Research Square

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Gestational diabetes mellitus induces sex-specific neonatal metabolomic signatures associated with early metabolic programming

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Abstract

Background

Metabolic signatures in newborns can serve as indicators of the intrauterine conditions to which they were exposed. Furthermore, they provide relevant information regarding metabolic status later in life. The aim of this study was to identify metabolic signatures in newborns exposed to gestational diabetes mellitus (GDM). Additionally, predictive models were developed and validated to identify offspring exposed to GDM.

Methods

This retrospective cohort study included 110 mother-infant dyads. GDM was diagnosed between 24 and 28 weeks of gestation using the criteria established by the International Association of Diabetes and Pregnancy Study Groups (IADPSG). Targeted metabolomics was used to determine circulating metabolites in blood samples obtained from the newborns' heels three days after birth.

Results

GDM-exposed girls had lower carnitine and valine, whereas exposed boys had lower dodecanoylcarnitine than unexposed peers. Across sexes, GDM exposure was associated with higher acetylcarnitine and propionylcarnitine. Pathway enrichment differed by sex, with lower lysine degradation, CoA/pantothenate biosynthesis, and valine pathways in exposed girls, and higher glycine, lipoic acid/porphyrin metabolism and primary bile acid biosynthesis in exposed boys. Exploratory ROC analyses showed that metabolite panels distinguished GDM-exposed from healthy infants with AUCs of 0.766 (95% CI: 0.532–0.917) in females and 0.810 (95% CI: 0.636–0.920) in males.

Conclusion

Our findings suggest that intrauterine exposure to GDM induces sex-specific metabolic programming, resulting in distinct neonatal metabolic signatures in male and female offspring. These metabolomic profiles may facilitate the identification of newborns exposed to GDM while highlighting the importance of considering fetal sex when evaluating the biological consequences of maternal hyperglycemia.

PLAIN ENGLISH SUMMARY

Gestational diabetes mellitus is the most common metabolic disorder, affecting millions of pregnancies worldwide and increasing the risk of offspring developing obesity and type 2 diabetes in adulthood. However, not all newborns respond to this prenatal exposure in the same way. One important factor that has received relatively little attention is biological sex. In this study, we examined blood samples from

newborns collected three days after birth to determine whether newborns exposed to gestational diabetes mellitus had distinct metabolic profiles depending on their biological sex. Girls exposed to gestational diabetes mellitus showed lower levels of metabolites involved in energy production and amino acid metabolism, while boys showed changes in metabolites related to fatty acid utilization and bile acid metabolism. We also generated metabolomic models to identify girls and boys exposed to gestational diabetes mellitus. These models accurately distinguished exposed newborns from healthy ones, suggesting that neonatal metabolomics could help identify those who require further monitoring. Our results suggest that biological sex influences the early metabolic response to gestational diabetes. Considering these differences from birth could improve our understanding of disease risk and support more personalized strategies for preventing later metabolic disorders.

HIGHLIGHTS

- GDM induces distinct sex-specific neonatal metabolomic signatures at birth.
- Female offspring exposed to GDM showed reduced carnitine and valine metabolism.
- Male offspring exposed to GDM exhibited altered acylcarnitines and bile acid-related pathways.
- Sex-specific metabolite panels identified GDM-exposed newborns with good predictive performance.

INTRODUCTION

Gestational diabetes (GDM) is defined as diabetes diagnosed during the second or third trimester of pregnancy, provided it is not overt diabetes before gestation or another type of diabetes existing during pregnancy [1]. It is the most common metabolic disorder during this period, affecting between 7% and 20% of pregnancies worldwide [2]. Its clinical significance lies in the short- and long-term complications that can affect both the mother and the offspring [3]. According to the Developmental Origins of Health and Disease (DOHaD) theory, GDM may predispose offspring to an increased risk of developing chronic metabolic diseases, such as type 2 diabetes and obesity [4].

Metabolomics quantitatively and qualitatively evaluates the metabolites present in biological samples. This approach allows for the study of an individual's metabolic state at a specific time [5]. In newborns, it also contributes to understanding the possible origins and early mechanisms related to disease development [6]. Most metabolomic studies in newborns have been performed using umbilical cord blood. These studies suggest that various factors (maternal body mass index (BMI), sex of the newborn, type of delivery, and exposure to GDM) are associated with higher concentrations of ketone bodies, valine, and glycerol. However, the available findings remain inconsistent [7, 8].

The early postnatal period is a critical window for metabolic adaptation, during which neonates rapidly transition from continuous transplacental nutrient supply to intermittent feeding. In this transition, fatty acid oxidation becomes an essential energy source, compensating for the abrupt cessation of placental glucose supply [9]. In the first few days of life, energy metabolism is catabolic. As the newborn adapts to

extrauterine life and begins feeding, metabolism gradually shifts toward an anabolic state. During the preceding catabolic phase, proteins are broken down, releasing glucogenic amino acids that are used to generate energy [10]. Neonatal blood allows us to evaluate how the neonate's body responds to the sudden transition from fetal life (e.g., placental nutrient supply) to extrauterine life [11].

The present study aims to identify metabolic signatures in newborns exposed to GDM. Additionally, predictive models were developed and validated to identify offspring exposed to GDM.

METHODS

Study population and clinical data

Between January 1, 2024, and December 31, 2025, pregnant women were randomly recruited from the Obstetrics Service of the General Hospital of Mexico “Dr. Eduardo Liceaga,” located in Mexico City, who met the following inclusion criteria: healthy singleton pregnant women, between 18 and 40 years of age, with no history of type 2 diabetes, hypertension, dyslipidemia, polycystic ovary syndrome, and/or gestational complications (preeclampsia and eclampsia). Between weeks 24 and 28 of gestation, an oral glucose tolerance test was performed to establish the diagnosis of GDM. The diagnosis of GDM was established according to IADPSG criteria (fasting plasma glucose ≥ 5.1 mmol/L, one-hour postprandial plasma glucose ≥ 10.0 mmol/L, or two-hour postprandial plasma glucose ≥ 8.5 mmol/L). All participants provided written informed consent. Dried blood samples were collected from the newborn screening cards of 110 newborns between 2024 and 2025, including 44 cases of GD and 66 healthy controls. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the General Hospital of Mexico “Dr. Eduardo Liceaga” (DI/24/303/05/3).

