Tokatly RT, Sharaf BM, Abuhelwa AY, Soliman S, El-Huneidi W, Alqudah MAY, Alzoubi KH, Abu-Gharbieh E, Albsoul A, Bulatova N, Kasabri V, AlQuoqa R, Khawaja N, Hyassat D, Abujbara M, Basha A, El- Khateeb M, Bustanji Y, Semreen MH. Enhancing Clinical Decision-Making in Gestational Diabetes Through Metabolomics: A Pharmacy Practice Outlook. Pharmacy Practice. 2026 Jan-Marc;24(1):3344.

https://doi.org/10.18549/PharmPract.2026.1.3344

Original Research

Enhancing Clinical Decision-Making in Gestational Diabetes Through Metabolomics: A Pharmacy Practice Outlook

Rimas Taher Tokatly, Basma Majed Sharaf, Ahmad Y. Abuhelwa, Sameh M. Soliman, Waseem El-Huneidi, Mohammad A. Y. Alqudah, Karem H. Alzoubi, Eman Abu-Gharbieh, Abla Albsoul, Nailya Bulatova, Violet Kasabri, Reem AlQuoqa, Nahla Khawaja, Dana Hyassat, Mousa Abujbara, Asma Basha, Mohammad El- Khateeb, Yasser Bustanji, Mohammad H. Semreen

Received (first version): 15-Jul-2025 Accepted: 12-Aug-2025 Published online: 06-Jan-2026

Abstract

Gestational diabetes mellitus (GDM) is a pregnancy complication associated with glucose intolerance and an increased risk of type 2 diabetes. Metabolic alterations, including changes in amino acids, fatty acids, and glycolysis, have been linked to GDM. However, comprehensive metabolomics analyses, particularly in Middle Eastern women cohort, are lacking. This study aims to identify unique metabolic pathways to enhance understanding of disease progression and guide diagnosis and targeted therapeutic strategies. Blood samples were collected from 32 women with GDM and 21 healthy pregnant women. Metabolomic analysis was performed using trapped ion mobility spectrometry time-of-flight mass spectrometry. Statistical analysis included a two-tailed independent Student's t-test, with a significance threshold of $p < 0.05$. Out of 108 identified metabolites student's t-test analysis revealed 33 statistically significant metabolites ($P < 0.05$) in GDM group compared to healthy pregnant women. Of them, citramalic acid, creatinine, D-arginine, and glutamine were significantly reduced in GDM, while 4-aminohippuric acid, homovanillic acid, alpha-aspartyl-lysine, L-aspartyl-L-phenylalanine, L-valine, L-leucine, and normetanephrine were increased. Pathway analysis further highlighted phenylacetate metabolism as a key pathway upregulated in GDM. This underscores the potential significance of phenylacetate metabolism in the metabolic alterations associated with GDM. A comprehensive understanding of metabolic alteration in GDM provides valuable insights into the factors influencing the metabolic environment of pregnant women with GDM. This knowledge not only enhances our understanding of the molecular mechanisms underlying GDM but also paves away for developing diagnostic and targeted therapeutic strategies. By addressing dysregulated metabolomic pathways, these findings hold the potential for improving the management and prevention of GDM.

Keywords: Gestational Diabetes Mellitus; MetaboAnalyst; UHPLC-ESI-QTOF-MS; metabolic pathways; metabolic profiling; metabolites; untargeted metabolomics

Rimas Taher Tokatly. Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates.
Basma Majed Sharaf. Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates.
Ahmad Y. Abuhelwa. Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Pharmacy Practice and Pharmacotherapeutics, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates.
Sameh Soliman. Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates ; Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates.
Waseem El-Huneidi. Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates.
Mohammad A. Y. Alqudah. Department of Pharmacy Practice and Pharmacotherapeutics, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan.
Karem H. Alzoubi. Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Pharmacy Practice and Pharmacotherapeutics, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates.
Eman Abu-Gharbieh. Department of Clinical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates. Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates, School of Pharmacy, The University of Jordan, Amman 11942, Jordan.
Abla Albsoul. School of Pharmacy, The University of Jordan, Queen Rania Street Amman, 11942, Jordan
Nailya Bulatova. School of Pharmacy, The University of Jordan, Queen Rania Street Amman, 11942, Jordan
Violet Kasabri. School of Pharmacy, The University of Jordan, Queen Rania Street Amman, 11942, Jordan
Reem AlQuoqa. School of Pharmacy, The University of Jordan, Queen Rania Street Amman, 11942, Jordan
Nahla Khawaja. National Center of Diabetes, Endocrinology and Genetic Diseases, Queen Rania Street, Amman, Jordan.
Dana Hyassat. National Center of Diabetes, Endocrinology and Genetic Diseases, Queen Rania Street, Amman, Jordan.
Mousa Abujbara. National Center of Diabetes, Endocrinology and Genetic Diseases, Queen Rania Street,

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

https://doi.org/10.18549/PharmPract.2026.1.3344

Amman, Jordan.
Asma Basha. School of Medicine, The University of Jordan, Queen Rania Street Amman, 11942, Jordan.
Mohammad El- Khateeb. National Center of Diabetes, Endocrinology and Genetic Diseases, Queen Rania Street, Amman, Jordan.
Yasser Bustanji*. Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates and Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates and School of Pharmacy, The University of Jordan, Amman 11942, Jordan. ybustanji@sharjah.ac.ae
Mohammad H. Semreen*. Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates and Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates. msemreen@sharjah.ac.ae

INTRODUCTION

Currently, gestational diabetes mellitus (GDM) is one of the most common medical complications during pregnancy, GDM is defined as any degree of glucose intolerance with onset during pregnancy and typically resolves after childbirth[1]. It commonly first appears between weeks 24 and 28 of pregnancy[2]. Underlying maternal defects in β-cell response to insulin during pregnancy may establish GDM. Interestingly, a new strong antepartum predictor of prediabetes is polycystic ovarian syndrome (PCOS), which accounts for the impressively high prevalence of glucose intolerance in early postpartum period in women with previous GDM. Moreover, compared to those without GDM, women with a prior history of GDM have a more than 7-fold greater risk of developing postpartum diabetes. The body's hormonal balance naturally shifts during pregnancy, which can occasionally cause issues with insulin function[2].

The World Health Organization (WHO) lists the following as some variables that raise the chance of developing GDM[3]: the mother's age surpassing 25 or 30 years, being overweight or obese before becoming pregnant, diabetes in the family history, GDM's history, specific ethnic groups (women of South Asian, African, Caribbean, or Middle Eastern descent are more at risk), a past pregnancy that resulted in a large baby (over 4 kg). Proper management of GDM is essential because uncontrolled GDM leads to the potential risk of gestational hypertension, pre-eclampsia, and cesarean delivery. Furthermore, GDM elevates the likelihood of complications, such as obesity, cardiovascular disease, and impaired glucose metabolism, which can result in the development of type 2 diabetes mellitus (T2DM) in both the mother and the child[3-5]. Screening for GDM is required to be performed between weeks 24 and 28 of pregnancy. According to the American Diabetes Association Guidelines, the target for pregnant women blood glucose level should be 95 mg/dL or less before taking a meal, 140 mg/dL or less one hour after a meal, and 120 mg/dL or less two hours after a meal[6]. Several lifestyle interventional trials combining pharmacological and nonpharmacological interventions to improve modifiable risk factors, such as diet, exercise, and breastfeeding, have successfully lowered the incidence of obesity-related morbidities, including postpartum diabetes and weight retention. Medical nutrition therapy, weight control, and physical activity are the first-line treatment for GDM[5,6]. It has been proposed that in 70–85% of women diagnosed with GDM, lifestyle modification alone is adequate to control blood glucose levels[7,8].

Omics technologies, such as genomics, proteomics, metabolomics, and transcriptomics, have significantly contributed to disease diagnosis through biomarker discovery. In response to genetic variations or physiological and pathological states, cells, tissues, organs, systems, or entire organisms catalyze metabolic reactions that result in small intermediates or end products. These reactions are known as metabolic reactions, in which metabolomics is used to identify and quantify these changes. Metabolites function as direct regulators of biological processes and phenotypes[9,10]. High sensitivity and selectivity biomolecule detection and characterization in complex matrices can be achieved through innovative biomarker research using mass spectrometry. Ultra-high performance liquid chromatography, electrospray ionization, quadrupole time of flight, and mass spectrometry (UHPLC-ESI-QTOF-MS) are sophisticated analytical platforms used to identify biomarkers associated with GDM as well as side effects from associated illnesses[10,11]. Metabolomics research can improve the accuracy and effectiveness of clinical procedures and studies involving hyperglycemic pregnant women[12].

The current study examines pregnant women's metabolomics profile within cross-sectional research on a Jordanian cohort using UHPLC-ESI-QTOF-MS. Our aim is to identify biomarkers that may help diagnose GDM-related diseases at an early stage along with proposed target therapeutic strategies.

