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RESEARCH

Maternal-offspring brain and tissue cross-talk in preeclampsia: insights from a rat model

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Abstract

This study aimed to investigate the differential metabolic profiles across maternal and offspring brains, serum, and placental tissues in preeclampsia (PE), with a particular focus on elucidating the maternal-offspring brain and tissue cross-talk that may contribute to the complex pathophysiology of PE. PE was induced in rats using the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) to simulate both early-onset PE (EOPE) and late-onset PE (LOPE). We utilized non-targeted proton nuclear magnetic resonance (NMR) metabolomics to characterize the metabolic profiles of serum, placental tissue extracts, and brain tissues from both mothers and offspring. Multivariate analysis, Spearman correlation, Density-Based Spatial Clustering of Applications with Noise algorithm, Data-Driven Statistical Predictive Correlation network analysis and Tissue heterogeneity analysis were employed to explore tissue-specific metabolic signatures and their interactions. Following L-NAME induction, both EOPE and LOPE presented significant metabolic differences and shared traits across tissues, with distinct tissue-specific responses characterizing the metabolic profile of PE. Serum from both PE groups showed a decrease in tryptophan, isobutyrate, and lactate, with an increase in betaine. Lactate was upregulated in placental tissues, highlighting its metabolic role. Extensive intra-tissue metabolic correlations and inter-tissue metabolite exchanges were detected among the maternal brain, serum, placenta, and offspring brain across all three experimental groups. EOPE and LOPE exhibited distinctly different metabolic characteristics and trajectories of differential metabolites, along with diverse interaction patterns between the maternal/offspring brain and the placenta. This study uncovers the multi-tissue metabolic remodeling in response to preeclampsia, implying that addressing pathophysiological stress is crucial and may have potential implications for neurological outcomes. The comprehensive analysis highlights the pivotal role of the brain-placenta axis in preeclampsia, advocating for a classified diagnostic and management approach.

Keywords Preeclampsia · Metabolome · Brain-placenta axis · Metabolic reprogramming · Neurotransmitter

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Introduction

Preeclampsia (PE), a significant multisystemic disorder affecting 2–8% of pregnancies, stands as a predominant contributor to maternal and perinatal morbidity and mortality rates worldwide (Creswell et al. 2023). This condition is distinguished by extensive maternal endothelial dysfunction, accompanied by a spectrum of neurological manifestations including persistent headache, cortical blindness, and seizures in the form of eclampsia (Zhu and Huang 2023). The hypertension and endothelial disturbances associated with PE not only compromise maternal health but also have the potential to impact offspring neurodevelopment through placental transfer, thereby elevating the risk of neurodevelopmental disorders in offspring (Kong et al. 2022). Epidemiological data consistently demonstrate that infants born to mothers with PE are at an increased risk for respiratory distress syndrome, hypertension, cerebrovascular accidents, epilepsy, and autism in later life (Liu et al. 2016, 2023). Despite advancements in our understanding of PE's etiology, insights into its metabolic ramifications and molecular interplay within the maternal brain and the offspring's nervous system remain scarce. There is a compelling need to delineate these aspects through animal models to deepen our comprehension of PE's impact on cerebral function.

The nitric oxide synthase inhibitor, N-nitro-l-arginine methyl ester (L-NAME), has been extensively employed to elicit a PE-like syndrome in rats, exhibiting symptoms analogous to human PE, such as hypertension, proteinuria, renal impairment, and an anti-angiogenic state (Ramesar et al. 2011; Bakrania et al. 2022). The temporal administration of L-NAME can effectively simulate the early-onset PE (EOPE) and late-onset PE (LOPE) phenotypes observed clinically (Kasture et al. 2019). Studies have meticulously documented the neuroinflammatory responses, shifts in blood-brain barrier permeability, hippocampal structural changes, and cognitive deficits that arise from L-NAME induced PE (ShamsEldeen et al. 2021; Zheng et al. 2021; Liu et al. 2023). In parallel, an escalating area of inquiry is probing the enduring neurodevelopmental and functional effects on offspring, enriching our understanding of the intergenerational impact of PE. However, to date, no studies have concurrently investigated the metabolic implications of L-NAME induced PE in both maternal and offspring brains, and their correlation with serum and placental metabolic profiles.

In this study, rat models of EOPE and LOPE were established using L-NAME. Non-targeted proton nuclear magnetic resonance (NMR) metabolomics was employed to analyze metabolic profiles across the brains, serum, and placentas of pregnant rats, as well as the brains of their offspring. The investigation meticulously elucidated the intricate interaction networks and metabolic pathways of differentially expressed metabolites across the examined tissues, culminating in the discovery of novel biomarkers and the characterization of their interrelationships.

Methods

Animals

The animal experiments adhered to the protocols outlined in the NIH Guide for the Care and Use of Laboratory Animals. A total of 36 female Sprague-Dawley rats, weighing 250 to 300 g, were individually housed under controlled conditions at a temperature of 25 °C and subjected to a 12-hour light/dark cycle with ad libitum access to food and water. Following acclimatization, the female rats were co-housed with male rats in a 1:1 ratio, and the presence of a vaginal plug was designated as gestational day (GD) 0. All the rats were kept alone during pregnancy.

Model of experimental PE

The induction of PE was achieved by administering L-NAME at a dose of 50 mg/kg/day. On GD 7, pregnant rats were randomly assigned to three groups: the control group ($n=12$), EOPE ($n=12$), and LOPE ($n=12$). The procedures for each group were as follows: (i) The control group received daily subcutaneous injections of an equal volume of 0.9% normal saline from GD 7 to GD 19; (ii) The EOPE group was subcutaneously injected with L-NAME from GD 7 to GD 19; (iii) The LOPE group received an equal volume of 0.9% normal saline from GD 7 to GD 13 and then was subcutaneously injected with L-NAME starting from GD 14 to GD 19 (Kasture et al. 2019).

Model verification

On GD 6, 11, and 18 of pregnancy, blood pressure, body weights, and 24-hour urine total protein were measured. On GD 19, blood flow within the left uterine artery was assessed using a MyLab™ 9VET ultrasound system (Esaote, Italy) equipped with a multifrequency linear transducer (10–22 MHz). Peak systolic velocity (PSV), end diastolic velocity (EDV), uterine artery resistance index (RI), and pulsatility index (PI) were measured over three cardiac cycles and then averaged.

On GD 19, following anesthesia induction with 3% pentobarbital sodium (50 mg/kg) via intraperitoneal injection, blood samples were collected via abdominal aorta puncture. The rats were humanely euthanized post-sampling. Blood was centrifuged at 2000 rpm for 10 min to separate serum,

which was then assayed for serological indicators: soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF) and pregnancy-associated plasma protein A (PAPP-A). These were measured using respective rat ELISA kits (Jianglai Biological, China, catalog numbers JL48077, JL11559, and JL10729) according to the manufacturer's protocols.

Tissue samples collection and preparation

Concurrently, the maternal brain and placental tissues were carefully extracted post-decapitation and processed for further analysis. Each fetus was weighed, anesthetized with the same regimen, and humanely euthanized. Offspring brains were harvested by decapitation, with every set of five pooled to form a composite sample. The harvested tissues were rinsed in cold saline to eliminate excess fluids and blood, then flash-frozen in liquid nitrogen. All samples were archived at −80 °C for future analyses.

NMR spectra acquisition

To prepare serum samples, 500 µL of rat serum was centrifuged at 13,000 rpm for 2 min. The supernatant was carefully collected. For tissue homogenization, frozen and weighed brains (both offspring and maternal) and placentae were pulverized and suspended in 1000 µL of purified water. The suspension was sonicated using an in-solution ultrasonic processor (Sonics VX-130, USA) with an eight-cycle sonification program of 4 s on and 3 s off. Following sonication, the mixture was centrifuged at 13,000 rpm for 15 min to pellet the debris. The supernatant was then transferred to a 0.5 mL ultrafiltration device with a 3 kDa molecular weight cutoff (Millipore, USA) and centrifuged at 13,000 rpm for 45 min to concentrate the metabolites. A total of 450 µL of the filtrate was collected for each sample. Prior to NMR analysis, the sample was mixed with a DSS standard solution (50 µL, Anachrom Canada) to ensure spectral alignment and quantification.

The acquisition of NMR spectra was performed using a Bruker AV III 600 MHz spectrometer. Serum samples were analyzed using the Carr-Purcell-Meiboom-Gill (CPMG) with a free relaxation duration of 100 ms and an echo time of 250 µs. For tissue samples, 1D Nuclear Overhauser Effect Spectroscopy (1D NOESY) was used with a water suppression of 100 ms mixing time along with a 990 ms presaturation (~80 Hz γB1). For data acquisition, a total of 128 scans were performed with 64 K data points under a line-broadening function of 0.3 Hz for each free induction decay (FID). All acquired NMR spectra were manually phase- and baseline-corrected and then referenced to the doublet at 1.32 ppm.

Metabolomics data processing

The collected FID signal was automatically zero-filled and Fourier transformed using the processing module in Chenomx NMR Suite 8.1 software (Chenomx Inc., Canada). Subsequently, experienced technicians using the Chenomx Processor carefully performed phase and baseline correction. All spectra were referenced to the internal DSS standard at 0.0 ppm and analyzed by experienced analysts against the Chenomx Compound Library.