Metabolomics analysis

Serum concentrations of acylcarnitines, free carnitine, and amino acids were measured by targeted metabolomics using electrospray ionization tandem mass spectrometry (Quattro Micro API tandem MS, Waters Inc., Milford, MA, USA). Serum metabolites were analyzed using the NeoBase Non-derivatized MS/MS Kit (Perkin Elmer, Waltham, MA, USA) [5].

Measurements of Covariates

Both maternal covariates (age, parity, weight, height and type of delivery) and neonatal covariates (birth weight and length, gestational age and sex) were obtained from medical records.

Statistical analysis

A descriptive statistical analysis was performed. Parametric variables were expressed as mean and standard deviation, and non-parametric variables as median and 25th and 75th percentiles. Normality

was assessed using the Kolmogorov-Smirnov test. For clinical data, t-tests were applied to metric variables and χ^2 tests to nominal variables.

In metabolomic analyses, data were normalized to the median, log10-transformed, and scaled using Pareto analysis. Differential metabolites were identified using the t-test, expression change (EC), and AUROC. Differences between groups were assessed using a one-way PLS-DA, validated with 1000 bootstrap permutations. Furthermore, random forests, with frequency and mean criteria, were used to estimate the contribution of each variable to identify the GDM-exposed group versus the control group.

For biomarker analysis, the dataset was randomly split into two subsets: training (80%) and validation (20%). To generate independent training-validation pairs, this process was repeated 10 times. Subsequently, univariate receiver operating characteristic (ROC) curve analyses were performed to identify metabolites with the greatest discriminatory capacity within each training subset. To capture this discriminatory capacity, the 10 highest-ranked metabolites from each training subset were recorded based on their p-values. The frequency of each metabolite was then assessed across the top ten lists of all iterations. This procedure was repeated in the validation subsets to evaluate the consistency of the final frequency of each metabolite. Candidates that were consistently identified among the top 5 across both subset types were selected for further multivariate ROC curve-based exploratory analysis.

Multivariate ROC analysis was performed using metabolic signatures of 2, 3, 4, and 5 metabolites. Classification and ranking methods employed algorithms such as Support Vector Machines (SVM), Partial Least Squares Discriminant Analysis (PLS-DA), Univariate AUROC, T-statistic, and Random Forests (RF). ROC curves were generated using Monte Carlo Cross-Validation (MCCV) with balanced sampling, using two-thirds of the samples in each iteration to evaluate metabolite relevance and validating with the remaining one-third. This process was replicated 2,000 times to assess model performance, and the predictive accuracies across the different features were reported. Statistical analyses were performed on MetaboAnalyst 6.0 (McGill University, Toronto, ON, Canada). For all statistical analyses, significance was assumed when the p-value was less than 0.05.

RESULTS

Characteristics of study population

Table 1 summarizes maternal and neonatal characteristics by GDM status. Mothers with GDM were significantly older than healthy controls (29.64 ± 7.02 vs. 23.72 ± 4.87 years, $p < 0.001$). They also had higher pregestational BMI (27.92 ± 5.39 vs. 24.45 ± 4.82 kg/m², $p < 0.001$), and higher BMI post-pregnancy (31.68 ± 5.02 vs. 28.52 ± 4.54 kg/m², $p < 0.001$). No significant difference was observed in maternal height between groups ($p = 0.632$). Women with GDM also had greater parity than healthy mothers (2.43 ± 1.32 vs. 1.62 ± 1.03, $p = 0.007$). Regarding family history, the prevalence of type 2 diabetes and hypertension was similar between groups. However, a family history of dyslipidemia tended

to be more frequent among women with GDM than among healthy mothers, reaching a borderline non-significant result. (p = 0.050).

Table 1
Maternal and offspring characteristics

Maternal age, years (SD)		**Healthy**	**GDM**	**p-values**
		23.72 (4.87)	**29.64 (7.02)**	**< 0.001**
Weight, kg (SD)		70.33 (11.71)	78.36 (14.95)	**< 0.001**
Height, m (SD)		1.57 (0.06)	1.57 (0.07)	0.632
Pregestational BMI, kg/m^2 (SD)		24.45 (4.82)	27.92 (5.39)	**< 0.001**
BMI, kg/m^2 (SD)		28.52 (4.54)	31.68 (5.02)	**< 0.001**
Parity (SD)		1.62 (1.03)	2.43 (1.32)	**0.007**
Family history	T2D (%)	28 (30%)	25 (32%)	0.777
	Hypertension (%)	20 (22%)	17 (22%)	0.958
	Dyslipidemia (%)	5 (5.5%)	11 (14%)	**0.05**
Newborn		Healthy	GDM	p-values
Gestational weeks (SD)		38.93 (1.49)	38.55 (1.43)	0.089
Weight, grams (SD)		3025.6 (409.47)	3098.96 (379.87)	0.234
Height, cm (SD)		49.38 (2.19)	49.14 (2.07)	0.477
Sex (Female %)		41 (45%)	39 (50.6%)	0.544
C-section (%)		14 (16%)	37 (49%)	**< 0.001**

Metrical variables are presented with mean and standard deviation; statistical testing was calculated with a T-test and effect size with Cohen's d. Nominal variables are presented in frequency and percentages; statistical testing was calculated with Chi square and effect size with Cramer's V.

