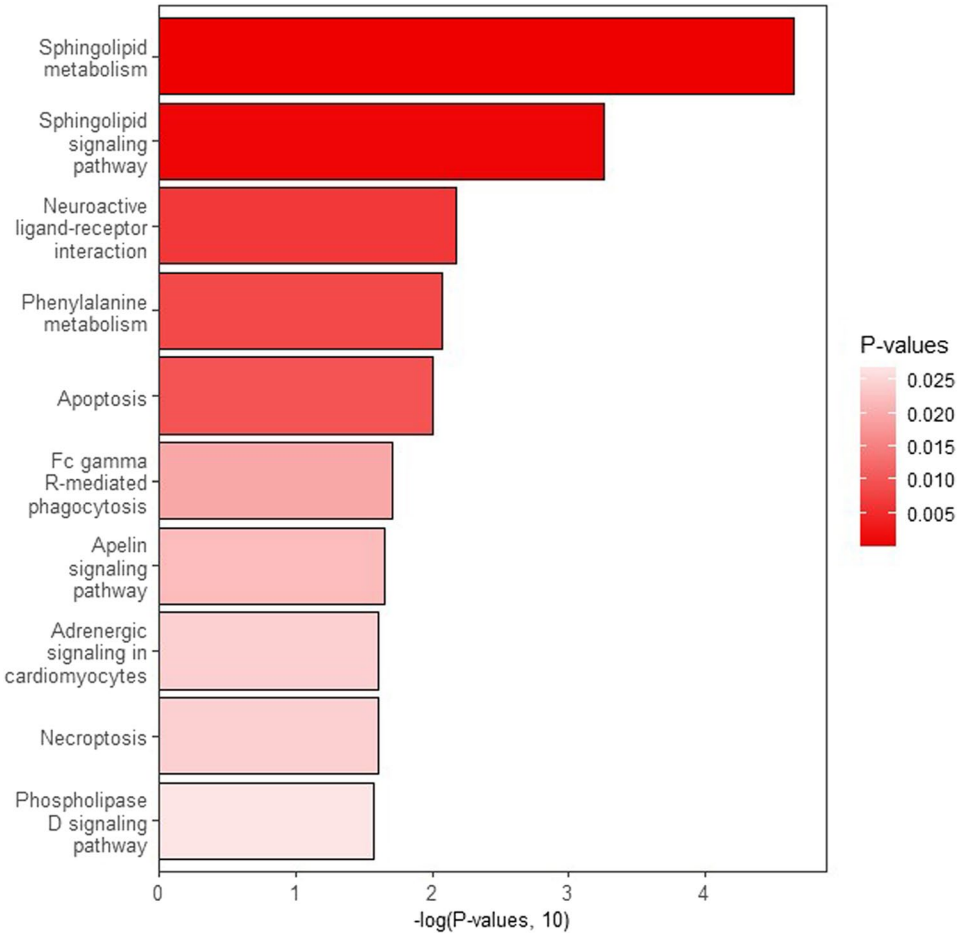

Fig. 3 Pathway enrichment analysis of differential serum metabolites in PCOS. (Bar plot representing the top enriched metabolic pathways identified using the KEGG database. The x-axis shows the – log10 transformed *p*-values, and the y-axis lists the significantly enriched pathways. Color intensity indicates the level of significance, with darker red representing lower *p*-values)

[42]. In individuals with type 2 diabetes mellitus and diet-induced obesity, the accumulation of acyl-coenzyme A intermediates in the mitochondria has been shown to lead to an increase in serum long-chain acylcarnitine levels [43]. Recognizing PCOS as a key metabolic dysfunction, recent findings show increased serum levels of long-chain acylcarnitines, including palmitoyl-L-carnitine, stearoyl-L-carnitine, and oleoyl-L-carnitine, aligning well with existing literature (Table 2 [42, 44]). However, recent studies have produced conflicting results regarding serum levels of total and free carnitine, as well as its derivatives, indicating a notable decrease [38, 45]. One of these studies suggested that decreased serum carnitine levels result from abnormalities in carnitine biosynthesis during the gestational period, which may lead to the development of PCOS [32]. It was also stated that lower serum carnitine levels indicate impairments in beta oxidation processes, leading to the accumulation of fatty acids in the cytosol, which affects oocyte quality [38]. It is essential to acknowledge that the contradictory results reported have shown a decrease in total serum carnitine levels, as opposed to the long-chain acylcarnitines identified in our study. To gain a clearer understanding, further rigorous research is necessary to accurately quantify the individual species of carnitine in the context of PCOS.