Next, the quantified NMR data were imported into the MetaboAnalyst 6.0 online platform (www.metaboanalyst.ca) for multivariate data analysis (Pang et al. 2024). Log transformation was applied to normalize all metabolites, followed by scaling using the Pareto scaling method. Partial least squares-discrimination analysis (PLS-DA) models were built to provide an overview of data distributions (Mora-Ortiz et al. 2019). Model validation was performed with a 5-fold stratified cross-validation approach and permutation testing (Herbert et al. 2023). Methods based on fold-change (FC) were also utilized to evaluate log ratio concentrations among groups and identify significant metabolite variations above a cut-off value. Metabolites with variable importance in the projection (VIP) values > 1.0 and adjusted *P*-values < 0.05 were selected for further investigation of their quantitative changes across all three studied groups (Van De Wetering et al. 2023). Metabolic pathways were calculated based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9. For all multiple comparison analyses, the Kruskal-Wallis test was employed to assess differences among groups. Corrections for multiple comparisons were made using the Dunn method for the Kruskal-Wallis test. A *P*-value < 0.05 was set as the threshold for statistical significance. The clustering analysis employed the Density- Based Spatial Clustering of Applications with Noise (DBSCAN) algorithm. For the correlation distance analysis among tissues, the Euclidean distance and Manhattan distance algorithms were respectively utilized. Additionally, Spearman correlation and the Debiased Sparse Partial Correlation (DSPC) network were employed to investigate the relationships between metabolites across different tissues (Mohammadi Ballakuti and Ghanati 2023). A correlation is considered strong when Spearman's correlation coefficient $|r| > 0.7$ and the *P*-value < 0.05.

Results

Establishment of the PE-like rat model

The pre-clinical rat model of PE, induced by L-NAME administration on gestational day, triggers a series of maternal and offspring physiological responses (Supplementary Fig.S1 and Fig.S2). Before induction, no significant differences in blood pressure or proteinuria were noted among groups. After L-NAME treatment from GD7 to GD19, the EOPE group showed significant increases in blood pressure, urine protein excretion, and embryonic mortality, along with elevated RI, PI, and sFlt-1 levels ($P<0.05$), and decreases in PSV, EDV, PAPP-A, and PLGF levels ($P<0.05$). The LOPE group, treated from GD14 to GD19, exhibited similar blood pressure and EDV changes with statistically significant differences from the control group ($P<0.05$).

NMR spectra of multiple samples

Utilizing NMR-based metabolomics, we systematically characterized the metabolomic features of brain, serum, and placental tissues, as depicted in Fig. 1A and B, and Supplementary Tab.S1. We comprehensively identified a total of 68 metabolites in samples from various tissue types. Specifically, 41 metabolites were identified in the brain tissue of maternal rats, 49 in the serum, 38 in the placental tissue, and 43 in the brain tissue of offspring rats. Notably, 27 metabolites were detected across all tissue types. Moreover, our study revealed the predominance of amino acid, carbohydrate, and branched-chain amino acid metabolic pathways. Serum demonstrated remarkable metabolic diversity, including 18 unique metabolites pivotal to the urea cycle, creatine metabolism, ammonia metabolism, and glycolipid pathways. Both maternal and offspring brain tissues were replete with neurotransmitter-associated compounds like 4-Aminobutyrate, alongside their precursors Choline and

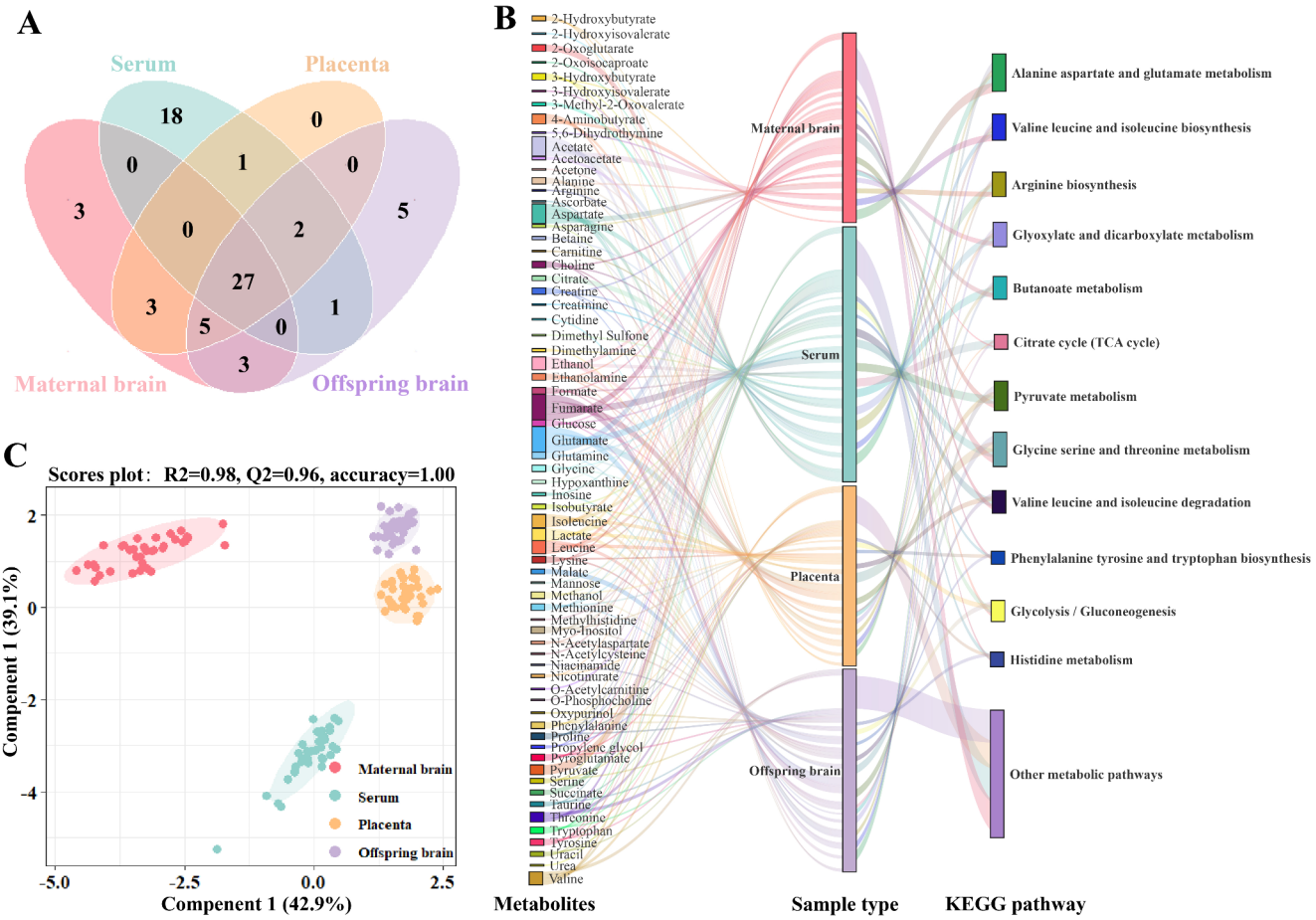


Fig. 1 Metabolic Landscape and Discriminative Analysis in Maternal and Offspring Tissues in PE. (**A**) A Venn diagram illustrated the overlap and specificity of metabolic alterations across maternal brain, serum, placenta, and offspring brain, revealing distinct and shared metabolic features. (**B**) The Sankey diagram portrayed the distribution of metabolites among the sample types and their flux through the KEGG metabolic pathways, indicating the metabolic interconnectivity and pathway involvement. (C) PLS-DA score plots delineated the metabolic separation between maternal brain, serum, placenta, and offspring brain groups, underscoring the discriminatory power of the model. PE preeclampsia, KEGG Kyoto Encyclopedia of Genes and Genomes, PLS-DA partial least squares-discrimination analysis

Ethanolamine, which are integral to neurotransmitter synthesis. Notably, maternal brain tissue specifically harbored N-acetylcysteine and O-phosphocholine, implicated in neural signal transduction and repair, whereas offspring brain tissue contained Ascorbate and Niacinamide, key players in oxidative stress regulation. The placenta was distinguished by an enrichment of amino acids, Taurine, Hypoxanthine, and Nicotinurate, which were extensively involved in placental cell proliferation, differentiation, energy production, and neural conduction. The metabolic distinctiveness of these tissues was corroborated by the PLS-DA model, which also highlighted a notable similarity between placental and offspring brain tissue metabolic profiles, underpinned by robust statistical measures with an R^2 value of 0.98 and a Q^2 value of 0.96, as depicted in Fig. 1C.