No significant differences were observed in neonatal gestational age at delivery (38.55 ± 1.43 vs. 38.93 ± 1.49 weeks, p = 0.089), birth weight (3098.96 ± 379.87 vs. 3025.60 ± 409.47 g, p = 0.234), birth length (49.14 ± 2.07 vs. 49.38 ± 2.19 cm, p = 0.477), or the proportion of female newborns (50.6% vs. 45.0%, p = 0.544). In contrast, C-section was significantly more frequented among women with GDM compared with healthy mothers (49% vs. 16%, p < 0.001).

Sex-specific metabolic alterations associated with prenatal exposure to GDM

Metabolic differences associated with exposure to GDM were observed and varied by sex. Girls exposed to GDM exhibited significantly lower concentrations of free carnitine (C0) and valine compared to those who were unexposed (Fig. 1A **and C**). In contrast, boys exposed to GDM showed lower concentration of dodecanoylcarnitine (AC12) compared to those who were unexposed (Fig. 1B **and D**). On the other hand, it was found that, regardless of sex, offspring of mothers with GDM exhibited higher concentrations of acetylcarnitine (C2) and propionylcarnitine (C3) (Fig. 1C-D).

Metabolic pathway enrichment analysis revealed sex-specific patterns. Girls exposed to GDM showed lower enrichment in pathways related to lysine degradation, coenzyme A (CoA) and pantothenate biosynthesis, and valine biosynthesis and degradation (Fig. 2A). Conversely, boys exposed to GDM exhibited greater enrichment in pathways involving in glycine, lipoic acid/porphyrin metabolism and primary bile acid biosynthesis (Fig. 2B).

Discriminatory potential of metabolite panels for distinguishing GDM from healthy subjects

Our results indicated sexual dimorphism in the metabolic programming induced by exposure to GDM; therefore, an exploratory multivariate analysis using ROC curves was conducted to evaluate the discriminatory potential of metabolite panels to distinguish between male and female infants exposed to GDM and healthy subjects. Figure 3A shows that, in females, the optimal model consisted of two metabolites, yielding an AUC of 0.766 (95% CI: 0.532–0.917). In males, (Fig. 3B) the optimal model consisted of five metabolites, achieving an AUC of 0.810 (95% CI: 0.636–0.920), indicating that the selected metabolites had good prediction performance. Predicted class probability plots showed partial discrimination between healthy offspring and those exposed to GDM in both sexes (Figs. 3C and 3D). In males, healthy individuals tended to display higher predicted probabilities than GDM-exposed participants, although considerable overlap remained between groups. A comparable pattern was observed in females, where healthy participants also tended to present higher predicted probabilities; however, group overlap was more extensive, indicating lower classification certainty than in males.

An analysis of model predictive accuracy based on the number of key features used to discriminate between groups showed that, for females, a two-variable prediction model achieved 74.0% accuracy in distinguishing girls exposed to GDM from the healthy group (Fig. 3E). In males, the four-metabolite model provides a prediction accuracy of 72.6% to discriminate the boys exposed to GDM from the healthy group (Fig. 3F). To select the candidate metabolites, the criteria of medium importance were used. In females, the most important metabolites were butyrylcarnitine (C4) and C2 (Fig. 3G), whereas in males they were 3-hydroxytetradecanoylcarnitine (C14OH), dodecanoylcarnitine (C12), valine, and glycine (Fig. 3G). These findings suggest that discrimination in females was driven more strongly by a small number of highly influential metabolites, whereas in males the discriminatory contribution was distributed more evenly across multiple metabolites.

DISCUSSION

In the present study, mothers with GDM were older, had a history of dyslipidemia, and had a higher body mass index (BMI) than women with uncomplicated pregnancies. Razo-Azamar indicates that gestational BMI was positively associated with GDM, with each 1 kg/m² increase corresponding to a 12.6% increase in the odds of GDM ($p = 0.033$). Additionally, the combined contribution of maternal age, parity, and gestational BMI significantly predicted GDM risk ($p = 0.0001$) [5].

Girls exposed to GDM exhibited significantly lower concentrations of C0 and valine, together with reduced enrichment of pathways involved in lysine degradation, coenzyme A (CoA) and pantothenate biosynthesis, and valine biosynthesis and degradation. These coordinated metabolite- and pathway-level alterations suggest an early disruption of mitochondrial energy metabolism and amino acid homeostasis.

C0 plays a central role in mitochondrial energy metabolism by facilitating the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation. In addition, C0 acts as a buffer for acyl residues generated during amino acid catabolism by forming acylcarnitines, thereby regenerating free coenzyme A (CoA) and maintaining an adequate free CoA/acyl-CoA ratio required for efficient mitochondrial fatty acid oxidation [12]. Accordingly, the concomitant reduction in C0 concentrations and enrichment of the CoA and pantothenate biosynthesis pathway may reflect impaired CoA availability and mitochondrial fatty acid oxidation, potentially predisposing female offspring exposed to GDM to later metabolic dysfunction.

Likewise, the lower valine concentrations observed in girls were accompanied by reduced enrichment in valine biosynthesis and degradation pathways, supporting early perturbations in branched-chain amino acid metabolism. Although elevated circulating BCAAs are frequently associated with insulin resistance and obesity later in life [13–15], reduced neonatal values have also been linked to adverse growth trajectories. In a prospective study from four European cohorts, lower cord blood valine concentrations were associated with an increased risk of childhood overweight/obesity, and including valine in predictive models substantially improved prediction of overweight compared with models using traditional risk factors alone [16]. These findings suggest that altered valine metabolism at birth may represent an early metabolic signature of developmental programming associated with adulthood obesity.