MATERIALS AND METHODS

Population and Study Design

The study is cross-sectional with samples and data collected from Amman, Jordan National Center for Diabetes, Endocrinology and Genetics (NCDEG) (No.1/2015) and the School of Pharmacy in the University of Jordan (JU) between December 2016 and December 2022 by Amman, Jordan's NCDEG Institutional Review Board, participants signed a consent form. Patients with GDM who were 20–28 weeks pregnant were employed in the study. The participant population was divided into two groups: Group 1 (control) was pregnant ladies with normal glucose blood levels, and Group 2 was hyperglycemic pregnant women (Gestational diabetes).

Collection of Samples

For this study, 53 women in total were recruited. Samples of plasma were taken from 21 pregnant ladies with normal

https://doi.org/10.18549/PharmPract.2026.1.3344

glucose blood levels and 32 hyperglycemic pregnant women in which they were classified according to the plasma glucose (FPG) that is carried out following an overnight fast with 75-g Oral Glucose Tolerance Test (OGTT), with blood glucose levels recorded at fasting, one hour and two hours after: Fasting glucose ≥ 92 mg/dL, 1-hour glucose ≥ 180 mg/dL, 2-hour glucose ≥ 153 mg/dL; if one or more values exceed these thresholds then a pregnant women is diagnosed with GDM. Blood samples were collected into heparinized tubes, and then centrifugation was performed for 5 minutes at 14,000 rpm. The obtained plasma samples were sent to the Research Institute University of Sharjah for additional analysis after being kept at –80 °C. Measurements of the results without fasting plasma glucose, changes in plasma levels of cardiometabolic risks, and associated pharmacotherapy in the second and third trimesters of pregnancy served as the foundation for this investigation.

Preparation of the Samples for Metabolomics Analysis

The samples were aliquoted into 100 µL Eppendorf tubes, and 300 µL of methanol (from Wunstorfer Strasse, Seelze, Germany) were added and mixed well by vortexing. The samples were then incubated for 2 hours at -20 °C. The samples underwent another vortex and were centrifuged at 14,000 rpm for 15 minutes. The resultant supernatants were evaporated at 35–40 °C.

To ensure the consistency and reliability of the analysis, a quality control (QC) sample was created by combining an equal volume (10 µL) from each sample. To assess the reproducibility of the analysis, this QC sample was added to the system every nine to ten samples. Using Honeywell's LC-MS CHROMASOLV in Wunstorfer Strasse, Seelze, Germany, the extracted samples were reconstituted in 250 µL of 0.1% formic acid in deionized water before injection. The supernatant was filtered after sample preparation to prepare it for LC-MS/MS analysis. A 0.45 µm pore size hydrophilic nylon syringe filter was used for this filtration. To preserve the integrity of the filtered sample for subsequent analysis, it was carefully collected and placed inside LC glass vials using a specialized insert.

Metabolomics Analysis Using Ultra-High Performance Liquid Chromatography Coupled With Electrospray Ionization And Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-ESI-QTOF-MS)

Ultra-high-performance liquid chromatography system (UHPLC) from Bremen, Germany's Bruker Daltonik GmbH, was used for LC-MS/MS analysis. Essential parts of this system were an autosampler, a solvent delivery systems pump (HPG 1300), an electrospray ionization (ESI) source, a quadrupole time-of-flight mass spectrometer (QTOF), and a thermostat column compartment. Utilizing Bruker Compass HyStar 5.0 SR1 Patch1 (5.0.37.1), Compass 4.1 for timsTOF Series was used along with Control Version 6.2 software for data acquisition, the system ran on Windows 10 Enterprise 2016 LTSB.

Mobile phases A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) were used in the analysis. 0–2 min, 99% A: 1% B; 2–17 min, 99–1% A: 1–99% B; 17–20 min, 99% B: 1% A was the gradient program. 0.25 ml/min was the flow rate. Next, 99% B to 99% A for 20–20.1 min; 99% A: 1% B at 0.35 ml/min flow rate for 20.1–28.5 min; and 99% A: 1% B at 0.25 ml/min for 28.5–30 min. Throughout the analysis, the flow rate was kept the same. For separation, the sample was injected into a Hamilton® Intensity Solo 2 C18 column (2.1 mm × 100 mm, 1.8 µm) in the form of a 10 µL aliquot. It was decided to use a column oven at 35 °C. The ESI source's parameters were set up as follows for every injection: The nitrogen drying gas flow rate was 10.0 l/min at 220 °C, the capillary voltage was adjusted to 4500 V, and the nebulizer pressure was maintained at 2.2 bar. During the MS2 acquisition stage, the collision energy stepping was conducted between 100 and 250% while keeping the end plate offset at 500 V and the value of 20 eV constant similar to our previous research[13].

Sodium formate was the calibrant used in the external calibration procedure. The two parts of the acquisition procedure were the auto MS scan, which took 0 to 0.3 min, and the auto MS/MS, which took 0.3 to 30 min and included fragmentation. At 12 Hz, both segments were run in the positive mode. The automatic in-run mass scan range was ±0.5 for the precursor ion width and covered 20–1300 m/z. For every cycle of 0.5 secs, three precursors were selected, and 400 counts served as the threshold. Following three spectra, active exclusion was started, and it was lifted after 0.2 minutes equivalent to our earlier studies[14].

Data Processing and Analysis

The MetaboScape® 4.0 software (Bruker Daltonics, Billerica, MA, USA) was used to analyze the collected data. The T-ReX 2D/3D workflow used bucketing parameters for the processed data, which included a peak length spanning 7 spectra, an intensity threshold of 1000, and peak area for quantification. Mass spectra calibration was performed in the range of 0-0.3 min, using features from at least 50 to 186 samples. With a mass range of 50 to 1300 m/z and a retention time range of 0.3 to 25 min.

For LC-QTOF analysis, duplicate samples from 52 individuals in each group were collected. Following the combination of these samples, a dataset with 3625 distinct features was produced. By matching the retention duration and MS/MS spectra to the HMBD 4.0 database—carefully designed to meet the unique requirements of the metabolomics community—metabolites were identified. After filtering with MetaboScape®, an extensive collection of unique metabolites was selected. The quantitative data matrix was created using metabolite›s peak intensities. The metabolite datasets contained only those metabolites that were statistically significant (p-value < 0.05*) and listed in the human metabolome database 4.0 (HMDB). The human metabolites were filtered using the HMDB online database (https://hmdb.ca/metabolites/HMDB0059911). After HMDB filtration, a total of 108 distinct metabolites remained from the original list. The MetaboAnalyst 5.0 software, developed by McGill University in Montreal, QC, Canada, is a comprehensive platform for metabolomics data analysis. The metabolite datasets were exported as CSV files and then imported into the program. The most distinctive characteristics within the group under study were chosen using MetaboAnalyst's sparse

https://doi.org/10.18549/PharmPract.2026.1.3344

partial least squares-discriminant analysis (sPLS-DA) method for sample classification. By applying the false discovery rate (FDR) approach, multiple hypothesis testing corrections were applied to minimize the rate of false positives. A two-tailed independent Student's t-test was employed for stastical analysis. As a result, a volcano plot was made to show the fold change (p<0.05*, FC=1.25) and statistical significance. Pathway analysis and metabolite set enrichment were conducted using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca).

Metabolomics data are deposited in Metabolomics Workbench with (datatrack_id:5511study_id: ST003671). The DOI for this project (PR002276) is: http://dx.doi.org/10.21228/M8F53S .

RESULTS

Participant and Blood Sample Characteristics

From the 53 recruiters 32 GDM women were 32.1±4.6 years old significantly older than the normal glucose level pregnant (control) women 28.7±3.8 years old. High FPG; 80.5+/-22.7 mg/dL; p=0.022 and 100.3±25.3 mg/dL; p=0.022 was accompanied in the GDM group with higher glycated hemoglobin (HbA1c% 5.85±1.07) versus control group of (4.63±0.29; p=0.014) respectively. There was no significance difference in BMI between both groups. These characteristics are summarized in Table 1.

Metabolic Changes

A total of 108 metabolites were identified, and 33 showed statistical significance (p<0.05) when compared between both groups using student t-test analysis (Figure 1). The metabolites were clustered into two groups with some of them shared between them. Figure 1 of the sPLS-DA model successfully demonstrated partial dataset separation between the two groups with minimal overlap when comparing the GDM group with the control group, indicating significant dissimilarities overcoming specific difficulties related to the dataset's complexity.

A two-tailed independent Student's t-test was performed using MetaboAnalyst 5.0 software to determine the fold change (p<0.05, FC=1.25) between groups. The following paths were taken in conducting the comparative analysis: GDM/Control. Table 2 shows that of the 108 identified metabolites, 31 showed significant differences with 27 metabolites increased in level vs. 4 metabolites decreased in GDM group compared to the control.