Metabolic profile changes in maternal brain tissue of preeclamptic rats

We employed the PLS-DA model to evaluate the differentiation effect of the maternal brain metabolic profile across different groups following L-NAME induction. As depicted in Fig. 2A, despite some overlap between groups, the metabolic profiles effectively distinguished between them, with a cross-validation Q2 of 0.74, an R2 of 0.90, and an accuracy of 89%; permutation testing yielded a P-value < 0.001. The differential metabolites for EOPE and LOPE, characterized by VIP values > 1.0 and adjusted P-values < 0.05, were presented in Fig. 2B C, and Supplementary Tab.S2. Among these, three metabolites—Choline, Glutamine, and Tyrosine—exhibited similar expression trends across both groups. However, EOPE and LOPE also displayed distinct expression patterns. For instance, Ethanol and Lactate were highly expressed in the EOPE group, while the essential amino acids Leucine and Phenylalanine were significantly reduced. In the LOPE group, Threonine, Glutamate, and Myo-Inositol were found to be differentially expressed. Furthermore, there were two metabolites, Ethanol and Myo-Inositol, that exhibited differential expression between the EOPE and LOPE groups. The differential metabolites in both the EOPE and LOPE groups were associated with several enriched pathways, including Phenylalanine, Tyrosine, and Tryptophan Biosynthesis, Phenylalanine Metabolism, Nitrogen Metabolism, and Valine, Leucine, and Isoleucine

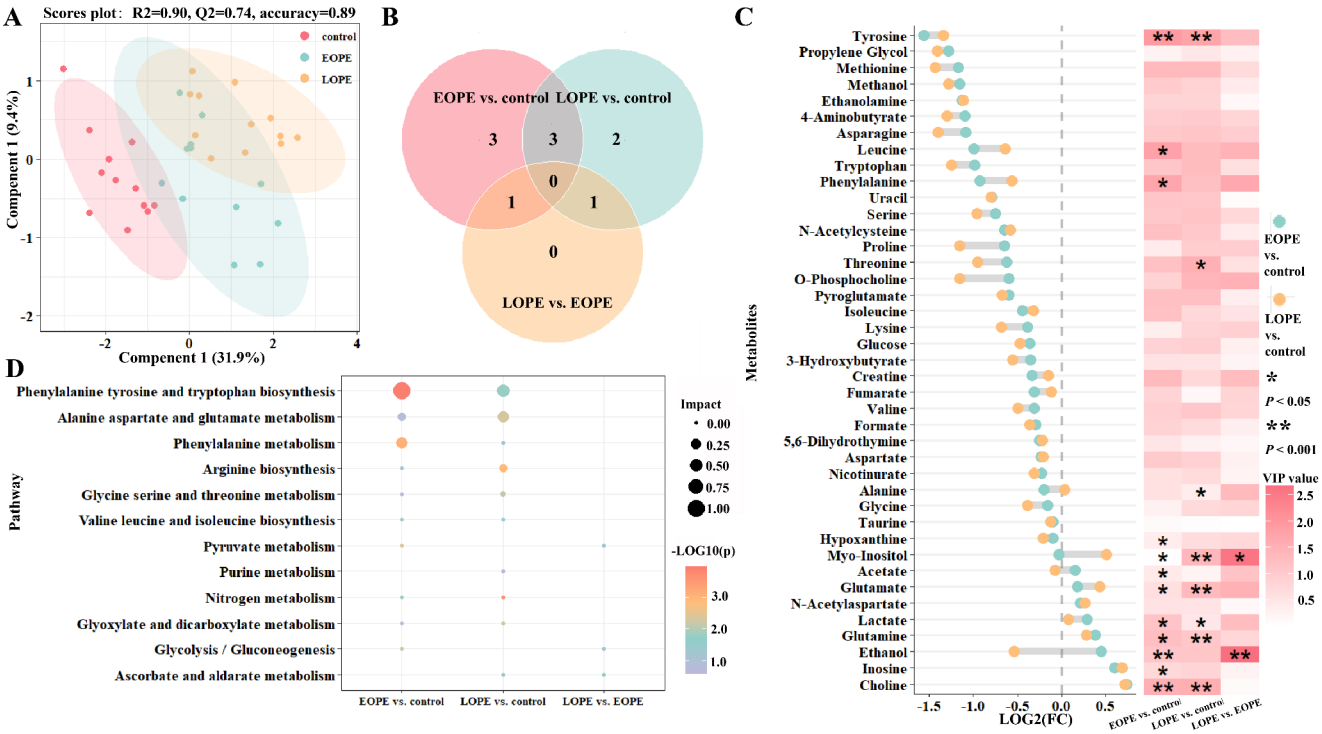


Fig. 2 Comparative maternal brain metabolic profiles induced by L-NAME in EOPE and LOPE rat models. (**A**) PLS-DA score plots revealed distinct metabolic profiles between the control, EOPE, and LOPE groups, with a focus on maternal brain tissue. (**B**) The Venn diagram vividly displays the shared and distinct significant metabolic shifts within the maternal brain across different PE groups. These significant metabolites are identified based on VIP > 1.0 and adjusted P < 0.05. (**C**) Fold-change differences, VIP values, and inter-group significance of metabolic products in the maternal brain tissues of three groups. (**D**) KEGG pathway enrichment analysis illustrated the enriched pathways in maternal brain tissue, with bubble size and color intensity representing the extent of impact and level of significance, respectively. L-NAME N-nitro-L-arginine methyl ester, EOPE early-onset preeclampsia, LOPE late-onset preeclampsia, PLS-DA partial least squares-discrimination analysis, VIP variable importance in the projection, KEGG Kyoto Encyclopedia of Genes and Genomes, FC fold-change

Biosynthesis. Additionally, the differential metabolites in the EOPE group were involved in Glycolysis/Gluconeogenesis and Pyruvate Metabolism (Fig. 2D). The metabolites unique to the LOPE group were implicated in Arginine Biosynthesis, Alanine, Aspartate, and Glutamate Metabolism, Glyoxylate and Dicotboxylate Metabolism, and Glycine, Serine, and Threonine Metabolism (Fig. 2D).

Serum metabolic profile variations in PE rats

Serum, the primary medium for inter-tissue communication, was analyzed to identify metabolic differences at various stages in response to L-NAME, with the aim of determining whether these changes mirrored those observed in the maternal brain. PE-induced serum metabolites exhibited significant group discrimination in the PLS-DA model, with excellent cross-validation accuracy (1.00), validated by permutation testing for statistical significance ($P<0.001$), as shown in Fig. 3A. Figure 3B and C, and Supplementary Tab.S3 detailed the differential serum metabolites, where the EOPE and LOPE groups shared four common ones: Tryptophan, Isobutyrate, and Lactate, which were down-regulated, while Betaine was up-regulated. Reflecting the brain's metabolic profile, distinct differential metabolite expression patterns were observed, including the significant down-regulation of the essential amino acid Lysine in the EOPE group and the up-regulation of Tyrosine. In the LOPE group, non-essential amino acids Methionine and Aspartate were notably down-regulated. KEGG pathway enrichment analysis revealed shared metabolic pathways between the EOPE and LOPE groups, encompassing Phenylalanine metabolism, Tyrosine and Tryptophan biosynthesis, Butanoate metabolism, Glycolysis/Gluconeogenesis, and Nitrogen metabolism. In the LOPE group, Pyruvate metabolism, Cysteine and Methionine metabolism, and Alanine, Aspartate, and Glutamate pathways were significantly down-regulated, indicating the TCA cycle was also affected (Fig. 3D). Interestingly, except for Phenylalanine, which showed consistent expression, brain and serum differential metabolites in PE rats often exhibited opposite regulation trends. For example, Glutamine, Lactate, and Tyrosine exhibited contrasting expression patterns between the brain and serum in the EOPE group. In the LOPE group, Glutamate and Choline were up-regulated in the maternal brain but were significantly down-regulated in the serum.