Finally, the lower enrichment of the lysine degradation pathway may further support impaired tricarboxylic acid (TCA) cycle, reduce ATP production, and limit fatty acid oxidation, driving metabolic inflexibility and fat accumulation associated with obesity and insulin resistance. Lysine is an essential ketogenic amino acid with its main catabolism via saccharopine to α- aminoadipic acid, ultimately resulting in acetoacetyl-CoA, which enters the TCA in liver mitochondria [17]. Lysine is also an essential precursor of carnitine via trimethylation to trimethyllysine, which provides substrates for eventual conversion to carnitine [18]. Recently, a study revealed elevated serum lysine levels in children with obesity [19]. Additionally, the authors demonstrated that a lysine-restricted diet may effectively

ameliorate childhood obesity [19]. Regarding the relation between lysine and insulin, a study found that lysine concentration increased during hyperinsulinemia in the insulin-resistant individual due to decreased lysine metabolic clearance rate [20]. Interestingly, regarding lysine degradation, boys exposed to GDM showed greater enrichment in this pathway, suggesting a distinct metabolic adaptation to the intrauterine environment in male offspring.

Boys exposed to GDM showed lower concentrations of C12, compared with unexposed boys. This finding was accompanied by greater enrichment of pathways related to primary bile acid biosynthesis and glycine, serine, and threonine metabolism. The lower concentration of C12, a medium-chain acylcarnitine generated during β-oxidation of long-chain fatty acids, may reflect alterations in fatty acid oxidative flux. Experimental evidence has shown that impaired tricarboxylic acid (TCA) cycle flux is sufficient to reduce complete lipid oxidation, resulting in a metabolic shift toward greater reliance on glucose oxidation [21]. Consistent with this mechanism, skeletal muscle from adults with type 2 diabetes exhibits an increased fasting respiratory quotient, indicative of preferential glucose utilization over fatty acid oxidation [22, 23]. Under these conditions, the production of medium-chain acylcarnitines derived from long-chain fatty acid β-oxidation, including C12, is reduced. Although these observations were reported in adult skeletal muscle, the lower C12 concentrations observed in boys exposed to GDM may similarly reflect early alterations in mitochondrial substrate utilization and fatty acid oxidative flux induced by the intrauterine diabetic environment.

Evidence from pediatric populations further supports the relevance of this finding. In a metabolomic study of adolescents, lower concentrations of medium- and short-chain acylcarnitines were observed in individuals with type 2 diabetes compared with their normal-weight and obese peers, suggesting that reduced acylcarnitine availability may accompany early disturbances in metabolic homeostasis [24]. The authors proposed that these metabolites may reflect impaired oxidative metabolism and reduced metabolic flexibility. Interestingly, the association was particularly evident in males, raising the possibility that sex-specific metabolic pathways contribute to the development of insulin resistance during growth and maturation. Although the neonatal period differs substantially from adolescence, the lower C12 concentrations observed in boys exposed to GDM may represent an early manifestation of altered mitochondrial substrate partitioning.

Among the sex-specific metabolic alterations identified in the present study, boys exposed to GDM exhibited greater enrichment of the primary bile acid biosynthesis, lipoic acid, porphyrin, and glycine pathways. Primary bile acids are synthesized in the liver and predominantly conjugated with glycine (75%) and taurine (25%) [25]. Beyond its role in bile acid synthesis, glycine is a precursor for several metabolites, such as porphyrins, purines, glutathione, heme, and creatine. Furthermore, lipoic acid serves as an essential cofactor in the glycine cleavage system, linking glycine catabolism to mitochondrial energy metabolism [26]. Collectively, these metabolic relationships suggest that the higher glycine concentrations observed in boys exposed to GDM may contribute to the coordinated enrichment of these interconnected pathways.

Interestingly, several studies indicate that glycine concentrations are lower in obese subjects compared to age-matched healthy controls [27, 28]. In contrast, we observed higher glycine concentrations in boys exposed to GDM, suggesting that neonatal metabolic adaptations to an intrauterine hyperglycemic environment may differ substantially from the metabolic disturbances described in obesity later in life. This interpretation is supported by previous studies showing that small-for-gestational-age newborns exhibit higher circulating concentrations of glycine, alanine, tyrosine, citrulline, ornithine, and proline than appropriate-for-gestational-age newborns [10, 29]. Liu et al. suggest that these concentrations could be due to neonates born small for gestational age experiencing normal nutrient absorption but insufficient nutrient utilization [29]. Likewise, the increased glycine concentration observed in GDM-exposed newborns may represent an adaptive response to the altered intrauterine metabolic environment, although the underlying mechanisms remain to be elucidated.

Regardless of sex, offspring of mothers with GDM exhibited higher concentrations of acetylcarnitine (C2) and propionylcarnitine (C3). C2 is the most abundant short-chain acylcarnitine previously shown to be elevated in insulin-resistant states and positively associated with cord blood leptin, a surrogate marker of adiposity [30]. Elevated C2 concentrations may indicate increased mitochondrial acetyl-CoA generation or reduced oxidative capacity, leading to the accumulation of acetyl groups that are buffered as C2. Also, C2 could be a marker of metabolic inflexibility. Excess acetyl-CoA favors the formation of malonyl-CoA, which limits mitochondrial alternation between glucose and fatty acids during fasting or postprandial periods. This alteration favors the accumulation of acylcarnitines in the blood, such as C2, and can affect insulin action [31].

Similarly, C3, which is derived primarily from the catabolism of branched-chain amino acids and odd-chain fatty acids, may reflect increased flux through amino acid oxidation or incomplete mitochondrial substrate utilization. Elevated circulating C3 and C4 have been associated with insulin resistance, body fat, impaired mitochondrial function, and an increased risk of future cardiometabolic disease [32–35]. This aligns with evidence that offspring of mothers with GDM are at higher risk of metabolic syndrome, cardiovascular disease, and obesity [32].