Metabolites increased in GDM group are 4-aminohippuric acid, homovanillic acid, alpha-Aspartyl-lysine, L-aspartyl-L-phenvlalanine, L-valine, L-leucine, and normetanepherine, while citramalic acid, creatinine, D-arginine, and L-glutamine were decreased (Table 2 and Figure 2).

Functional Analysis Pathway Changes

Using MetaboAnalyst 5.0 along with PubChem, KEGG, and HMDB databases, phenylacetate metabolic pathway significantly differed (p value) in GDM from the control group (Figure 3). Other enriched pathways include ammonia recycling, tyrosine metabolism, pyruvaldehyde degradation.

DISCUSSION

Influence of metabolic dysregulation on biomarker profiles in gestational diabetes

Studies state that (GDM) is recognized as one of the most significant risk factors for the development of type 2 diabetes. Women with prior GDM have a 20% - 50% probability of progressing type 2 diabetes[15]. GDM has been positively associated with an elevated risk of acute coronary syndrome (ACS)[16]. GDM, which is referred to as glucose intolerance, first manifests during pregnancy. Type 2 diabetes is more common in GDM women than in non-GDM women, according to a prospective cohort study[17]. Additionally, babies born from GDM mothers have higher body weights and are at higher risk of contracting infectious diseases[18].

Our study demonstrated a metabolic profile change between normal pregnant women and pregnant women having GDM. Out of identified metabolites, 31 were significantly related to GDM compared to control group. A significant decrease was found with citramalic acid, D-arginine, creatinine and L-glutamine, whereas a significant increase was shown with aminohippuric acid, alpha-aspartyl-lysine, homovanillic acid, L-aspartyl-L-phenylalanine, L-leucine, normetanephrine and L-valine.

Table 1. Patients' Clinical Characteristics

Characteristics	Normoglycemic pregnant group	GDM pregnant group	
	Mean ± SD	Mean ± SD	*p-Value*
Age (years)	28.7 ±3.8	32.1 ±4.6	**0.032**
BMI (kg/m2)	28.2 ±3.1	29.9 ±5.4	**1**
FPG (mg/dL)	80.5 ±22.7	100.3±25.2	**0.022**
HbA1c%	4.63 ±0.29	5.85 ±1.07	**0.014**
SBP (mm Hg)	111.5 ±9.0	115.7 ±11.7	-
DBP (mm Hg)	72.8 ±7.2	71.1 ±7.5	-

Comparisons of means and p-values were obtained by the t-test.
Abbreviations: SD: standard deviation; BMI: body mass index; FPG: fasting plasma glucose; HbA1C%: percent glycosylated haemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure.

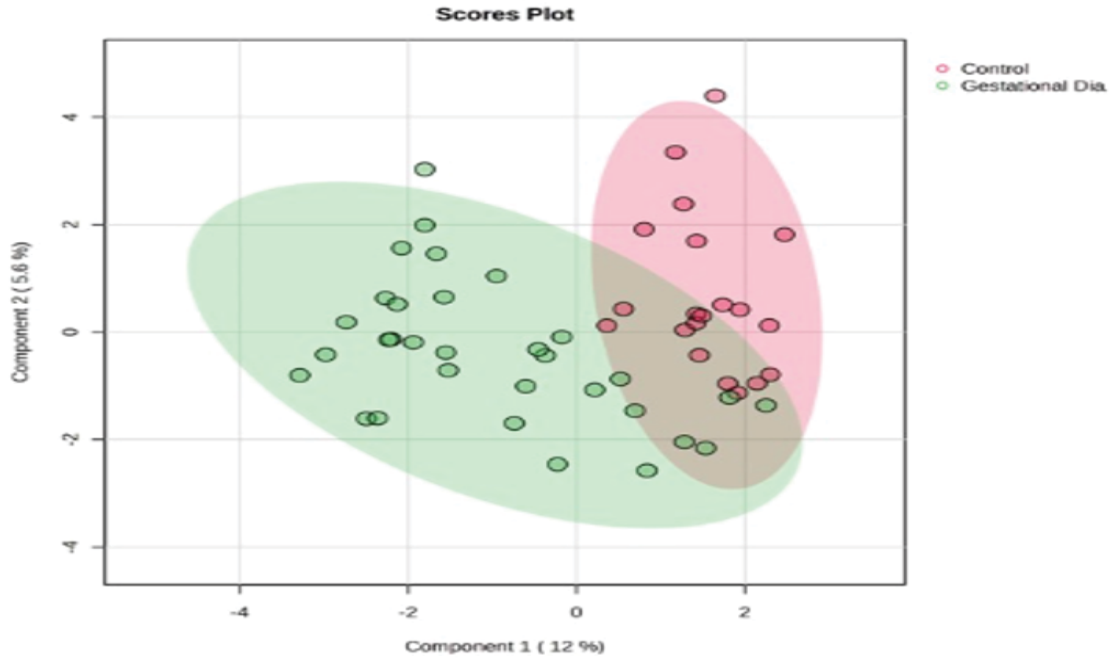

Figure 1. Sparse Partial Least Squares-Discriminant Analysis (sPLS-DA); Group 1: Control (Normal glucose level pregnant); Group 2: Gestational Diabetes

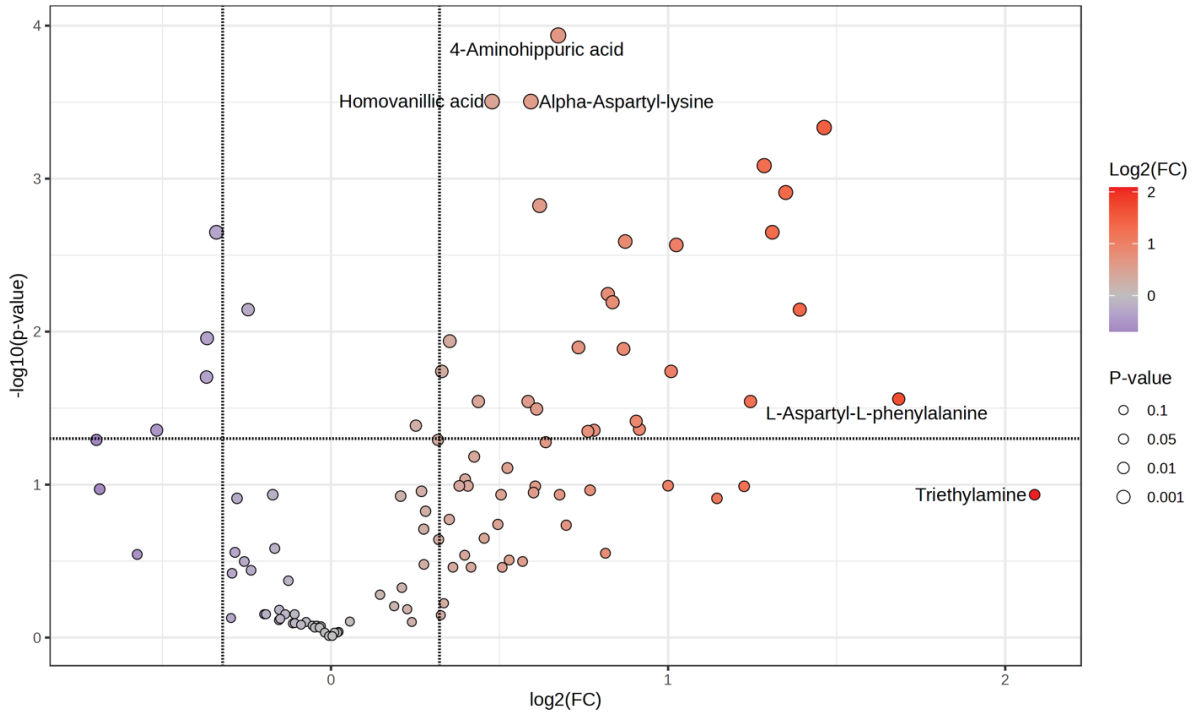

Figure 2. Metabolite volcano plot analysis between GDM and control groups.