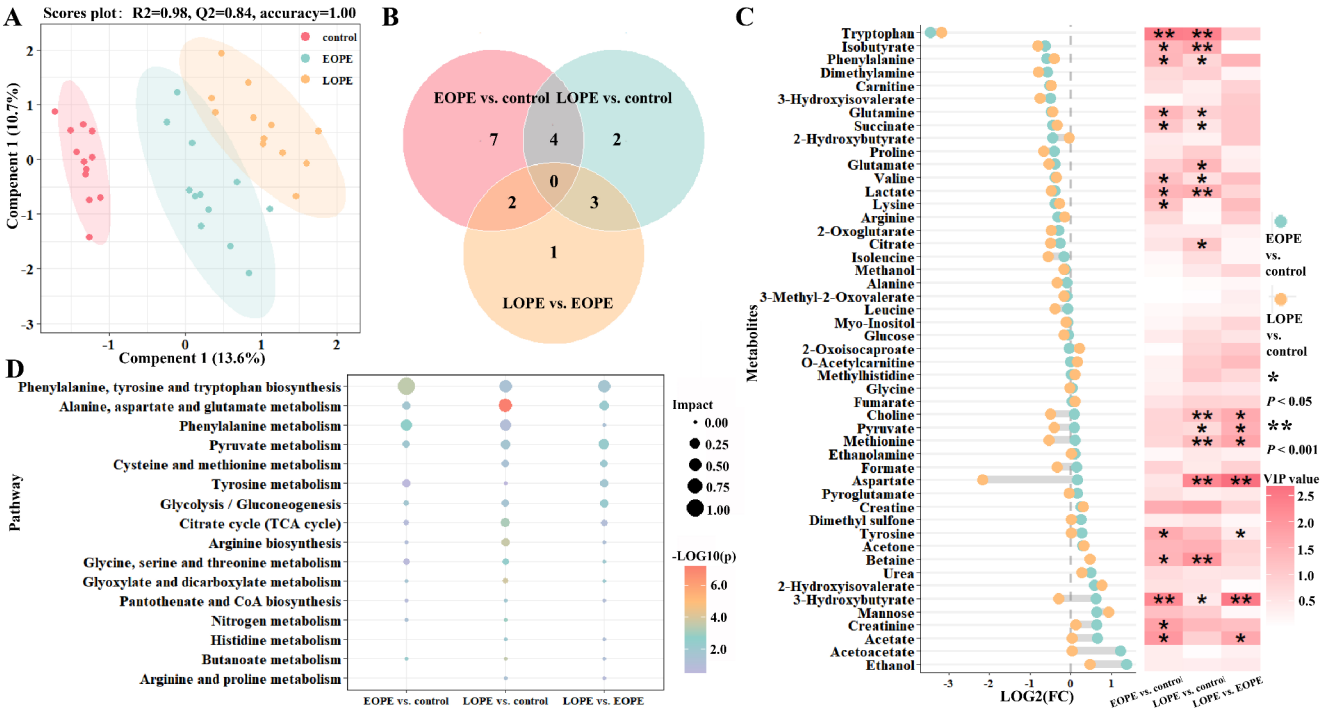


Fig. 3 Comparative serum metabolic profiles induced by L-NAME in EOPE and LOPE rat models. (**A**) PLS-DA score plots distinguished the control, EOPE, and LOPE groups based on serum metabolites. (**B**) The Venn diagram clearly demonstrates the common and distinct significant metabolic alterations in the serum across diverse PE groups. The significant metabolites here are identified based on VIP>1.0 and an adjusted $P<0.05$. (**C**) The fold-change differences, VIP values, and inter-group significance of the metabolic products in the serum of three groups are presented. (**D**) KEGG pathway analysis displayed affected pathways, with bubble size and color indicating impact and significance. L-NAME N-nitro-L-arginine methyl ester, EOPE early-onset preeclampsia, LOPE late-onset preeclampsia, PLS-DA partial least squares-discrimination analysis, VIP variable importance in the projection, KEGG Kyoto Encyclopedia of Genes and Genomes, FC fold-change

Changes of placental metabolic profile in PE rats

PE, a placental-origin disease, may involve placental metabolic disorders that play a significant role in its onset and in maternal-fetal neurological complications (Hu et al. 2024). Our analysis of the placental metabolic profiles in PE rats revealed distinct patterns. The PLS-DA scores plot showed significant overlap between the EOPE group and the control group, while the LOPE group was markedly separated from the others, with cross-validation for a one-component model yielding an accuracy of 0.81 and permutation testing indicating significance ($P=0.007$) (Fig. 4A). Similarly, the discrepancies in differential metabolites between the EOPE and LOPE groups were more pronounced in the placenta, with Lactate being the only metabolite significantly upregulated in both groups, aligning with its expression trend in maternal brain tissue (Fig. 4B&C; Supplementary Tab.S4). The unique differential metabolites in the EOPE group—Ethanol, Creatine, and Lysine—are primarily involved in energy metabolism and essential amino acid metabolism. In contrast, the unique differential metabolites in the LOPE group—Phenylalanine, Glycine, Isoleucine, and Tyrosine—are mainly engaged in the metabolism of both essential and non-essential amino acids. Disruptions in energy metabolism, specifically Pyruvate metabolism and Glycolysis/Gluconeogenesis, are common metabolic pathway characteristics of both EOPE and LOPE placentae (Fig. 4D). Additionally, there are divergences in the metabolic pathways of amino acids and non-essential amino acids between the two groups.

Changes in the offspring brain metabolic profile in PE

The offspring brain metabolism is a significant focus of our study. On the PLS-DA plot, we can see a clear distinction between different groups, but the model's performance in cross-validation (Q2 = 0.43, R2 = 0.79, accuracy = 0.59) and permutation testing with a *P*-value of 0.01 is not ideal, indicating a certain degree of overfitting (Fig. 5A). Although the offspring brain PLS-DA model cannot be used to screen for molecular markers related to offspring brain damage, it can still be used to assess whether metabolites remain significant in multivariate analysis. We observed that, compared to the control group, only Isoleucine was significantly downregulated in the EOPE group. The metabolic differences

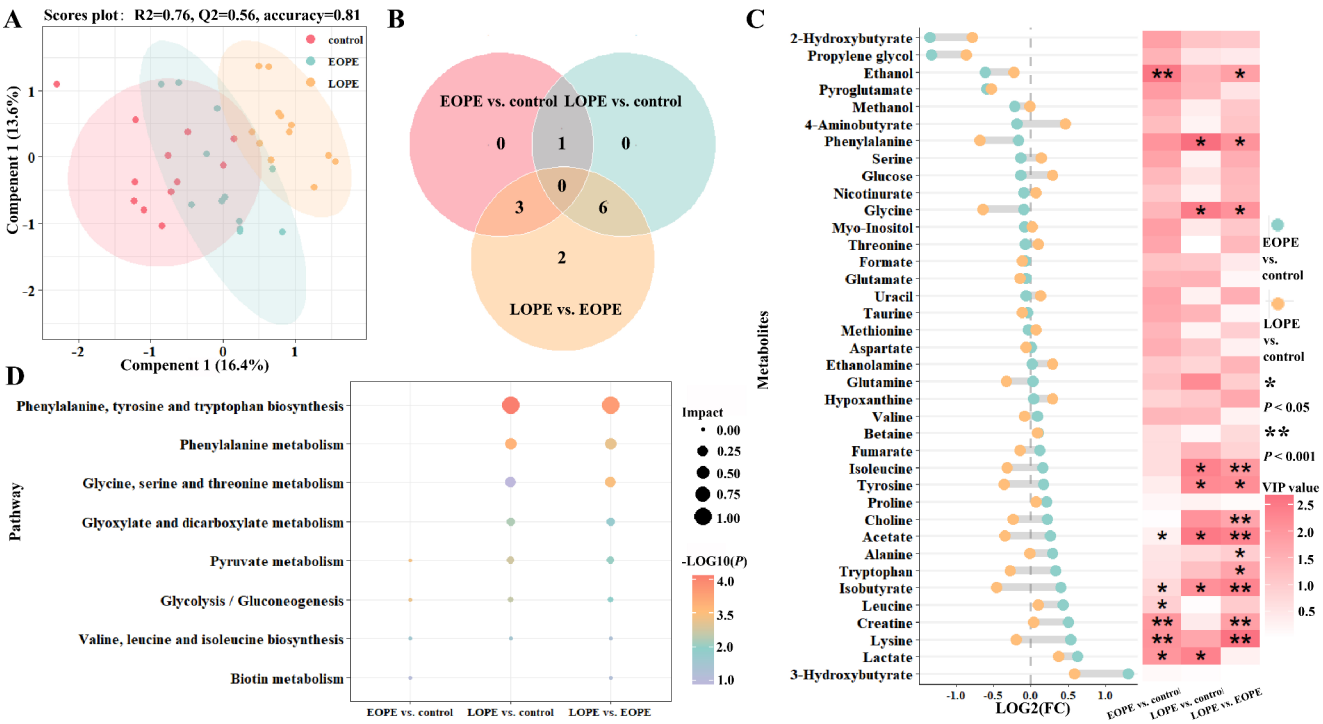


Fig. 4 Placental metabolic profiles in response to L-NAME-induced PE in rats. (**A**) OPLS-DA score plots revealed distinctions in placental metabolites among control, EOPE, and LOPE groups. (**B**) The Venn diagram effectively exhibits the shared and unique significant metabolic changes within the placenta among various PE groups. Metabolites with VIP > 1.0 and adjusted $P<0.05$ were considered significant. (**C**) Presents fold-change differences, VIP values, and inter-group significance of placental metabolic products in three rat groups. (**D**) KEGG pathway enrichment analysis identified affected pathways, using bubble size and color to denote pathway impact and significance. L-NAME N-nitro-L-arginine methyl ester, EOPE early-onset preeclampsia, LOPE late-onset preeclampsia, PLS-DA partial least squares-discrimination analysis, VIP variable importance in the projection, KEGG Kyoto Encyclopedia of Genes and Genomes, FC fold-change

between the LOPE group and the EOPE group did not overlap; 3-Hydroxybutyrate, Choline, Glutamine, Glycine, and Lysine were the differential metabolites in the LOPE group. Upon comparing the LOPE and EOPE groups, we found that the metabolic differences were primarily concentrated in the changes of essential amino acids (Isoleucine, Phenylalanine, Valine) and non-essential amino acids (Glutamine, Proline), as well as the corresponding metabolic pathway alterations (Fig. 5B, C&D; Supplementary Tab.S5).

Cross-talk of metabolic correlations within and between maternal and offspring tissues in EOPE and LOPE rats

To explore metabolite interactions within and between tissues in PE rats, we conducted Spearman correlation analysis on the control, EOPE, and LOPE groups (Fig. 6A). The three groups showed distinct intra-and inter-group metabolic correlation characteristics. In the EOPE group, there were 741 significantly correlated metabolite pairs (295 intra-tissue and 446 inter-tissue), more than in the control group, indicating extensive metabolic exchanges and reprogramming. Conversely, in the LOPE group, the numbers dropped to 169 and 176, respectively.

Intra-tissue correlation analysis

We used the DBSCAN algorithm for intra-tissue correlation analysis. Under normal pregnancy, maternal brain metabolic correlations mainly involved energy metabolism (Cluster 2: Lactate, Creatine) and amino acid synthesis (Lysine, Glycine), while the offspring brain focused on amino acid synthesis regulation (Cluster 11: Asparagine, Serine, Threonine, Tryptophan). In EOPE, maternal brain Cluster 3's up-regulated Glutamate was significantly correlated with 5,6-Dihydrothymine, hinting at potential DNA damage. In the offspring brain, Cluster 11's Ethanolamine-Tryptophan correlation increased, both related to neural development. In LOPE, the maternal brain clustered into energy metabolism related clusters (Cluster 1: Lactate, Creatine; Cluster 5: Lysine, Taurine, Glycine) and an oxidative stress/DNA damage related cluster (Cluster 6: 5,6-Dihydrothymine, Nicotinurate).