Finally, in Mexico, the prevalence of GDM has risen from 4% to 30% over the last thirty years. Unfortunately, most data collected on the incidence of GDM are retrospective and based on maternal self-reporting; consequently, the true incidence of GDM in the country likely remains unknown [36]. Furthermore, the lack of screening programs results in many women going undiagnosed and untreated [37]. In the absence of a prenatal GDM diagnosis, identifying children exposed to this adverse intrauterine environment would enable the early detection of individuals at higher risk of developing metabolic diseases in adulthood. Therefore, we developed and validated a predictive model using machine learning and circulating metabolites to identify offspring of mothers exposed to GDM. Given that our results indicated sexual dimorphism in the metabolic programming induced by exposure to GDM, our models were adjusted according to the sex of the neonate. Exploratory ROC analyses showed that metabolite panels distinguished GDM-exposed from healthy infants. In females, the optimal model consisted of two metabolites (C4 and C2), yielding an AUC of 0.766 (95% CI: 0.532–0.917). In males, the

optimal model consisted of five metabolites (C14OH, C12, valine, and glycine), achieving an AUC of 0.810 (95% CI: 0.636–0.920). Evidence on metabolomic models for identifying children exposed to GDM is limited, as most published GDM metabolomics studies focus on compounds in maternal fluids (amniotic fluid, plasma, serum, urine) associated with or predictive of GDM. In the study by Perng et al., although no AUC was reported, a metabolomic signature associated with GDM exposure and cardiometabolic risk during childhood (ages 6–14) and adolescence was identified. Interestingly, this metabolomic signature was associated with greater adiposity, higher leptin concentration, and lower insulin sensitivity in girls, but not in boys. The authors note that GDM exposure is associated with earlier onset of puberty, with stronger or exclusive effects observed in female offspring [38]. We believe our findings indicate that sexual dimorphism mediated by GDM exposure is observable from birth; longitudinal studies are required to determine whether these metabolite differences have long-term impacts depending on the sex of the offspring. Finally, in the study by Yin et al., although samples from the neonate or umbilical cord blood were not used, metabolites with the best predictive capacity for identifying neonatal hypoglycemia (AUC = 0.871) and macrosomia (AUC = 0.943) were evaluated using maternal serum obtained at the time of GDM diagnosis[39].

CONCLUSION

Our findings suggest that intrauterine exposure to GDM induces sex-specific metabolic programming, resulting in distinct neonatal metabolic signatures in male and female offspring. These metabolomic profiles may facilitate the identification of newborns exposed to GDM while highlighting the importance of considering fetal sex when evaluating the biological consequences of maternal hyperglycemia. Such sex-dependent metabolic signatures may improve early risk stratification and provide insight into the mechanisms underlying differential susceptibility to metabolic disorders later in life.

Abbreviations

AC12 (C12)
Dodecanoylcarnitine
AUC
Area Under the Receiver Operating Characteristic Curve
AUROC
Area Under the Receiver Operating Characteristic Curve
BMI
Body Mass Index
C0
Free Carnitine
C2
Acetylcarnitine
C3
Propionylcarnitine

C4
Butyrylcarnitine
C14OH
3-Hydroxytetradecanoylcarnitine
CI
Confidence Interval
CoA
Coenzyme A
DOHaD
Developmental Origins of Health and Disease
EC
Expression Change
GDM
Gestational Diabetes Mellitus
Gly
Glycine
IADPSG
International Association of Diabetes and Pregnancy Study Groups
MCCV
Monte Carlo Cross-Validation
MS/MS
Tandem Mass Spectrometry
PLS-DA
Partial Least Squares Discriminant Analysis
RF
Random Forest
ROC
Receiver Operating Characteristic
SVM
Support Vector Machine
TCA
Tricarboxylic Acid
Val
Valine
VIP
Variable Importance in Projection

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the General Hospital of Mexico “Dr. Eduardo Liceaga” (DI/24/303/05/3).

Consent for publication

Not applicable

Availability of data and material

The raw metabolite data are included in the Supporting Information. Further datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request

Competing interests

The authors declare no competing interests

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Authors' contributions

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References

1. Diabetes* A. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes—2026. Diabetes Care. 2025;49(Supplement1):S27–49.
2. Frankevich NA, et al. Amino Acid Dysregulation in the Mother–Fetus Unit: Multi-Compartment Metabolomic Signatures of Gestational Diabetes Mellitus and Fetal Macrosomia. Int J Mol Sci. 2026;27(8):3346.
3. Li W et al. Early pregnancy biomarkers for gestational diabetes mellitus prediction: a systematic review and meta-analysis of routine laboratory, metabolic, and inflammatory markers. Front

Endocrinol, 2026. 17.
4. Thornton JM et al. Multigenerational diabetes mellitus. Front Endocrinol, 2024. 14.
5. Razo-Azamar M, et al. An early prediction model for gestational diabetes mellitus based on metabolomic biomarkers. Volume 15. Diabetology & Metabolic Syndrome; 2023. 1.
6. Wang S, et al. Investigation of newborn blood metabolomics in varying intrauterine growth conditions. J Pediatr (Rio J). 2025;101(1):74–81.
7. Lowe WL Jr., et al. Maternal BMI and Glycemia Impact the Fetal Metabolome. Diabetes Care. 2017;40(7):902–10.
8. Mansell T, et al. The newborn metabolome: associations with gestational diabetes, sex, gestation, birth mode, and birth weight. Pediatr Res. 2022;91(7):1864–73.
9. Demirel A et al. Early metabolic biomarkers in neonates of diabetic mothers: predictive insights from amino acid and acylcarnitine profiling. J Maternal-Fetal Neonatal Med, 2025. 38(1).
10. Beken S et al. Early Postnatal Metabolic Profile in Neonates With Different Birth Weight Status: A Pilot Study. Front Pead, 2021. 9.
11. Clinck B, Allison C, Patino F. The transition to life outside the womb: a review of neonatal physiology. Volume 26. Anaesthesia & Intensive Care Medicine; 2025. pp. 719–29. 11.
12. Nambo-Venegas R et al. A predictive model for neoadjuvant therapy response in breast cancer. Metabolomics, 2025. 21(2).
13. Gumus Balikcioglu P, et al. Branched-chain α-keto acids and glutamate/glutamine: Biomarkers of insulin resistance in childhood obesity. Volume 6. Endocrinology, Diabetes & Metabolism; 2023. 1.
14. Jachthuber Trub C, et al. Impact of lifestyle Intervention on branched-chain amino acid catabolism and insulin sensitivity in adolescents with obesity. Volume 4. Endocrinology, Diabetes & Metabolism; 2021. 3.
15. Öge Enver E et al. Plasma amino acid profiles in pediatric obesity: potential biomarkers for the early assessment of metabolic risk. Front Pead, 2025. 13.
16. Handakas E, et al. Cord blood metabolic signatures predictive of childhood overweight and rapid growth. Int J Obes. 2021;45(10):2252–60.
17. Tan Y, Chrysopoulou M, Rinschen MM. Integrative physiology of lysine metabolites. Physiol Genomics. 2023;55(12):579–86.
18. Kalogeropoulou D, et al. Lysine ingestion markedly attenuates the glucose response to ingested glucose without a change in insulin response. Am J Clin Nutr. 2009;90(2):314–20.
19. Zhao F et al. A lysine-restricted diet ameliorates obesity via enrichment of Parabacteroides goldsteinii and 1,4-methylimidazoleacetic acid. Nat Commun, 2025. 16(1).
20. Chang AY, et al. Insulin Regulation of Lysine and α-Aminoadipic Acid Dynamics and Amino Metabolites in Women With and Without Insulin Resistance. Diabetes. 2024;73(10):1592–604.
21. Bene J, et al. Similarities in serum acylcarnitine patterns in type 1 and type 2 diabetes mellitus and in metabolic syndrome. Ann Nutr Metab. 2013;62(1):80–5.