https://doi.org/10.18549/PharmPract.2026.1.3344

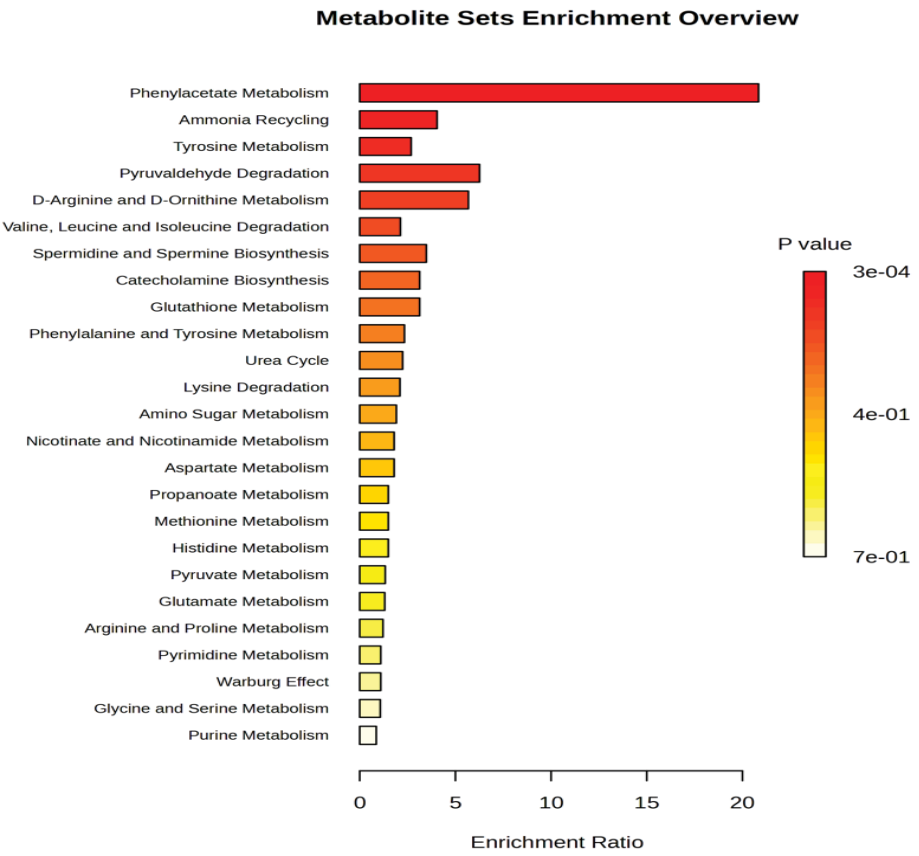

Figure 3. Enrichment Pathway analysis of GDM group vs control group.

Table 2. Metabolites with statistically significant difference between GDM and control groups.

Metabolite name	*t*.stat	*p*.value	FDR	Fold Change	Log2(FC)
4-Aminohippuric acid	-5.5536	1.07E-06	0.0001158	1.5956	0.67409
Homovanillic acid	-5.0177	7.02E-06	0.0003133	1.3926	0.47783
Alpha-Aspartyl-lysine	-5.0053	8.70E-06	0.0003133	1.5083	0.59295
L-Valine	-4.8495	1.72E-05	0.0004632	2.7569	1.463
L-Leucine	-4.6102	3.80E-05	0.0008218	2.437	1.2851
Normetanephrine	-4.422	6.84E-05	0.0012315	2.5471	1.3489
L-Dopa	-4.2432	9.72E-05	0.0015002	1.5357	0.61888
L-Alloisoleucine	-4.0977	0.00017793	0.0022413	2.4778	1.3091
Citramalic acid	4.0354	0.00018678	0.0022413	0.79012	-0.33986
Phenylacetic acid	-4.0322	0.00023846	0.0025754	1.8313	0.87287
L-Homoserine	-3.9194	0.00027618	0.0027116	2.0341	1.0244
Saccharopine	-3.6483	0.00063119	0.0056807	1.767	0.82132
N-Methyl-D-aspartic acid	-3.5908	0.00077311	0.0064228	1.7842	0.83529
Pyruvaldehyde	3.6148	0.00098432	0.0071819	-	-
N-Acetyl-L-phenylalanine	-3.5046	0.00099748	0.0071819	2.622	1.3906
Creatinine	3.3954	0.0016382	0.011058	0.77507	-0.3676
L-Phenylalanine	-3.297	0.0018164	0.011539	1.2766	0.35227

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

https://doi.org/10.18549/PharmPract.2026.1.3344

Tryptophanol	-3.2903	0.0021142	0.012685	1.6634	0.73411
Methylglutaric acid	-3.2182	0.0022816	0.012969	1.8249	0.86786
2-Hydroxy-3-methylbutyric acid	-3.0773	0.0033915	0.018194	1.2559	0.32876
2,4-Diaminobutyric acid	-3.0761	0.0035378	0.018194	2.0126	1.009
D-Arginine	3.0652	0.0040354	0.01981	0.77421	-0.3692
L-Aspartyl-L-phenylalanine	-2.9198	0.0058731	0.027578	3.2133	1.6841
L-Proline	-2.8445	0.0065441	0.028617	2.3692	1.2444
2-Furoylglycine	-2.8342	0.0067266	0.028617	1.354	0.4372
o-Tyrosine	-2.8421	0.0068893	0.028617	1.4997	0.58463
Pipecolic acid	-2.7621	0.0080139	0.032056	1.5263	0.61
Pyroglutamic acid	-2.7146	0.0099789	0.03849	1.873	0.90538
Traumatic acid	-2.6407	0.01105	0.041151	-	-
Alpha-N-phenylacetyl-L-glutamine	-2.6339	0.012086	0.04351	1.8854	0.91489
L-Glutamine	2.6233	0.012903	0.044099	0.69889	-0.51686
Urocanic acid	-2.6023	0.013066	0.044099	1.7177	0.78045
Isovalerylcarnitine	-2.5768	0.013709	0.044867	1.6959	0.76204

Dysregulation of aspartylphenylalanine in GDM samples, identified in our study, is known as one of the key metabolites implicated in severe cardiovascular condition (ACS). ACS tends to present at an earlier age in individuals with diabetes and is linked to increased mortality. This heightened risk is attributed to the proinflammatory and prothrombotic states commonly observed in diabetic patients. Additionally, diabetes is associated with a worsened cardiovascular risk profile, a higher prevalence of comorbidities, and an increased incidence of bleeding complications in elderly ACS patients, collectively contributing to a significant rise in mortality rates[16]. Aspartylphenylalanine is produced through the enzymatic cleavage catalyzed by angiotensin-converting enzyme (ACE). ACE is a critical component of the renin-angiotensin system, where it converts angiotensin-I to angiotensin-II, a process essential for blood pressure regulation, cardiovascular homeostasis, and the modulation of neural and endocrine functions[19]. A Mendelian randomization study has demonstrated a positive correlation between circulating aspartylphenylalanine levels and an increased risk of ACS[20].

Beyond serving as a marker of ACE activity[21], aspartylphenylalanine is also a degradation byproduct of the gut-brain peptide cholecystokinin-8[22], which plays diverse physiological roles, including delaying gastric emptying to regulate food intake and enhancing dietary triglyceride absorption in white adipose tissue[20,23,24]. In individuals with type 1 diabetes, prolonged hyperglycemia acts as an independent risk factor for delayed gastric emptying, further complicating the metabolic challenges associated with the disease[25].

This imbalance may worsen metabolic and cardiovascular outcomes in diabetic patients. Additionally, aspartylphenylalanine's role in promoting dietary triglyceride absorption in white adipose tissue could further aggravate metabolic dysfunction, amplifying the associated cardiovascular risks[20]. Phenylalanine, a metabolite of L-aspartyl-L-phenylalanine, has been associated with impaired insulin signaling and the development of insulin resistance[26].

This study also identified a significant elevated level of homovanillic acid in individuals with GDM compared to control. Homovanillic acid, a metabolite of dopamine and its precursors tyrosine and 5-hydroxytryptophan (5-HT), is predominantly localized in the amacrine and bipolar cells of the retina[27].

Elevated homovanillic acid levels may suggest neurodegenerative changes in diabetic macular edema (DME), a prevalent sight-threatening complication of diabetic retinopathy (DR), affecting approximately 15% of diabetic patients within the first 15 years post-diagnosis[28]. These findings are consistent with previous studies that reported elevated homovanillic acid levels in GDM patients compared to healthy control[29]. The observed elevation of homovanillic acid in our study may serve as a potential biomarker for the increased risk of developing diabetic retinopathy in GDM, particularly DME[29]. Further research is needed to explore its role in early detection and risk assessment of diabetic retinopathy in GDM patients. The increased homovanillic acid levels may reflect increased catecholamine activity, which has been associated with insulin resistance in certain models[30].

4-aminohippuric acid (4-AHA) was found to be elevated in GDM patients this study. AHA, also known as para-aminohippuric acid (PAH), is a metabolite found in human plasma and urine specifically in urinary extracellular vesicles (EVs). It is non-toxic, does not bind to plasma proteins, and is not able to cross erythrocyte membranes[31]. 4-AHA is widely used as a marker to assess kidney function in both clinical and laboratory settings[32]. Normally, a person with healthy kidney function excretes about 92% of PAH through urine, making urinary PAH clearance an effective measure of renal plasma flow (ERPF)[33,34].