Inter-tissue metabolite interactions

Compared to intra-tissue interactions, all three groups had extensive inter-tissue metabolite exchanges between the maternal brain and serum, placenta, and offspring brain.

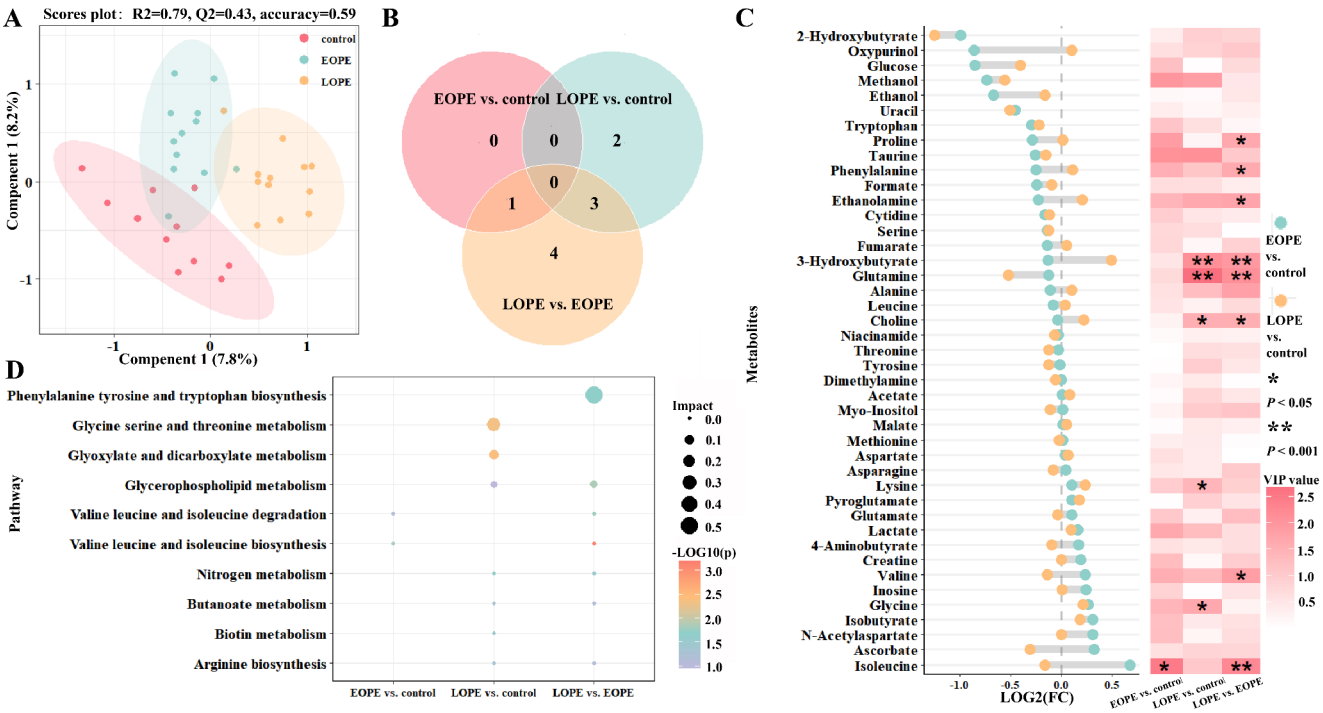


Fig. 5 Metabolic alterations induced by L-NAME in the offspring brain of EOPE and LOPE rat models. (**A**) OPLS-DA score plots differentiated between the control, EOPE, and LOPE groups. (**B**) Venn diagram showcases common and specific significant brain metabolic shifts in offspring rats across PE groups. Metabolites discerned by VIP > 1.0 and adjusted $P < 0.05$. (**C**) Compares fold-change of metabolite contents in 3 groups of offspring rat brain tissues and shows statistical values. (**D**) KEGG pathway enrichment analysis indicated the affected pathways, with bubble size and color reflecting the impact and significance of the pathways, respectively. L-NAME N-nitro-L-arginine methyl ester, EOPE early-onset preeclampsia, LOPE late-onset preeclampsia, PLS-DA partial least squares-discrimination analysis, VIP variable importance in the projection, KEGG Kyoto Encyclopedia of Genes and Genomes, FC fold-change

In the control group, we identified 4 inter-tissue interaction clusters. Maternal brain metabolites (Valine, Ethanol, Tyrosine, Nicotinurate) were significantly correlated with others. In dominant Cluster 1, maternal Valine and Ethanol interacted with 7 serum, 2 placental, and 14 offspring brain metabolites. In Cluster 3, Tyrosine interacted with offspring brain Glutamate and Inositol, and in Cluster 5, Nicotinurate correlated with placental Ethanolamine.

In EOPE, 7 maternal brain metabolites (Valine, Alanine, Nicotinurate) changed inter-tissue interactions, interacting with 6 serum, 13 placental, and 8 offspring brain metabolites. In dominant Cluster 2, the placental metabolite proportion (41.38%) was significantly higher than in normal-group Cluster 1 (8.00%, Chi-square: 9.978, $P<0.05$). Cluster 5 suggested a close maternal brain Methanol-N-Acetylcysteine-offspring brain Choline relationship. The EOPE group's dominant Cluster 8 (3 serum and 8 offspring brain metabolites) may imply that the offspring brain seeks external help via serum during placental dysfunction.

In the LOPE group, maternal brain metabolites Glutamine, Ethanol, Choline, and Glutamate correlated with others in three clusters. In dominant Cluster 3, Ethanol and Choline correlated with 22 offspring brain, 6 placental, and 3 serum metabolites, similar to the control-group Cluster 1's offspring-derived components. Notably, when comparing the LOPE group with the EOPE and control groups, we observed that in the inter-tissue interaction clusters of LOPE, placental components seemed to be isolated. Specifically, 17 placental metabolites independently formed an intra-tissue Cluster 15, which further suggested the isolated state of placental metabolism in the LOPE condition.

DSPC network analysis

We used DSPC network analysis to show metabolite connection patterns and intensities across tissues (Fig. 6B). In the normal group, intra-and inter-tissue interactions had clear boundaries. In Cluster 1, offspring brain Lysine-Methionine, serum Betaine, and placental Choline were crucial for cross-tissue metabolism. The network also showed maternal brain Ethanol and Valine correlations with placental metabolites in normal-group Cluster 1. In EOPE, inter-tissue metabolite interactions were more complex. Maternal brain metabolites were affected by multiple tissues; e.g., Cluster 2's Nicotiniurate was influenced by offspring brain 4-Aminobutyrate, N-Acetylaspartate, and placental Glutamate. Alanine was affected by serum Lactate, and Aspartate was affected by placental Glutamine. In LOPE, inter-group interaction nodes and edges decreased, and placental metabolite numbers were lower, supporting reduced placental metabolism influence.

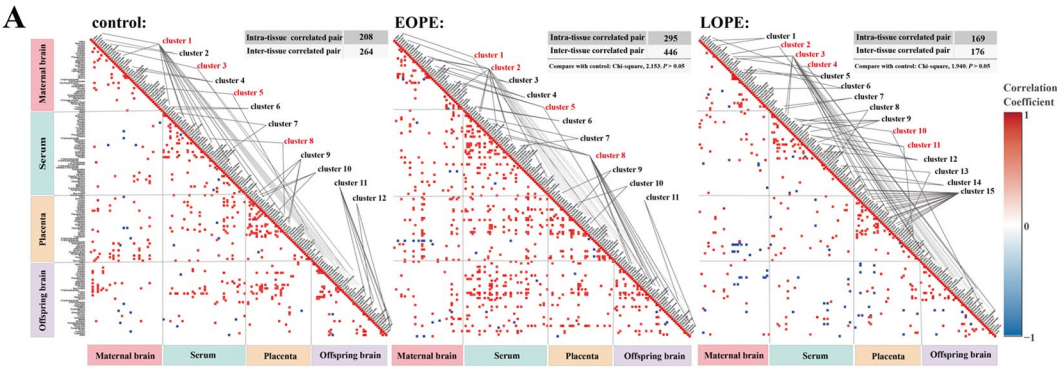
Verification of placental involvement in cross-talk

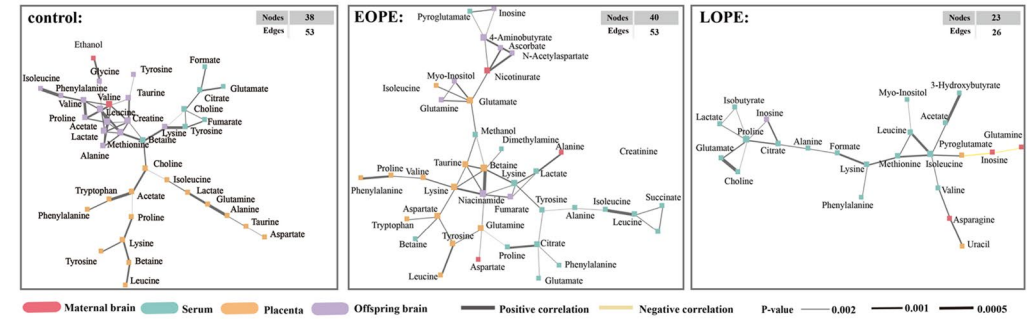
To verify placental involvement in maternal-offspring brain cross-talk and its isolation in LOPE, we calculated Euclidean and Manhattan distances between tissues (Supplementary Fig.S3). During normal pregnancy, the maternal brain and placenta had similar metabolic patterns (Euclidean: 9.51, Manhattan: 52.73), and the offspring brain was also close (Euclidean: 10.78, Manhattan: 70.36). In EOPE, placental metabolic changes affected the maternal (Euclidean: 9.99, Manhattan: 58.68) and offspring (Euclidean: 11.97, Manhattan: 66.51) brains. In LOPE, the placenta's role weakened; the maternal and offspring brains were more similar to serum in Euclidean distance, and most similar to each other in Manhattan distance (61.37).