22. Gaster M. Reduced TCA flux in diabetic myotubes: A governing influence on the diabetic phenotype? Biochem Biophys Res Commun. 2009;387(4):651–5.
23. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes. 2000;49(5):677–83.
24. Newbern D, et al. Sex differences in biomarkers associated with insulin resistance in obese adolescents: metabolomic profiling and principal components analysis. J Clin Endocrinol Metab. 2014;99(12):4730–9.
25. Alves A et al. Glycine Metabolism and Its Alterations in Obesity and Metabolic Diseases. Nutrients, 2019. 11(6).
26. Wang W, et al. Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids. 2013;45(3):463–77.
27. Yan-Do R, Macdonald PE. Impaired Glycine-mia in Type 2 Diabetes and Potential Mechanisms Contributing to Glucose Homeostasis. Endocrinology. 2017;158(5):1064–73.
28. Bugajska J, Sztefko K. Plasma amino acid profile in obese children, adolescents, and adults: similarities or differences — a narrative review. Endokrynologia Polska. 2025;76(6):611–8.
29. Liu Q, et al. Analysis of amino acids and acyl carnitine profiles in low birth weight, preterm, and small for gestational age neonates. J Matern Fetal Neonatal Med. 2017;30(22):2697–704.
30. Kadakia R, et al. Cord Blood Metabolites Associated with Newborn Adiposity and Hyperinsulinemia. J Pediatr. 2018;203:144–e1491.
31. Kadakia R, et al. Cord Blood Metabolomics: Association With Newborn Anthropometrics and C-Peptide Across Ancestries. J Clin Endocrinol Metab. 2019;104(10):4459–72.
32. Yang Y et al. *Associations Between Gestational Diabetes Mellitus and Neonatal Acyl Metabolic Profiles: An Empirical Study Based on a Birth Cohort.* Diabetes, Metabolic Syndrome and Obesity, 2026. Volume 19: pp. 1–13.
33. Lee YH, Park S. Genetic and Lifestyle-Related Factors Influencing Serum Hyper-Propionylcarnitine Concentrations and Their Association with Metabolic Syndrome and Cardiovascular Disease Risk. Int J Mol Sci, 2023. 24(21).
34. Mai M, et al. Serum Levels of Acylcarnitines Are Altered in Prediabetic Conditions. PLoS ONE. 2013;8(12):e82459.
35. Mastrangelo A, et al. Insulin resistance in prepubertal obese children correlates with sex-dependent early onset metabolomic alterations. Int J Obes. 2016;40(10):1494–502.
36. Dainelli L, et al. Screening and management of gestational diabetes in Mexico: results from a survey of multilocation, multi-health care institution practitioners. Volume 11. Metabolic Syndrome and Obesity: Targets and Therapy,: Diabetes; 2018. pp. 105–16.
37. Martinez-Juarez LA et al. A strategic framework for managing gestational diabetes in Mexico. Global Health Res Policy, 2025. 10(1).

38. Perng W, et al. A prospective study of associations between in utero exposure to gestational diabetes mellitus and metabolomic profiles during late childhood and adolescence. Diabetologia. 2020;63(2):296–312.
39. Yin X et al. Metabolic profiles in gestational diabetes mellitus can reveal novel biomarkers for prediction of adverse neonatal outcomes. Front Pead, 2024. 12.