4-AHA, an amide derivative of glycine and hippuric acid, serves as a sensitive diagnostic marker for toxicity and demonstrates

https://doi.org/10.18549/PharmPract.2026.1.3344

significant biological activity as a ligand due to its nanoparticle-binding ability[35,36]. Monitoring PAH levels in physiological fluids can aid in diagnosing renal diseases. Recent studies have shown that elevated 4-AHA levels are associated with individuals at high risk for cardiovascular diseases (CVDs), suggesting its role as a novel biomarker and utility in identifying signalling pathways linked to cardiovascular events[37]. In conclusion, we propose that elevated 4-AHA levels serve as a dual biomarker for renal dysfunction and cardiovascular risk, providing insights into the interconnected pathways of metabolic, renal, and cardiovascular complications, particularly in GDM. This underscores its potential in identifying and addressing key risk factors associated with diabetes and its related disorders. Furthermore, our study revealed that normetanephrine, a 3-O-methylated derivative of norepinephrine (NE), exhibited elevated levels in the GDM group. Its measurement provides insights into the extent of NE release during sympathetic nervous system activity. The enzyme catechol-O-methyltransferase (COMT) catalyses the formation of normetanephrine, and its metabolites have potential utility in identifying genetic and acquired disorders related to catecholamine metabolism that remain undiagnosed[38]. A previous study that investigated the association between obstructive sleep apnea (OSA) and sympathetic activation in GDM reported significantly elevated Plasma normetanephrine levels in women with both GDM and OSA compared to those without OSA. These findings indicate heightened sympathetic tone in GDM patients with OSA, as reflected by the increased normetanephrine levels (39). The study highlights a strong correlation between elevated normetanephrine and sympathetic activation in GDM. We hypothesize that increased normetanephrine levels in GDM reflect enhanced sympathetic nervous system activation and could serve as a biomarker for identifying patients with comorbidities, such as obstructive sleep apnea (OSA), which may further exacerbate the metabolic and cardiovascular complications associated with GDM.

L-leucine metabolite, and L-valine were also increased in GDM group. Both are branched-chain amino acids (BCAA), which function as substrates and regulators of protein and energy metabolism[40]. Increased plasma BCAA levels have been continuously linked to the risk of obesity, insulin resistance, and diabetes in numerous cross-sectional and cohort studies conducted in recent years[41]. Pervious research has also been done to examine the links between higher BCAA levels and a higher risk of GDM; these findings indicated a positive correlation of leucine and valine levels and the risk of GDM. Patients with diabetes showed high levels of these BCAAs since they are associated with insulin resistance[42]. We propose that the increased levels of L-leucine and L-valine in GDM patients reflect an underlying metabolic dysfunction related to impaired BCAA metabolism, which may contribute to insulin resistance and increased GDM risk. Additionally, dietary factors, particularly the source of protein intake, may further influence BCAA metabolism and GDM development[43]. Consequently, modulating BCAA metabolism could represent a promising strategy for the treatment or prevention of GDM. Leucine is vital for amino acid transport across the placenta and for fetal growth, with transport deficiencies being associated with growth impairments[44]. Furthermore, placental leucine metabolism is involved in synthesizing other key amino acids, like glutamine, essential for fetal development[45] .One study demonstrated that valine promotes insulin resistance by increasing fatty acid uptake in muscle, resulting in lipid accumulation and disrupted insulin signalling[46]. Altered valine levels have also been linked to pregnancy complications such as intrauterine growth restriction and GDM, potentially indicating placental dysfunction[47].

Other metabolites that showed significant decrease in GDM group include D-arginine, aligned with previous findings[48-50]. Diabetes insipidus (DI) a condition linked to pregnancy and labour, produced because of inadequate vasopressin (AVP, also called arginine vasopressin or antidiuretic hormone, ADH) activity, leading to excessive water loss and dehydration[51]. Likewise, the creatinine, which is the primary metabolite of creatine, is produced almost exclusively in skeletal muscle, with its production rate closely linked to overall skeletal muscle mass[52]. Some prospective cohort studies have shown a positive association between lower serum creatinine levels and a higher risk of developing type 2 diabetes (T2DM), particularly among Asian populations[53]. Emerging research suggests that insulin resistance may be related to specific metabolomic alterations, with evidence indicating that reduced serum creatinine levels during early pregnancy could be associated with a higher risk of developing abnormal glucose metabolism after childbirth in women diagnosed with GDM[54].

The reduced levels of glutamine observed in GDM patients may reflect altered metabolic processes. Glutamine plays a pivotal role in protein synthesis, energy metabolism, and fetal development, and its deficiency could lead to impaired fetal carbon and nitrogen metabolism. A previous study showed that in GDM pregnancies, significant increases in amino acids such as valine, phenylalanine, and leucine were observed, while glutamine levels were notably reduced in the umbilical vein and artery[55]. This pattern highlights disruptions in placental amino acid transport commonly associated with GDM. The observed reduction in glutamine levels may serve as an early biomarker of metabolic imbalance and compromised placental function, with possible consequences for both maternal and fetal well-being. Interestingly, L-glutamine has a dual role—promoting insulin resistance in adipose tissue while enhancing insulin sensitivity in the liver and muscle[56]. It has notable anti-inflammatory effects, particularly in adipose tissue and systemic inflammation, by restoring normal nitric oxide levels, thereby helping to reduce excessive inflammation[57]. Glutamine plays a vital role in placental amino acid transport and energy metabolism, which are essential for proper fetal growth and development[58].

The identification and alteration of specific biomarkers may provide valuable insights for developing targeted pharmacologic and lifestyle interventions in GDM. A common feature of GDM is impaired renal excretory function and tubular dysfunction. In this context, elevated levels of 4-AHA may serve as an early indicator of renal oxidative stress. This

https://doi.org/10.18549/PharmPract.2026.1.3344

suggests a potential benefit from dietary strategies aimed at reducing oxidative burden, such as increased consumption of antioxidant-rich foods (e.g., berries, green tea) and maintaining adequate hydration to support renal detoxification pathways. Additionally, HVA, a major metabolite of dopamine, reflects dopaminergic activity and may offer further insight into metabolic dysregulation in GDM. Altered dopamine metabolism has been associated with insulin resistance and impaired central appetite regulation. This raises the possibility of incorporating dopamine-modulating therapies, such as bromocriptine—a dopamine agonist approved for use in type 2 diabetes as a potential adjunct to improve insulin sensitivity in affected individuals[59]. Moreover, increased concentrations of BCAAs, including L-valine and L-leucine, have been consistently linked to insulin resistance. This underscores the potential benefit of limiting excessive protein consumption, especially from BCAA-rich foods like red meat. Furthermore, engaging in regular physical activity has been shown to promote BCAA breakdown, supporting improved metabolic flexibility and insulin sensitivity in individuals with or at risk for GDM[60]. Additionally elevated levels of normetanephrine suggest increased sympathetic activity**,** which correlates with insulin resistance and hypertension in GDM. Stress management techniques**,** moderate aerobic exercise, and sleep hygiene to normalize autonomic balance are recommended[61]. Together, these findings highlight the potential utility of biomarker-guided interventions to address the metabolic and renal disturbances characteristic of GDM.

Comparison of current metabolite findings with established GDM biomarkers

In our study, the metabolomic alterations observed in GDM are consistent with those reported in previous investigations, particularly with respect to metabolites involved in amino acid metabolism, neurotransmitter pathways, and renal function. These findings underscore the complex metabolic dysregulation associated with GDM and offer insights into potential biomarkers for early detection and therapeutic targets.

Aspartylphenylalanine, a dipeptide produced during protein catabolism, was found to be elevated in our study, which mirrors similar results from prior research. Increased levels of aspartylphenylalanine in these studies are thought to reflect enhanced proteolysis or altered peptide metabolism in response to insulin resistance, a characteristic feature of GDM[62,62].

Our findings also align with previous studies regarding **homovanillic acid,** a major dopamine metabolite. In our cross-sectional study, HVA levels were significantly altered, consistent with prior cohort studies indicating disrupted catecholamine metabolism in GDM[64]. Similarly, **normetanephrine**, a metabolite of norepinephrine involved in sympathetic nervous system regulation, was altered in our study, reflecting changes like those observed in earlier work. These changes in normetanephrine levels may suggest compensatory adjustments in stress response or vascular tone regulation, as observed in other GDM studies[38,39].

In terms of vascular function, **D-arginine**, an enantiomer of L-arginine involved in nitric oxide synthesis, was reduced in our study, confirming the findings of previous investigations. This decrease in D-arginine levels suggests impaired vascular homeostasis and increased oxidative stress, leading to endothelial dysfunction, a well-established feature of GDM[65,66].

Additionally, we observed **L-glutamine** levels to be lower in GDM patients, a result that is consistent with multiple studies linking decreased glutamine concentrations to impaired glucose and amino acid metabolism in GDM. This reduction in glutamine levels may contribute to dysfunctions in insulin secretion and sensitivity, as reported in earlier studies[67,68].

Overall, the metabolomic alterations observed in our study closely align with those documented in the existing literature, reinforcing the concept of systemic metabolic dysregulation in GDM. These consistent findings may provide a strong basis for the development of metabolite-based biomarkers and therapeutic strategies for GDM management.