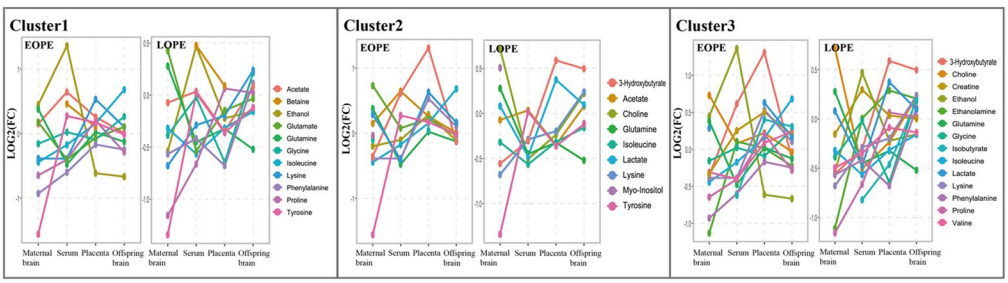
Exchange and trajectory changes of differential metabolites and their KEGG pathway associations in maternal-offspring tissues of EOPE and LOPE rats

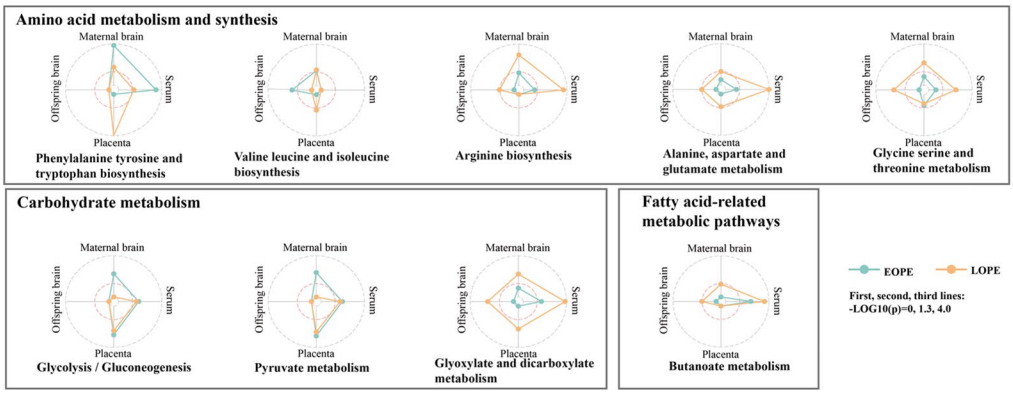
We identified the key metabolites that can distinguish the PE state from the normal state, as well as the EOPE and LOPE states, through the trajectory analysis of dominant clusters and differential metabolites enriched in KEGG pathways. First, we focused on the differential metabolites in the dominant clusters of the three groups in inter-tissue interactions (Cluster 1 in the normal group, Cluster 2 in the EOPE group, and Cluster 3 in the LOPE group) to observe the metabolic trajectory changes between different tissues in rats under different states and the co-regulated metabolites under different states (Fig. 6C). We identified a series of differential metabolites with correlated metabolic trajectories that exhibited similar patterns in both the LOPE and EOPE groups (correlation coefficient >0.07 and $P<0.05$). For example, in Cluster 1, Glutamine-Tyrosine-Glutamate and Phenylalanine-Lysine-Proline- Isoleucine-Ethanol showed such correlations; in Cluster 2, it was Lysine-3-Hydroxybutyrate and Glutamine-Choline-Tyrosine; and in Cluster 3, Choline-Ethanolamine-Glutamine-Creatine-3- Hydroxybutyrate-Proline-Lysine-Valine. Monitoring the correlations of the metabolic trajectories of these metabolites in different tissues may provide a method for detecting the onset of PE.

Secondly, the metabolic pathways of differentially-expressed metabolites in the EOPE and LOPE groups mainly included amino acid metabolism and synthesis, carbohydrate metabolism, and Fatty acid-related metabolic pathways (Fig. 6D). In terms of amino acid metabolism and synthesis, the EOPE and LOPE groups exhibited comparable distribution profiles across different tissues in several metabolic pathways, including arginine synthesis, alanine, aspartate, and glutamate metabolism, as well as glycine, serine, and threonine metabolism. However, they had different


A
control:
EOPE:
LOPE:
Maternal brain
Serum
Placenta
Offspring brain
Correlation Coefficient


B
control:
EOPE:
LOPE:
Positive correlation
Negative correlation
P-value


C
Cluster1
Cluster2
Cluster3
EOPE
LOPE
LOG2(FC)


D
Amino acid metabolism and synthesis
Phenylalanine tyrosine and tryptophan biosynthesis
Valine leucine and isoleucine biosynthesis
Arginine biosynthesis
Alanine, aspartate and glutamate metabolism
Glycine serine and threonine metabolism
Carbohydrate metabolism
Glycolysis / Gluconeogenesis
Pyruvate metabolism
Glyoxylate and dicarboxylate metabolism
Fatty acid-related metabolic pathways
Butanoate metabolism
EOPE
LOPE
First, second, third lines: -LOG10(p)=0, 1.3, 4.0

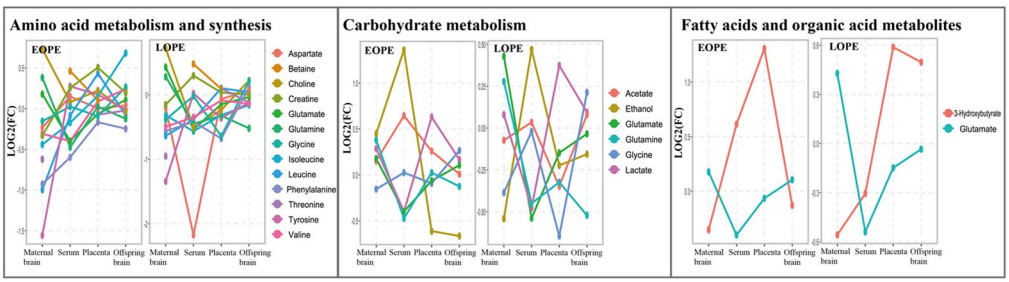

E
Amino acid metabolism and synthesis
Carbohydrate metabolism
Fatty acids and organic acid metabolites
EOPE
LOPE
LOG2(FC)

Fig. 6 Comprehensive analysis of metabolite correlations and metabolic pathway interactions among different tissue groups. (**A**) Spearman correlation distribution and correlation clustering of all metabolites among and within three groups of tissues. The correlation heatmap depicts strongly correlated metabolites among and within different tissues, with an absolute value of the correlation coefficient > 0.07 and $p < 0.05$. Red squares indicate positive correlations between metabolites, while blue squares represent negative correlations. Lines represent the DBSCAN clustering of metabolites. Cluster names in red denote that the metabolites in the cluster are from different tissues, and those in black indicate that the metabolites are from the same tissue. The table in the upper-right corner shows the number of strongly correlated metabolite pairs among tissues within different groups, the number of strongly correlated metabolite pairs within tissues, and the corresponding statistical values. (**B**) DSPC network analysis of different tissues in the EOPE and LOPE groups, which reveals the interactions of metabolites within and between tissue types. (**C**) Metabolic trajectory changes of the dominant metabolite clusters in the EOPE and LOPE groups. (**D**) The radar chart shows the tissue-specific enrichment KEGG trajectory changes of differential metabolites between the EOPE and LOPE groups. (**E**) Metabolic trajectory changes of three types of significantly enriched differential metabolites in the EOPE and LOPE groups. The y axis indicates the $\log_2$ fold - change (FC) of the levels of each differential metabolite in the EOPE or LOPE group compared to the control groups. DBSCAN Density-Based Spatial Clustering of Applications with Noise, DSPC Debiased Sparse Partial Correlation, EOPE early-onset preeclampsia, LOPE late-onset preeclampsia, FC fold-change, KEGG Kyoto Encyclopedia of Genes and Genomes

metabolic profiles in phenylalanine, tyrosine and tryptophan biosynthesis and valine, leucine and isoleucine biosynthesis. In EOPE, the former was mainly enriched in the maternal brain and serum, while in LOPE, it was enriched in the placenta. The latter was mainly enriched in the offspring brain in EOPE and in the placenta in LOPE. At the level of carbohydrate metabolism, distinct differences were observed between the EOPE and LOPE groups. In the EOPE group, the maternal brain exhibited pronounced glycolysis/gluconeogenesis and pyruvate metabolism, accompanied by the up-regulation of differentially expressed metabolites ethanol and lactate. These phenomena suggest that EOPE is associated with more severe energy metabolism disorders and neural damage in the brain compared to LOPE. Conversely, the LOPE group showed a different metabolic profile. It had more abundant glyoxylate and dicarboxylate metabolism in various tissues. This difference in glyoxylate and dicarboxylate metabolism further distinguishes the carbohydrate metabolism patterns between the two groups. Moreover, the LOPE group also had a relatively higher presence of fatty acids and organic acid metabolites, indicating a shift towards different energy-related metabolic pathways in comparison to the EOPE group.