Figures

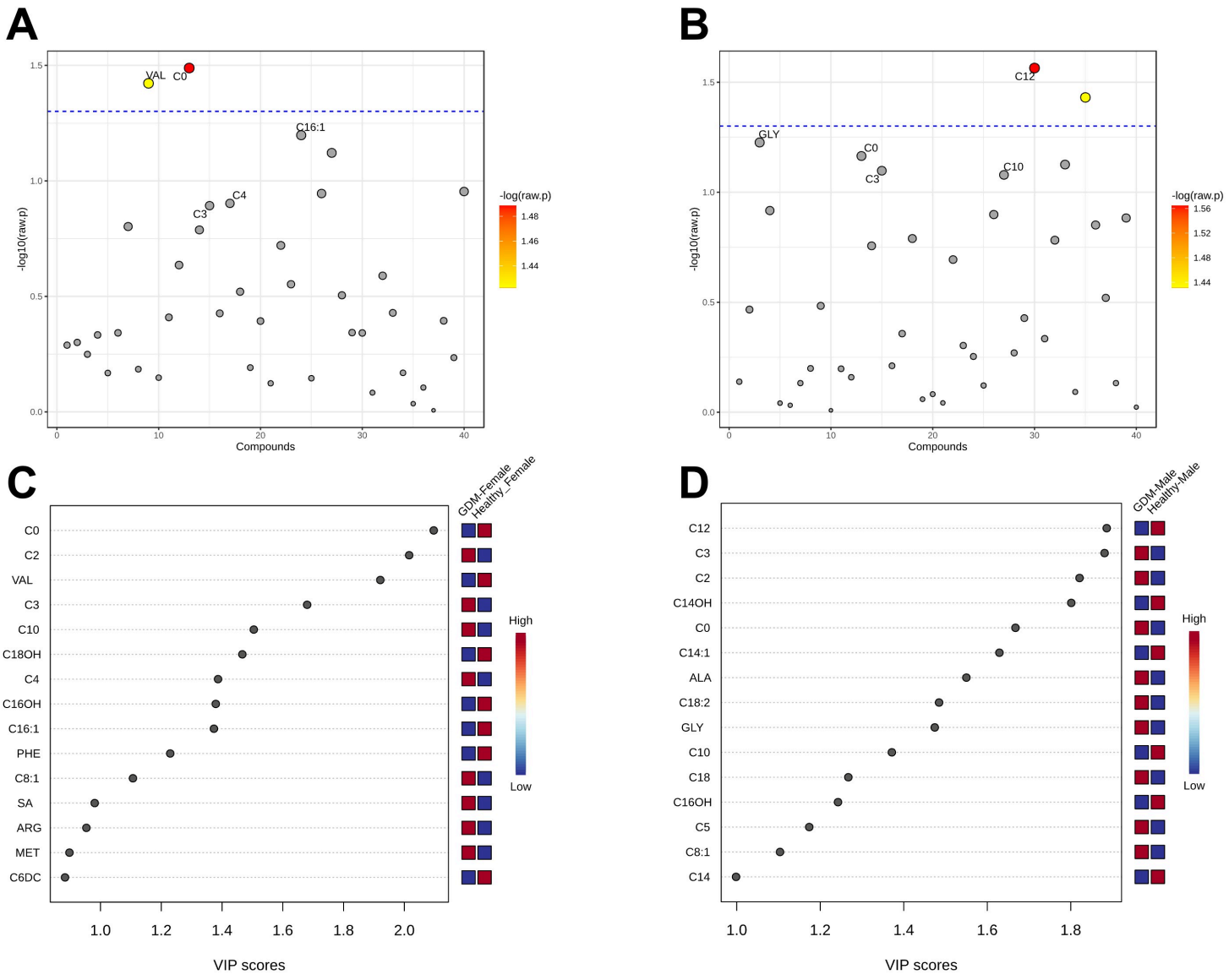


Figure 1

One factor analysis results. **A)** Raw T-test results between GDM and healthy females showing that Val and C0 achieved a p value below 0.05 and thus significance. **B)**The PLSDA Biplot between GDM females /red circles) and healthy females (green circles). The explained variances are shown in parentheses C1 with 13.9% and C2 with 5.4%. **C)** Top 15 metabolites with highest VIP scores to identify between GDM

females and healthy Females. **D)** Raw T-test results between GDM and healthy males showing that C12 and C14OH achieved a p value below 0.05 and thus significance. **E)** The PLSDA Biplot between GDM males (red circles) and healthy males (green circles). The variances explained are shown in parentheses C1 with 12.4% and C2 with 10%. **F)** Top 15 metabolites with highest VIP scores to identify between GDM males and healthy males.