Insights into Metabolic Pathways of Gestational Diabetes

Over recent decades, there has been a comprehensive exploration of the pathophysiology of GDM, uncovering an escalating involvement of a metabolic pathway in this condition[69].

Phenylacetate Metabolism

Enriched metabolic pathway analysis revealed that phenylacetate metabolism, particularly the overexpression of phenylacetate, has the highest impact score in GDM. Phenylacetate, a phenylalanine metabolite, is a carboxylic acid ester detected in the biofluids of individuals with phenylketonuria (PKU), a genetic metabolic disorder[52]. Phenylacetylglutamine (PAG) is primarily derived from dietary phenylalanine (Phe). Any unabsorbed Phe is metabolized by gut microbiota into phenylacetate, which enters the bloodstream. In the liver, phenylacetate reacts with glutamine to form PAG[70]. This process is illustrated in Figure 4.

Interestingly, the findings of our study are consistent with previous research, which reported elevated serum phenylacetic acid levels in pregnant women with GDM during early pregnancy[71]. This suggests a compensatory rise in phenylacetic acid levels at this stage. Furthermore, our results support that phenylacetic acid plays a critical role in metabolic dysregulation. It has been shown to significantly reduce gluconeogenesis and increase blood glucose levels by inhibiting pyruvate carboxylase, an enzyme essential for promoting islet cell activity[71]. These findings highlight the complex interplay between liver function, gut microbiota, and phenylacetate metabolism in GDM pathophysiology.

Clinical Implications and Decision-Making Pathways

The application of metabolomic biomarkers in GDM care provides an emerging opportunity for clinical pharmacists to play a more active role in personalized healthcare delivery. Pharmacists can utilize these biomarkers during patient counseling sessions to tailor lifestyle and dietary

https://doi.org/10.18549/PharmPract.2026.1.3344

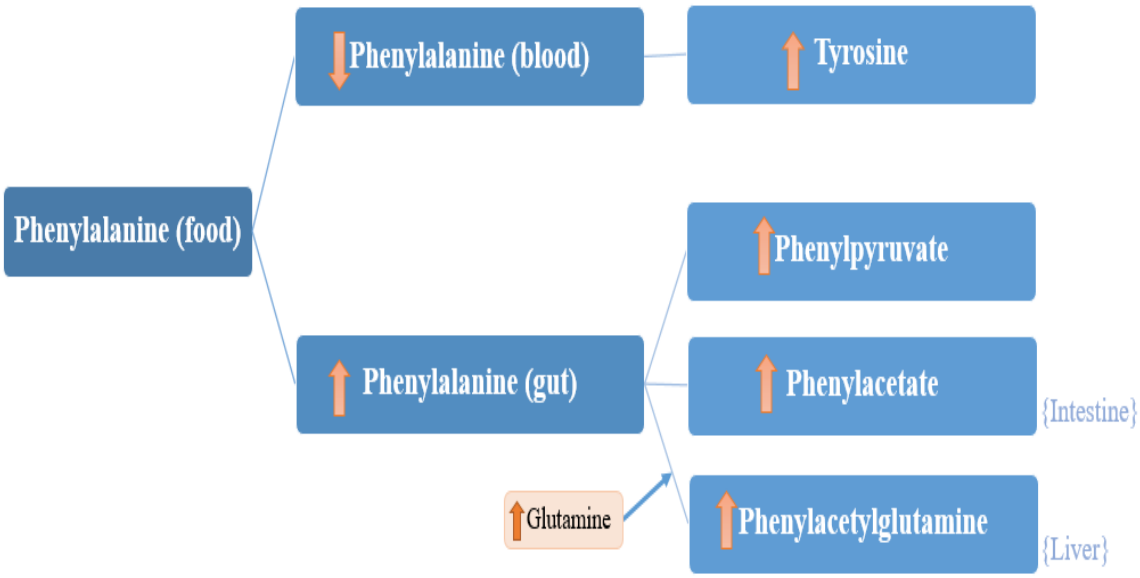

Figure 4. Pathway of PAG formation from phenylacetate.

recommendations based on individual metabolic profiles.

For instance, elevated levels of branched-chain amino acids (BCAAs) may signal an increased risk of insulin resistance, while high normetanephrine concentrations could indicate heightened sympathetic activity and a predisposition to hypertension. Recognizing these patterns enables pharmacists to support timely therapeutic decisions, such as initiating insulin sensitizers like metformin or antihypertensives like beta-blockers.

Similarly, biomarkers such as homovanillic acid and 4-aminohippuric acid may offer insights into dopaminergic activity and renal oxidative stress, respectively, informing non-pharmacologic interventions like stress reduction, antioxidant-rich dietary changes, and adequate hydration. In clinical practice, metabolomic data can be integrated into electronic health records and interpreted in conjunction with conventional parameters—including oral glucose tolerance test results, body mass index and blood pressure to guide personalized treatment algorithms.

Furthermore, in the context of therapeutic drug monitoring, pharmacists can collaborate with other healthcare professionals to adjust pharmacologic regimens based on metabolic pathway activity, particularly when altered profiles suggest hormonal or neurotransmitter dysregulation.

By incorporating metabolomic insights into decision-making processes, clinical pharmacists can contribute to early risk stratification, individualized therapy optimization, and enhanced patient education—ultimately supporting a more proactive, precise, and patient-centered model of GDM management.

Limitations of the Study and Suggestions for Future Research

This study provides valuable insights into the metabolomic alterations associated with GDM. However, its cross-sectional design limits the ability to draw definitive conclusions about causal relationships. Moreover, the relatively small sample size (n = 53) restricts statistical power, which may hinder the detection of subtle metabolic differences and reduce the generalizability of the findings. This study type also increases the risk of type I and type II errors, potentially leading to false-positive or false-negative associations.

To address these limitations and strengthen the clinical relevance of the identified biomarkers, future research should prioritize expanding the sample size and including larger, more diverse populations across multiple geographic and ethnic backgrounds. Longitudinal study designs will be particularly valuable for assessing the temporal stability of metabolic markers and their predictive utility regarding GDM progression, treatment response, and postpartum outcomes.

In addition, integrating metabolomic data with genomics, and dietary assessments could provide a comprehensive systems biology approach, thereby optimizing personalized interventions for GDM. Employing prospective study designs will allow researchers to track metabolic changes over time, facilitating a more robust exploration of causal associations. Advanced statistical methods will be utilized to adjust for potential confounding variables, further enhancing the validity and reproducibility of the results. A critical next step involves transitioning from untargeted to targeted metabolomics to enable focused validation of the biomarkers identified in this study and to gain deeper insights into specific metabolic pathways. Prospective validation in independent cohorts will

https://doi.org/10.18549/PharmPract.2026.1.3344

also be essential to confirm the reproducibility and clinical applicability of these findings. Furthermore, combining proteomics and genomics data will offer a more comprehensive understanding of the molecular mechanisms underlying GDM, supporting the development of precision medicine strategies.

Finally, planned lipidomics studies will explore lipid and amino acid metabolism in greater detail, further elucidating the metabolic dysfunctions associated with GDM and their potential clinical implications.

CONCLUSION

Our study examined metabolite changes in GD women and associated metabolic pathways compared to control. Of the 108 identified metabolites, 31 differed significantly between the GDM and the control groups. In particular, the GDM group showed higher levels of 4-aminohippuric acid, homovanillic acid, alpha-aspartyl-lysine, L-aspartyl-L-phenylalanine, L-valine, L-leucine, and normetanepherine. In contrast, the GDM group had significantly lower levels of citramalic acid, creatinine, D-arginine, and L-glutamine metabolites. Phenylacetate metabolism was the major metabolic pathway affected in GDM group. This is in addition to the ammonia recycling, tyrosine metabolism, pyruvaldehyde degradation pathways. These findings can be employed for further analysis in the development of diagnostic and therapeutic strategies.

AUTHOR CONTRIBUTION

Conceptualization and study design, M.H.S., Y.B., and R.T.T.; Sample collection, A.A., N.B., V.K., R.A., N.K., D.H., M.A., A.B., M.E.; methodology, M.H.S., Y.B., and B.M.S.; Analysis and interpretation of data, M.H.S., Y.B., R.T.T and B.M.S.; writing—original draft preparation, R.T.T., B.M.S.; writing—review and editing, N.B., R.T.T., B.M.S., A.A., S.S., W.E., M.A.Y., K.H.A., E.A.-G., Y.B., and M.H.S.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this study.