Further, we conducted a more in-depth trajectory analysis of the differentially-expressed metabolites in the above-mentioned metabolic pathways (Fig. 6E). In amino acid metabolism, the metabolic trajectories of Choline-Creatine-Aspartate-Tyrosine-Glutamine-Glutamate exhibited a consistent pattern across the PE groups. The main difference between the two groups was that the EOPE group showed a correlation in the metabolic trajectories of branched-chain amino acids Valine-Leucine-Isoleucine and amino acids Phenylalanine-Glycine, while the LOPE group did not. In terms of carbon metabolism, both groups had the same Ethanol-Glutamate-Lactate-Glutamine, and the correlated metabolic trajectory changes of Acetate-Ethanol and Acetate-Glycine were the key to distinguishing EOPE from LOPE.

Discussion

The development of a rat model resembling PE has uniquely positioned researchers to delve into the metabolic shifts that transpire within the brains of both mother and offspring, as well as in other tissues. This offers profound insights into the intricate pathophysiology of PE.

The brain-placenta axis in PE

The brain-placenta axis is a pivotal concept in reproductive biology, highlighting the indispensable interaction between the maternal brain's stress-responsive neuroendocrine system and the placenta for fetal development and pregnancy maintenance (Behura et al. 2019a, b; Islam and Behura 2024). The placenta, an endocrine organ, influences maternal adaptation to pregnancy by secreting neuroactive molecules that regulate endocrine gland functions, particularly through the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-thyroid (HPT), and hypothalamic- pituitary-gonadal (HPG) axes (Behura et al. 2019a, b). A deeper understanding of gene expression and metabolic coupling in the maternal and offspring brains under stress is essential for assessing the impact of stress on the maternal-fetal nervous system, especially in PE. Our meticulous cluster analysis, network analysis, and tissue heterogeneity analysis indicate that an interaction pattern exists among the maternal brain, serum, placenta, and offspring brain in rats, regardless of whether they are in a normal pregnancy or PE state. Additionally, these analyses have revealed a series of characteristics related to the maternal brain-placenta and placenta-fetal brain that are associated with the onset of PE.

There are multiple mechanisms underlying the interaction between the placenta and the maternal brain, including hormonal regulation, cytokine and chemokine regulation, metabolic transmission, neurotransmitter transmission, and neural signal conduction (Rosenfeld 2021). During pregnancy, the placenta plays a crucial role in regulating the maternal blood-brain barrier. It can establish maternal immune tolerance to the fetus through various mechanisms,

reducing the attack of the maternal immune system on the fetus. For example, steroids secreted by the placenta, including estradiol and progesterone, along with adrenal glucocorticoids and leptin, activate specific transport mechanisms of the blood-brain barrier. This activation not only enhances the immune adaptability of the maternal brain but also physiologically facilitates the information transmission of the blood-brain barrier regarding immunity, hormones, and metabolites (Grattan and Ladyman 2020). Growth factors such as sFLT and PlGF, as well as exosomal vesicles secreted by the placenta, have the potential to directly augment the permeability of the blood-brain barrier, thereby promoting communication between the brain and placental tissues (Sandoval et al. 2024; Bergman et al. 2021). In the normal group, our DSPC interaction network, cluster analysis, and sample heterogeneity analysis have all confirmed extensive metabolic exchanges between the maternal brain and the placenta. In the PE state, in addition to the alterations of neurotransmitter - related substances such as Choline, Glutamate, 4-Aminobutyrate, Glutamine, Tyrosine, Phenylalanine, Glycine, and Tryptophan in EOPE and LOPE, we found that in EOPE, rats exhibited more extensive inter- and intra-tissue interactions and metabolic dysfunctions. The proportion of placental function in the dominant metabolite clusters of rats increased significantly, disrupting the expression of metabolites related to neural damage in the maternal brain, such as Alanine, Myo-Inositol, Hypoxanthine, and Nicotinate. This may be due to the brain's excessive exposure to placental-derived metabolites and inflammatory factors, which induce the disruption of the blood-brain barrier, leading to the entry of fluids, ions, and metabolites into the brain parenchyma (Fishel Bartal and Sibai 2022; Sandoval et al. 2024).

The interaction between the fetal brain and the placenta is another important research area of the placenta-brain axis. The placenta's response to various internal and external stresses may affect fetal brain development, thereby influencing the long-term health of the offspring. Neurotransmitters produced by the placenta, such as serotonin, norepinephrine, and dopamine, may affect the fetal brain via the bloodstream, participating in processes such as cell division, neural migration, and synapse formation. Dopamine and norepinephrine in the placenta also contribute to the regulation of fetal physiological processes (Freedman et al. 2022; Rosenfeld 2021). Our study focuses on the characteristics of metabolic profile changes to comprehensively explore the interaction between the placenta and the fetal brain and compare the differences among normal pregnancy, EOPE, and LOPE. Under normal pregnancy conditions, the metabolic association between the placenta and the fetal brain is well-organized. The placenta provides sufficient nutrients and signaling molecules to support the normal development of the fetal brain. Neurotransmitters produced by the placenta act precisely on the fetal brain through the circulatory system, promoting the growth and development of nerve cells and ensuring the proper construction of neural networks (Behura et al. 2019a, b). In the EOPE state, from the perspective of tissue correlation, the clustering distance between the offspring brain and the placenta is shorter than that between other tissues. Moreover, significant alterations occur in the placental metabolism of EOPE rats. Lactate is significantly upregulated in the placental tissue, and unique differential metabolites such as ethanol, creatine, and lysine are mainly involved in energy metabolism and essential amino acid metabolism. These changes not only reflect placental energy metabolism disorder, which may compromise nutrient supply and signal transmission to the fetal brain, but also suggest that the placenta may be attempting to alleviate fetal metabolic disorders. Additionally, our network analysis reveals that fetal brain neural-damage-related substances, including Niacinamide, Myo-Inositol, and Glutamine, are directly regulated by placental metabolites such as Glutamate, Isoleucine, Betaine, Lysine, and Glutamine. This finding offers new insights into assessing the neural damage status of EOPE fetuses by monitoring placental metabolites.

In our study of the metabolic profiles of the brain-placenta axis, we observed distinct manifestations of placental function in different PE subtypes. In LOPE, we noted a decrease in the proportion of placental metabolites in the dominant clusters, with most placental metabolites forming independent clusters. This may imply the existence of a potential metabolic compensation mechanism in LOPE, aiming to isolate the impact of placental abnormalities. We verified the tissue heterogeneity in LOPE using Euclidean and Manhattan distances, and the results also confirmed that the distances between the maternal and offspring brains, as well as between the maternal brain and serum, are shorter than that between the maternal brain and the placenta. The distinct placental manifestations in EOPE and LOPE are supported by the transcriptome analysis of placental samples from previously reported PE patients, revealing that EOPE is mainly associated with metabolic disorders while LOPE is primarily related to immune function abnormalities, which indicates their different molecular characteristics (Ren et al. 2021). This also supports the view that although EOPE and LOPE share similar pathological and clinical manifestations, they may lead to disease development through different molecular mechanisms.

Amino acid metabolism in PE

Amino acids are the fundamental building blocks of proteins and are essential for life. In recent years, numerous

studies have reported on the correlation between amino acid metabolism in women with PE and the occurrence of the disease, proposing the use of serum amino acids as predictive biomarkers for PE (Odibo et al. 2011; Weckman et al. 2019; Worton et al. 2019; Yao et al. 2022; Youssef et al. 2024). However, existing human metabolomic studies have primarily focused on blood, placenta, urine, and umbilical cord blood (Yao et al. 2022; Wang et al. 2022), with insufficient attention given to the brains of mothers and offspring, and the analysis across different stages of PE is also not comprehensive.

Our research has confirmed changes in amino acid metabolism in maternal blood and placenta, findings which are corroborated by human studies (Prameswari et al. 2022). For example, we observed a downward trend in Tryptophan and Isobutyrate in the serum of both EOPE and LOPE. In the EOPE group, there was a significant down-regulation of the essential amino acid Lysine and an up-regulation of Tyrosine. In the LOPE group, non-essential amino acids Methionine and Aspartate were notably down-regulated. Similarly, in placental metabolism, Lysine in the EOPE group showed a decrease. In contrast, the unique differential metabolites in the LOPE group—Phenylalanine, Glycine, Isoleucine, and Tyrosine—were down-regulated. All these indicate the presence of amino acid metabolic disorders in PE. The roles of these metabolic disorders in PE are multifaceted. For instance, disorders in Tryptophan metabolism may affect the function of regulatory T cells, thereby impacting the maternal immune response to the placenta (Saito et al. 2007; Nilsen et al. 2012); Tryptophan, 4-Aminobutyrate, Tyrosine, and Phenylalanine play crucial regulatory roles in vasodilation (Broekhuizen et al. 2020; Oketch-Rabah et al. 2021; Lin et al. 2022); Methionine is a precursor to various biomolecules, including the antioxidant glutathione and polyamines, and Aspartate is an important amino acid in the urea cycle and neurotransmitter synthesis (Jung 2015; Herring et al. 2015).