As a consequence of disruptions in carnitine metabolism, an increase in serum free fatty acid (FFA) concentrations is anticipated, particularly concerning long-chain fatty acids. This occurrence is primarily attributed to the inhibition of fatty acid oxidation processes [42]. In this context, the long-chain fatty acids 8(9)-Epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid (8,9-EPETE) and 9-octadecenoic acid (monoelaidin) were found in increased levels in the PCOS serums (Table 2). In support, a plasma metabolomics analysis showed that all identified long-chain fatty acids were upregulated in individuals with PCOS [24]. Another study based on LCMS indicated increased serum FFA 18:1/FFA 18:0 and FFA 20:3 molecules as part of a combined diagnostic model for PCOS [37]. FFAs of C14:0, C16:1, and C18:1 were also found to be elevated in the plasma of individuals with PCOS using an untargeted metabolomics approach [46].

The current body of research on 2-hexyldecanoic acid is limited, making the clinical significance of elevated serum levels uncertain. Furthermore, the increased levels of 2-linoleoylglycerol observed in individuals with PCOS have not been addressed in the literature, as this compound is not considered a natural metabolite and is instead categorized as part of the exposome (Table 2).

Amino acid metabolism

Despite not exceeding the fold change of 2, phenylalanine (Phe) was significantly elevated in the serum of PCOS patients, as determined by student t-test analysis (Table 2). Multiple studies have indicated that elevated Phe levels are present in the follicular fluids of patients with PCOS [9, 35]. A strong evidence indicates increased serum Phe in PCOS, suggesting a combined biomarker panel that includes Phe and several FFAs [37]. In a quantitative targeted LCMS study, the sum of the serum concentrations of phenylalanine and tyrosine was significantly higher in PCOS compared to metabolic syndrome [4]. A recent study indicates that elevated levels of Phe may serve as one of the most effective distinguishing parameters in the diagnosis of PCOS [11]. Elevated serum Phe levels are consistently observed in PCOS across metabolomics studies, indicating its potential as a reliable biomarker and a promising therapeutic target [47].

The majority of significantly altered amino acids, except for Phe, including L-methyladenosine, cystine, and the glutamate-glutamine (Glu-Gln) dipeptide, were observed at reduced levels in individuals with PCOS (Table 2; Fig. 2). Among the findings, the reduction in Gln-derivative dipeptide represented one of the most clinically relevant findings, as Gln is the most abundant amino acid in circulation and plays an essential role in various metabolic pathways [48]. A proton nuclear magnetic resonance metabolomics study has confirmed that the levels of serum Gln and Glu are down-regulated [49]. While earlier metabolomics studies predominantly reported reduced Gln levels in serum [49, 50] and follicular fluid [51] of PCOS, recent research has found elevated Gln levels in both sample types [46, 52]. Given that numerous studies have established Gln as a significant indicator of oocyte quality [53] and treatment outcome [54], its lower levels in the follicular environment may potentially exacerbate the underlying factors contributing to PCOS [53].

Bile acids

Alterations in bile acid metabolism were reflected by significantly decreased serum levels of 7-alpha-hydroxy-3-oxo-4-cholestenoic acid and glycocholic acid in patients. Similarly, previous LC-MS studies have demonstrated lower serum glycocholic acid levels in individuals with PCOS [32, 37]. It was suggested that the fat absorption abnormalities observed in women with PCOS may partially result from a decrease in essential bile acids.