References

1. Szmuilowicz ED, Josefson JL, Metzger BE. Gestational Diabetes Mellitus. Endocrinol Metab Clin North Am. 2019 Sep;48(3):479–93.
2. Capula C, Chiefari E, Vero A, Foti DP, Brunetti A, Vero R. Prevalence and predictors of postpartum glucose intolerance in Italian women with gestational diabetes mellitus. Diabetes Res Clin Pract. 2014 Aug;105(2):223–30.
3. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic Syndrome in Childhood: Association With Birth Weight, Maternal Obesity, and Gestational Diabetes Mellitus. Pediatrics. 2005 Mar 1;115(3):e290–6.
4. Muche AA, Olayemi OO, Gete YK. Prevalence and determinants of gestational diabetes mellitus in Africa based on the updated international diagnostic criteria: a systematic review and meta-analysis. Archives of Public Health. 2019 Dec 6;77(1):36.
5. TEH WT, TEEDE HJ, PAUL E, HARRISON CL, WALLACE EM, ALLAN C. Risk factors for gestational diabetes mellitus: Implications for the application of screening guidelines. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2011 Feb;51(1):26–30.
6. Kautzky-Willer A, Harreiter J, Winhofer-Stöckl Y, Bancher-Todesca D, Berger A, Repa A, et al. Gestationsdiabetes (GDM) (Update 2019). Wien Klin Wochenschr. 2019 May 12;131(S1):91–102.
7. Thayer SM, Lo JO, Caughey AB. Gestational Diabetes. Obstet Gynecol Clin North Am. 2020 Sep;47(3):383–96.
8. Yamamoto JM, Kellett JE, Balsells M, García-Patterson A, Hadar E, Solà I, et al. Gestational Diabetes Mellitus and Diet: A Systematic Review and Meta-analysis of Randomized Controlled Trials Examining the Impact of Modified Dietary Interventions on Maternal Glucose Control and Neonatal Birth Weight. Diabetes Care. 2018 Jul 1;41(7):1346–61.
9. Al-Daffaie FM, Al-Mudhafar SF, Alhomsi A, Tarazi H, Almehdi AM, El-Huneidi W, et al. Metabolomics and Proteomics in Prostate Cancer Research: Overview, Analytical Techniques, Data Analysis, and Recent Clinical Applications. Int J Mol Sci. 2024 May 7;25(10):5071.
10. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol. 2016 Jul 16;17(7):451–9.
11. Cui L, Lu H, Lee YH. Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases. Mass Spectrom Rev. 2018 Nov 27;37(6):772–92.
12. Ortmayr K, Causon TJ, Hann S, Koellensperger G. Increasing selectivity and coverage in LC-MS based metabolome analysis. TrAC Trends in Analytical Chemistry. 2016 Sep;82:358–66.
13. Abufares HI, Zenati RA, Soares NC, El-Huneidi W, Dahabiyeh LA, Al-Hroub HM, et al. A non-targeted metabolomics comparative study on plasma of pfizer and sinopharm COVID-19 vaccinated individuals, assessed by (TIMS-QTOF) mass spectrometry. Heliyon. 2024 Aug;10(15):e35443.
14. Hassanein MM, Hagyousif YA, Zenati RA, Al-Hroub HM, Khan FM, Abuhelwa AY, et al. Metabolomics insights into doxorubicin and 5-fluorouracil combination therapy in triple-negative breast cancer: a xenograft mouse model study. Front Mol Biosci. 2025 Jan 13;11.
15. Chen P, Wang S, Ji J, Ge A, Chen C, Zhu Y, et al. Risk Factors and Management of Gestational Diabetes. Cell Biochem Biophys.

2015 Mar 1;71(2):689–94.
16. Babes EE, Bustea C, Behl T, Abdel-Daim MM, Nechifor AC, Stoicescu M, et al. Acute coronary syndromes in diabetic patients, outcome, revascularization, and antithrombotic therapy. Biomedicine & Pharmacotherapy. 2022 Apr;148:112772.
17. Feleke BE, Feleke TE, Adane WG, Kassahun MB, Girma A, Alebachew A, et al. Maternal and newborn effects of gestational diabetes mellitus: A prospective cohort study. Prim Care Diabetes. 2022 Feb;16(1):89–95.
18. Yao D, Shen C, Zhang X, Tang J, Yu J, Tu M, et al. Untargeted metabolomics study of mature human milk from women with and without gestational diabetes mellitus. Food Chem. 2024 Dec;460:140663.
19. Miller AJ, Arnold AC. The renin–angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. Clinical Autonomic Research. 2019 Apr 9;29(2):231–43.
20. Qiu G, Lin Y, Ouyang Y, You M, Zhao X, Wang H, et al. Nontargeted Metabolomics Revealed Novel Association Between Serum Metabolites and Incident Acute Coronary Syndrome: A Mendelian Randomization Study. J Am Heart Assoc. 2023 Jul 4;12(13).
21. Altmaier E, Menni C, Heier M, Meisinger C, Thorand B, Quell J, et al. The Pharmacogenetic Footprint of ACE Inhibition: A Population-Based Metabolomics Study. PLoS One. 2016 Apr 27;11(4):e0153163.
22. Altmaier E, Fobo G, Heier M, Thorand B, Meisinger C, Römisch-Margl W, et al. Metabolomics approach reveals effects of antihypertensives and lipid-lowering drugs on the human metabolism. Eur J Epidemiol. 2014 May 10;29(5):325–36.
23. Plaza A, Merino B, Del Olmo N, Ruiz-Gayo M. The cholecystokinin receptor agonist, CCK-8, induces adiponectin production in rat white adipose tissue. Br J Pharmacol. 2019 Aug 20;176(15):2678–90.
24. Plaza A, Merino B, Cano V, Domínguez G, Pérez-Castells J, Fernández-Alfonso MS, et al. Cholecystokinin is involved in triglyceride fatty acid uptake by rat adipose tissue. Journal of Endocrinology. 2018 Mar;236(3):137–50.
25. Halland M, Bharucha AE. Relationship Between Control of Glycemia and Gastric Emptying Disturbances in Diabetes Mellitus. Clinical Gastroenterology and Hepatology. 2016 Jul;14(7):929–36.
26. Zhou Q, Sun WW, Chen JC, Zhang HL, Liu J, Lin Y, et al. Phenylalanine impairs insulin signaling and inhibits glucose uptake through modification of IRβ. Nat Commun. 2022 Jul 25;13(1):4291.
27. Ríos H, Brusco A, Saavedra JP. Development of serotoninergic chick retinal neurons. International Journal of Developmental Neuroscience. 1997 Oct 15;15(6):729–38.
28. Chu KO, Chan TI, Chan KP, Yip YW, Bakthavatsalam M, Wang CC, et al. Untargeted metabolomic analysis of aqueous humor in diabetic macular edema. Mol Vis. 2022;28:230–44.
29. Liu T, Li J, Xu F, Wang M, Ding S, Xu H, et al. Comprehensive analysis of serum metabolites in gestational diabetes mellitus by UPLC/Q-TOF-MS. Anal Bioanal Chem. 2016 Feb 16;408(4):1125–35.
30. ŠTOLBA P, OPLTOVÁ H, HUŠEK P, NEDVÍDKOVÁ J, KUNEŠ J, DOBEŠOVÁ Z, et al. Adrenergic Overactivity and Insulin Resistance in Nonobese Hereditary Hypertriglyceridemic Rats [a]. Ann N Y Acad Sci. 1993 Jun 17;683(1):281–8.
31. Rodríguez-Romero V, González-Villalva KI, Reyes JL, Franco-Bourland RE, Guízar-Sahagún G, Castañeda-Hernández G, et al. A novel, simple and inexpensive procedure for the simultaneous determination of iopamidol and p-aminohippuric acid for renal function assessment from plasma samples in awake rats. J Pharm Biomed Anal. 2015 Mar;107:196–203.
32. Karabacak M, Cinar Z, Cinar M. A structural and spectroscopic study on para-aminohippuric acid with experimental and theoretical approaches. Spectrochim Acta A Mol Biomol Spectrosc. 2012 Jan;85(1):241–50.
33. Raoof JB, Ojani R, Baghayeri M, Ahmadi F. Fabrication of a fast, simple and sensitive voltammetric sensor for the simultaneous determination of 4-aminohippuric acid and uric acid using a functionalized multi-walled carbon nanotube modified glassy carbon electrode. Analytical Methods. 2012;4(6):1825.
34. Chen H, Yuan B, Miao H, Tan Y, Bai X, Zhao YY, et al. Urine metabolomics reveals new insights into hyperlipidemia and the therapeutic effect of rhubarb. Analytical Methods. 2015;7(7):3113–23.
35. Sun R, Zhang J, Xiong M, Chen Y, Yin L, Pu Y. Metabonomics Biomarkers for Subacute Toxicity Screening for Benzene Exposure in Mice. J Toxicol Environ Health A. 2012 Sep 15;75(18):1163–73.
36. Jin W, Huang P, Chen Y, Wu F, Wan Y. Colorimetric detection of Cr3+ using gold nanoparticles functionalized with 4-amino hippuric acid. Journal of Nanoparticle Research. 2015 Sep 8;17(9):358.
37. Agudiez M, Martinez PJ, Martin-Lorenzo M, Heredero A, Santiago-Hernandez A, Molero D, et al. Analysis of urinary exosomal metabolites identifies cardiovascular risk signatures with added value to urine analysis. BMC Biol. 2020 Dec 14;18(1):192.
38. Oeltmann T, Carson R, Shannon JR, Ketch T, Robertson D. Assessment of O-methylated catecholamine levels in plasma and urine for diagnosis of autonomic disorders. Autonomic Neuroscience. 2004 Nov;116(1–2):1–10.
39. Serednytskyy O, Alonso-Fernández A, Ribot C, Herranz A, Álvarez A, Sánchez A, et al. Systemic inflammation and sympathetic activation in gestational diabetes mellitus with obstructive sleep apnea. BMC Pulm Med. 2022 Dec 18;22(1):94.
40. Holeček M. Why Are Branched-Chain Amino Acids Increased in Starvation and Diabetes? Nutrients. 2020 Oct 11;12(10):3087.
41. McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE, et al. Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. Pediatr Obes. 2013 Feb 7;8(1):52–61.
42. Holeček M. The Role of Skeletal Muscle in The Pathogenesis of Altered Concentrations of Branched-Chain Amino Acids (Valine, Leucine, and Isoleucine) in Liver Cirrhosis, Diabetes, and Other Diseases. Physiol Res. 2021 Jun 30;293–305.
43. Gadgil MD, Ingram KH, Appiah D, Rudd J, Whitaker KM, Bennett WL, et al. Prepregnancy Protein Source and BCAA Intake Are