Additionally, we have paid attention to the changes in metabolites in the brains of the mother rats and their offspring. We found that in the maternal brain, both groups exhibited alterations in amino acids related to neurotransmitter synthesis. For example, Glutamine, a principal energy substrate for the brain and a precursor in neurotransmitter synthesis, exhibited upregulation in PE. This increase may correlate with heightened energetic demands of neuronal cells, the challenges of oxidative stress, the activation of immune responses, and the escalated requirements for neurotransmitter synthesis (Eid et al. 2016; Dienel 2019; Wang et al. 2019). Tyrosine is a precursor for the catecholamine neurotransmitter dopamine. The downregulation of Tyrosine metabolism in PE may affect dopamine-induced blood pressure regulation and vascular reactivity (Wang et al. 2021; Channer et al. 2023). Glutamate is the main excitatory neurotransmitter, and its imbalanced release can lead to excessive neuronal excitation, which is an important cause of epileptic seizures (Sarlo and Holton 2021). We also found the downregulation of the inhibitory neurotransmitter 4-Aminobutyrate in Fig. 2C, with logFC values of −1.10 and −1.30 in the EOPE and LOPE groups, respectively, and an upregulation of its precursor Glutamine. In terms of metabolites in the offspring's brains, we found that EOPE and LOPE have different amino acid regulatory patterns for the development of the rat offspring's brains. In LOPE, the downregulation of Glutamine and the upregulation of Glycine and Lysine help regulate the learning and memory activities of the offspring's brain and promote brain development (Andersen et al. 2019; Limón et al. 2021; Serra et al. 2022).

Our enrichment analysis of amino acid metabolites presents evidence supporting both similarities and differences in the pathogenic mechanisms of EOPE and LOPE. There are common enrichment phenomena in certain amino acid metabolic pathways in both the EOPE and LOPE groups. For instance, the metabolic profiles of arginine synthesis, alanine, aspartate, and glutamate metabolism, as well as glycine, serine, and threonine metabolism, are similar across different tissues. These metabolic pathways in the body involve the coordinated expression of metabolites such as pyruvate, alanine, glutamate, glutamine, glycine, and serine. Under both physiological and pathological conditions, they are subject to the coordinated regulation of common hormones, cytokines, and transcription factors. For example, mTOR signaling may influence the activity or expression of arginine synthetase, alanine aminotransferase, aspartate aminotransferase, glycine synthetase, and serine hydroxymethyltransferase simultaneously by regulating these amino acid metabolic pathways, resulting in a similar metabolic profile (Ke et al. 2025). In different tissues, we observed similar metabolic trajectories of Choline-Creatine-Aspartate-Tyrosine-Glutamine-Glutamate in the PE group. These amino acid metabolic pathways work in synergy to maintain the body's nitrogen balance and are also closely associated with energy metabolism. The abnormalities in these common metabolic pathways indicate that both subtypes are similarly affected in basic metabolic processes such as amino acid synthesis, decomposition, and transformation. This may be attributed to the oxidative stress and inflammatory responses triggered by placental ischemia and hypoxia, which alter the body's physiological requirements for neurotransmitter synthesis, detoxification, and other functions, thereby influencing these amino acid metabolic pathways in a similar manner.

However, differences between EOPE and LOPE are also evident in the tissue distribution of amino acid metabolic

pathways. For example, in the biosynthesis pathway of phenylalanine, tyrosine, and tryptophan, EOPE is mainly enriched in the maternal brain and serum, while LOPE is enriched in the placenta. Moreover, EOPE shows characteristic changes in the metabolic trajectories of branched-chain amino acids (Valine, Leucine, Isoleucine) and amino acids (Phenylalanine, Glycine). The differences in the tissue distribution of amino acid metabolic pathways between EOPE and LOPE result from the combined effects of multiple factors, including metabolic pathways, enzyme activities, and regulatory mechanisms. In the case of EOPE, the mother may face more severe neuroregulatory stress. To cope with the neural stress caused by EOPE, the maternal brain enhances the biosynthesis of these amino acids to meet the demand for neurotransmitter synthesis, such as dopamine, norepinephrine, and serotonin, which are downstream products of amino acid synthesis (Miyajima 2020). Additionally, the imbalance of the maternal neuro-immune regulatory network, disordered energy metabolism, oxidative stress, and inflammatory mediators can alter the activity of enzymes related to branched-chain amino acid metabolism, such as branched-chain amino acid transaminase and branched-chain α-keto acid dehydrogenase complex (Lu et al. 2024; Nie et al. 2018). This leads to impaired catabolism of branched-chain amino acids and changes in their metabolic trajectories.

Metabolic reprogramming in PE

In addition to amino acids involved in energy metabolism, we identified differential changes in metabolites directly reflecting metabolic reprogramming alterations in EOPE and/or LOPE, such as Lactate, Acetate, Ethanol, and 3-Hydroxybutyrate, involving processes like glycolysis, the TCA cycle, and ketone body metabolism. In maternal placental and brain tissues, a significant increase in Lactate was observed, indicating that under preeclamptic conditions, there may be not only placental hypoxia but also maternal brain hypoxia. In the maternal brain tissue of the EOPE group, Acetate and Ethanol showed significant increases, leading to the upregulation of glycolytic pathways, suggesting different energy metabolic adaptation patterns between EOPE and LOPE. Our data indicate that, aside from changes in amino acids involved in energy metabolism, direct metabolite differences related to energy metabolism in the fetus are not significant, which may also be related to the placental regulatory role in fetal neurodevelopment (Parenti et al. 2024). Metabolites related to energy also participate in numerous metabolic reprogramming processes, involving adjustments in cellular metabolic pathways to adapt to various physiological or pathological states (Ye et al. 2022). For example, increased Lactate has functions in cellular signal transduction, can be involved in regulating immune responses and cell survival, and can directly promote placental growth and invasion through the lactylation modification process, as well as prevent hypoxic damage in the brain (Li et al. 2022, 2023; Certo et al. 2022).

There are marked differences in energy metabolic patterns between the EOPE and LOPE groups. The EOPE group is associated with more severe energy metabolic disorders and cerebral nerve damage. The maternal brain exhibits prominent glycolysis/gluconeogenesis and pyruvate metabolism, accompanied by the upregulation of metabolites such as ethanol and lactate. This aligns with recent findings from human studies on EOPE. In EOPE, abnormal vascular remodeling occurs in the placenta at an early stage, leading to insufficient placental perfusion. This ischemic and hypoxic state prompts the placenta to generate a substantial amount of reactive oxygen species (ROS). Neurons in the maternal brain are particularly sensitive to oxidative stress, as ROS can disrupt neuronal membrane integrity and interfere with mitochondrial function, thereby affecting energy metabolic processes (Muñoz-Mayorga et al. 2023). Consequently, this results in disorders of glycolysis/gluconeogenesis and pyruvate metabolism, as evidenced by the upregulation of metabolites like ethanol and lactate.

In contrast, the LOPE group presents a distinct metabolic profile characterized by more abundant glyoxylate and dicarboxylate metabolism, as well as fatty acid metabolism across various tissues. This indicates that, compared to EOPE, LOPE demonstrates adaptive adjustments in energy utilization. The glyoxylate cycle can conserve key intermediates in the TCA cycle to enhance energy utilization efficiency (Johnson et al. 2023). In LOPE rats, the placenta directly enhances glyoxylate and dicarboxylate metabolic pathways to more efficiently utilize limited nutrients and energy substrates, thereby maintaining the metabolic requirements of both the fetus and the mother. Furthermore, the LOPE placenta may increase the uptake and metabolism of fatty acids, producing substantial ATP through β-oxidation, with metabolic products such as ketone bodies providing additional energy to tissues, including the fetal brain (Mauro et al. 2022).

Conclusion

This research sheds light on the unique metabolic profiles within both the maternal and offspring brains during PE, uncovering intricate interactions with peripheral tissues. The observed metabolic shifts, particularly in neurotransmitters and their precursors, as well as in energy and amino acid metabolism, indicate that the neurometabolic response to PE could significantly influence maternal cognitive and

emotional well-being, stress resilience, and the neural development of the embryo (Coplan et al. 2018; Cruz-Carrillo and Camacho-Morales 2021).

The cross-tissue metabolic correlations highlight the systemic influence of PE, with the brain-placenta axis standing out as a pivotal regulatory nexus. Our results, in an exploratory manner, demonstrate the distinct metabolic pattern changes between EOPE and LOPE. They reflect the close associations between the maternal brain-placenta and offspring brain-placenta in normal and EOPE states, as well as the shift in metabolic characteristics with weakened placental metabolism in the LOPE state. This represents a complex and dynamic transformation of the metabolic signaling network, which warrants in - depth validation and mechanistic exploration.

However, our study acknowledges limitations. The cross-species applicability of our findings requires validation through human studies, and the precise mechanistic links between the metabolic changes and neurodevelopmental outcomes need further exploration. Additionally, while our correlational analyses hint at interconnected metabolic networks, the directionality and causality of these relationships are yet to be determined.

In conclusion, our research highlights the intricate metabolic landscape of the maternal and offspring brains in PE and emphasizes the importance of the brain-placenta axis. These findings pave the way for future investigations into the neurometabolic underpinnings of PE and the development of targeted interventions that consider the brain's role in maternal and fetal well-being. Moreover, the distinct metabolic profiles of EOPE and LOPE identified in our study offer valuable data - driven support for the classified diagnosis of PE.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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