Exogeneous metabolites

Increased exogeneous compounds in PCOS serums included di(2-ethylhexyl) phthalate (DEHP), tetrahydromagnolol, 5-Methyl-5-phenylhydantoin, butylparaben, erucamide, and DDAO. The only decreased compound was 2,2'-Methylene-bis (6-tert-butyl-4 methylphenol) (Table 2; Fig. 2). These exogenous metabolites belong to the human exposome and are not endogenously produced [55]. These molecules, mostly environmental pollutants, are expected to have increased in patient serums due to the multifactorial etiology of PCOS, where environmental exposures significantly impact menstrual and reproductive disruptions [56, 57]. The role and implications of these metabolites in PCOS pathophysiology remain unclear, with literature showing varied results [57, 58].

DEHP was the most impactful molecule in differentiating PCOS from controls and is the most commonly used phthalate found in cosmetic, pharmaceutical products, and medical devices [59]. Evidence suggests that increased urinary DEHP levels and phthalate exposure are positively related to the prevalence of PCOS [60]. The presence of phthalates in urine was previously shown in PCOS patients to be as high as 51.7% [61]. However, several studies found that serum levels of DEHP were not different between PCOS patients and controls [58, 62, 63].

Butylparaben, one of the main endocrine disrupting chemicals (EDCs), has potent estrogenic effects [64] and was identified as the highest impact serum metabolite in patients with PCOS (Fig. 2). Although multiple studies have previously shown a positive association between parabens and fertility problems [65–68], the literature lacks direct observations involving PCOS patients. In a limited number of studies, no correlation between parabens and the risk of developing PCOS was shown [69, 70], which contradicts the current findings.

Promethazine, a drug derivative, and valerenic acid, a plant constituent, are also exogenous blood molecules. Their increased levels were attributed to previous consumption and are not considered clinically significant.

While supervised multivariate methods such as OPLS-DA and VIP scoring are often used to enhance group separation, we did not include them in this study. PCA already revealed distinct clustering between PCOS and control groups (Fig. 1), and additional supervised modeling was considered unnecessary for the scope of the present work.

Conclusions

This untargeted UPLC–HRMS metabolomics study revealed pronounced alterations in lipid and amino acid metabolic pathways and serum metabolite profiles in women with PCOS. The most consistent perturbations were observed in sphingolipids, phospholipids, fatty acid derivatives, and amino acids, alongside elevated levels of exogenous compounds, including endocrine disruptors. These findings suggest that PCOS is characterized not only by intrinsic metabolic dysregulation but also by a distinct environmental contaminant signature, underscoring the multifactorial nature of the disorder.

Our results expand on previous metabolomics research by confirming and extending reported disruptions in lipid and amino acid metabolism, thereby contributing new evidence to the literature. At the same time, important knowledge gaps remain. As in other untargeted metabolomics studies, we were unable to directly correlate significant metabolites with routine clinical and hormonal parameters. Moreover, while PCA provided robust evidence of group separation, supervised approaches such as OPLS-DA and VIP scoring were not applied to avoid potential overfitting in this cohort. Finally, validation of candidate pathways and metabolites will require targeted molecular methods, including gene expression profiling and proteomic quantification, in larger, independent populations.

Taken together, our study provides novel biochemical insights into the pathogenesis of PCOS and reinforces the potential of serum metabolomics to support early diagnosis and personalized therapeutic strategies. Future integrative, multi-omics and longitudinal studies will be essential to further delineate the mechanistic links between metabolic disturbances, endocrine dysfunction, and environmental exposures in PCOS.

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Authors' contributions

ÖF Özer: Manuscript writing, statistical analysis, AZ Gül: Manuscript writing, M Demirel: Manuscript writing, data management and analysis, S Ateş: Diagnosis of PCOS, recording examination information, AZ İbrahimoğlu, HS Taha and M İbrahimoğlu: Data collection, Ş Selek: Project's development, manuscript preparation and editing. All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Approval for this study was obtained by the respective Institutional Review Board of the Bezmialem Vakif University (2022/367). The study was performed in accordance with the Declaration of Helsinki.

Competing interests

The authors declare no competing interests.

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