Associated with Gestational Diabetes Mellitus in the CARDIA Study. Int J Environ Res Public Health. 2022 Oct 29;19(21):14142.
44. Avagliano L, Garò C, Marconi AM. Placental Amino Acids Transport in Intrauterine Growth Restriction. J Pregnancy. 2012;2012:1–6.
45. Cleal JK, Lofthouse EM, Sengers BG, Lewis RM. A systems perspective on placental amino acid transport. J Physiol. 2018 Dec 7;596(23):5511–22.
46. Zheng HY, Wang L, Zhang R, Ding R, Yang CX, Du ZQ. Valine induces inflammation and enhanced adipogenesis in lean mice by multi-omics analysis. Front Nutr. 2024 May 13;11.
47. Calvo MJ, Parra H, Santeliz R, Bautista J, Luzardo E, Villasmil N, et al. The Placental Role in Gestational Diabetes Mellitus: A Molecular Perspective. touchREVIEWS in Endocrinology. 2024;20(1).
48. Zhou Y, Zhao R, Lyu Y, Shi H, Ye W, Tan Y, et al. Serum and Amniotic Fluid Metabolic Profile Changes in Response to Gestational Diabetes Mellitus and the Association with Maternal–Fetal Outcomes. Nutrients. 2021 Oct 18;13(10):3644.
49. Zhao H, Li H, Chung ACK, Xiang L, Li X, Zheng Y, et al. Large-Scale Longitudinal Metabolomics Study Reveals Different Trimester-Specific Alterations of Metabolites in Relation to Gestational Diabetes Mellitus. J Proteome Res. 2018 Dec 12;acs.jproteome.8b00602.
50. Meng X, Zhu B, Liu Y, Fang L, Yin B, Sun Y, et al. Unique Biomarker Characteristics in Gestational Diabetes Mellitus Identified by LC-MS-Based Metabolic Profiling. J Diabetes Res. 2021 Jun 9;2021:1–15.
51. Takeda R, Demura M, Sugimura Y, Miyamori I, Konoshita T, Yamamoto H. Pregnancy-associated diabetes insipidus in Japan-a review based on quoting from the literatures reported during the period from 1982 to 2019. Endocr J. 2021 Apr 28;68(4):375–85.
52. Heymsfield S, Arteaga C, McManus C, Smith J, Moffitt S. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. Am J Clin Nutr. 1983 Mar;37(3):478–94.
53. Hu H, Nakagawa T, Honda T, Yamamoto S, Okazaki H, Yamamoto M, et al. Low serum creatinine and risk of diabetes: The Japan Epidemiology Collaboration on Occupational Health Study. J Diabetes Investig. 2019 Sep 2;10(5):1209–14.
54. Chen N, Zeng R, Xu C, Lai F, Chen L, Wang C, et al. Low Serum Creatinine Levels in Early Pregnancy Are Associated with a Higher Incidence of Postpartum Abnormal Glucose Metabolism among Women with Gestational Diabetes Mellitus: A Retrospective Cohort Study. Nutrients. 2023 May 5;15(9):2193.
55. Wu X, Xie C, Zhang Y, Fan Z, Yin Y, Blachier F. Glutamate–glutamine cycle and exchange in the placenta–fetus unit during late pregnancy. Amino Acids. 2015 Jan 16;47(1):45–53.
56. Prada PO, Hirabara SM, Souza CT de, Schenka AA, Zecchin HG, Vassallo J, et al. RETRACTED ARTICLE:l-glutamine supplementation induces insulin resistance in adipose tissue and improves insulin signalling in liver and muscle of rats with diet-induced obesity. Diabetologia. 2007 Sep 29;50(9):1949–59.
57. Deters BJ, Saleem M. The role of glutamine in supporting gut health and neuropsychiatric factors. Food Science and Human Wellness. 2021 Mar;10(2):149–54.
58. McIntyre KR, Vincent KMM, Hayward CE, Li X, Sibley CP, Desforges M, et al. Human placental uptake of glutamine and glutamate is reduced in fetal growth restriction. Sci Rep. 2020 Oct 1;10(1):16197.
59. Ezrokhi M, Luo S, Trubitsyna Y, Cincotta AH. Neuroendocrine and metabolic components of dopamine agonist amelioration of metabolic syndrome in SHR rats. Diabetol Metab Syndr. 2014 Dec 25;6(1):104.
60. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance. Cell Metab. 2009 Apr;9(4):311–26.
61. Landsberg L, Aronne LJ, Beilin LJ, Burke V, Igel LI, Lloyd-Jones D, et al. Obesity-Related Hypertension: Pathogenesis, Cardiovascular Risk, and Treatment. The Journal of Clinical Hypertension. 2013 Jan 18;15(1):14–33.
62. Aleidi SM, Al Fahmawi H, AlMalki RH, Al Mogren M, Alwahsh M, Mujammami M, et al. Untargeted metabolomics profiling of gestational diabetes mellitus: insights into early diagnosis and metabolic pathway alterations. Front Mol Biosci. 2024 Dec 23;11.
63. Borges Manna L, Syngelaki A, Würtz P, Koivu A, Sairanen M, Pölönen T, et al. First-trimester nuclear magnetic resonance–based metabolomic profiling increases the prediction of gestational diabetes mellitus. Am J Obstet Gynecol. 2024 Dec;
64. Xu Z, Fang J, Wang Y, Wu X, Liu L, Cui X, et al. Targeted metabolomics reveals novel biomarkers for gestational diabetes mellitus. Diabetes Obes Metab. 2025 Apr 30;27(4):2163–72.
65. Razo-Azamar M, Nambo-Venegas R, Meraz-Cruz N, Guevara-Cruz M, Ibarra-González I, Vela-Amieva M, et al. An early prediction model for gestational diabetes mellitus based on metabolomic biomarkers. Diabetol Metab Syndr. 2023 Jun 1;15(1):116.
66. Burzynska-Pedziwiatr I, Jankowski A, Kowalski K, Sendys P, Zieleniak A, Cypryk K, et al. Associations of Arginine with Gestational Diabetes Mellitus in a Follow-Up Study. Int J Mol Sci. 2020 Oct 22;21(21):7811.
67. Yu J, Ren J, Ren Y, Wu Y, Zeng Y, Zhang Q, et al. Using metabolomics and proteomics to identify the potential urine biomarkers for prediction and diagnosis of gestational diabetes. EBioMedicine. 2024 Mar;101:105008.
68. Dudzik D, Zorawski M, Skotnicki M, Zarzycki W, Kozlowska G, Bibik-Malinowska K, et al. Metabolic fingerprint of Gestational Diabetes Mellitus. J Proteomics. 2014 May;103:57–71.
69. Huynh J, Xiong G, Bentley-Lewis R. A systematic review of metabolite profiling in gestational diabetes mellitus. Diabetologia. 2014 Dec 6;57(12):2453–64.

70. De Las Heras J, Aldámiz-Echevarría L, Martínez-Chantar ML, Delgado TC. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. Expert Opin Drug Metab Toxicol. 2017 Apr 3;13(4):439–48.
71. Tian M, Ma S, You Y, Long S, Zhang J, Guo C, et al. Serum Metabolites as an Indicator of Developing Gestational Diabetes Mellitus Later in the Pregnancy: A Prospective Cohort of a Chinese Population. J Diabetes Res. 2021 Feb 5;2021:1–13.