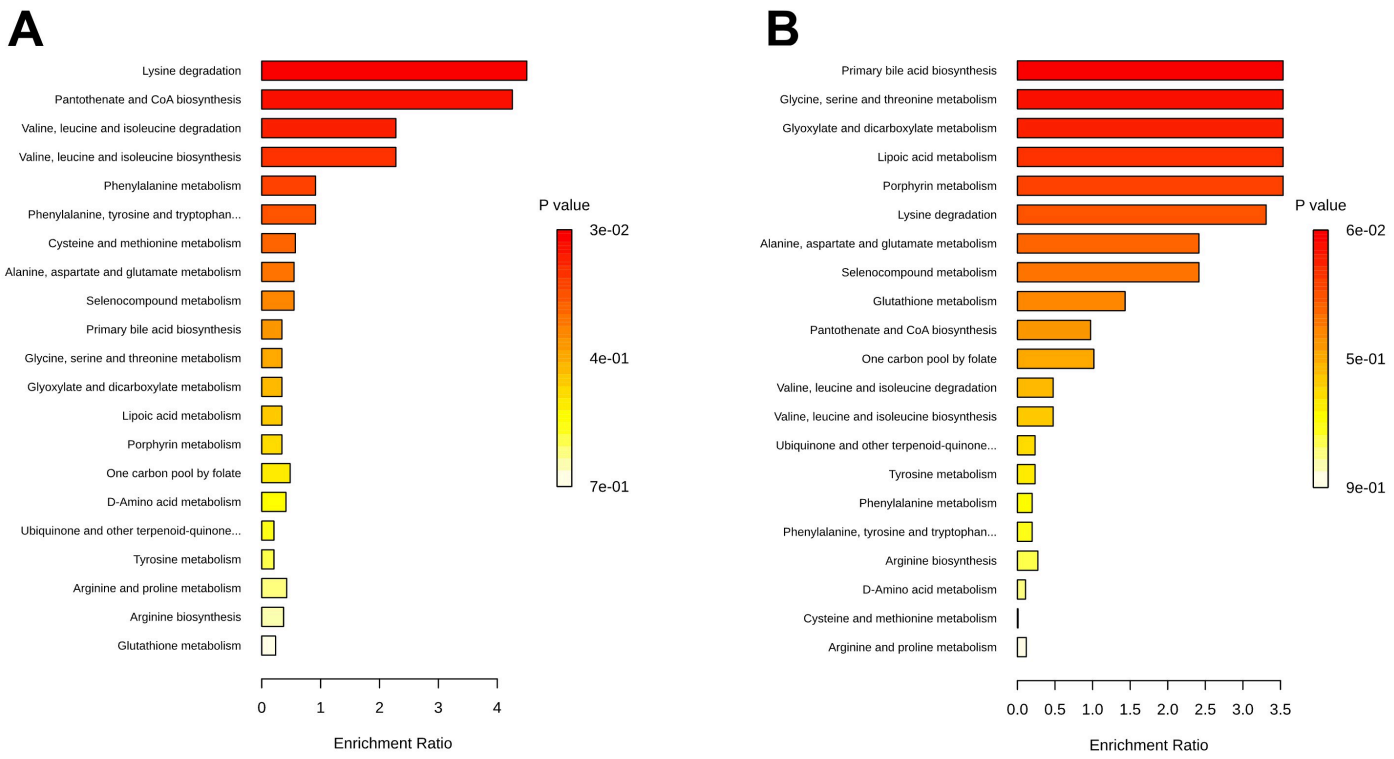


Figure 2

Quantitative enrichment analysis showing the top 21 metabolic pathways. **A)** The GDM vs healthy females graph shows that Lysine degradation and Pantothenate and CoA biosynthesis were the most enriched pathways. **B)** In GDM male vs healthy male it can be observed that the top metabolic pathways ways were more equitable with primary bile acid biosynthesis, glycine, serine and threonine metabolism and 3 other pathways having very similar enrichment ratios.

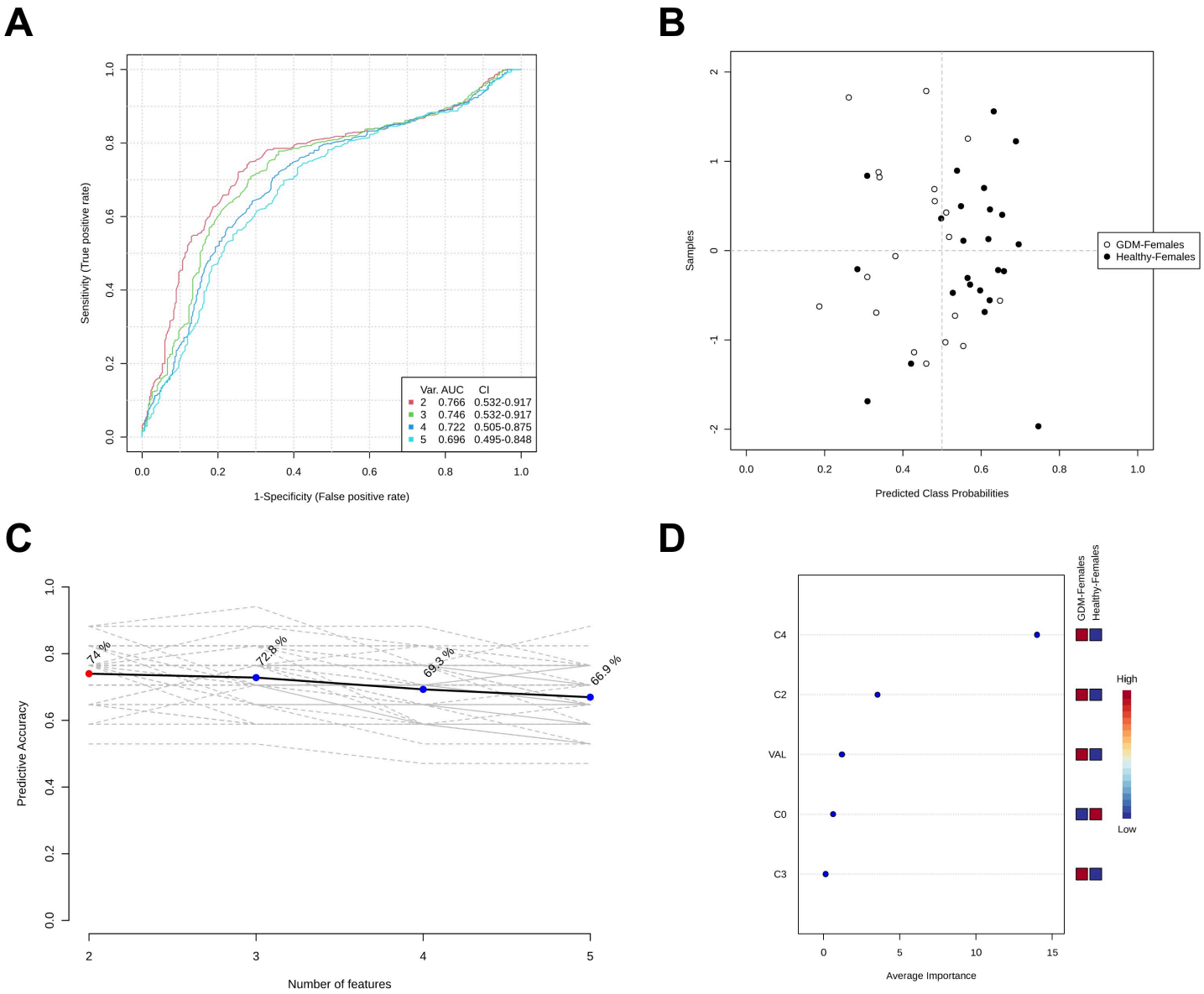


Figure 3

Multivariate ROC Curve based exploratory analysis results in females. **A)**Shows the AUC results for all possible combinations within the five metabolites with the 2 metabolite one having the highest AUC with 0.766. **B)**Predicted class probabilities for all samples with GDM females in white and healthy females in black. **C)** Predictive accuracy graph through Random Forest analysis with the 2-metabolite model having the highest accuracy with 74%. **D)** The ranking of the 5 metabolites through average mean importance.

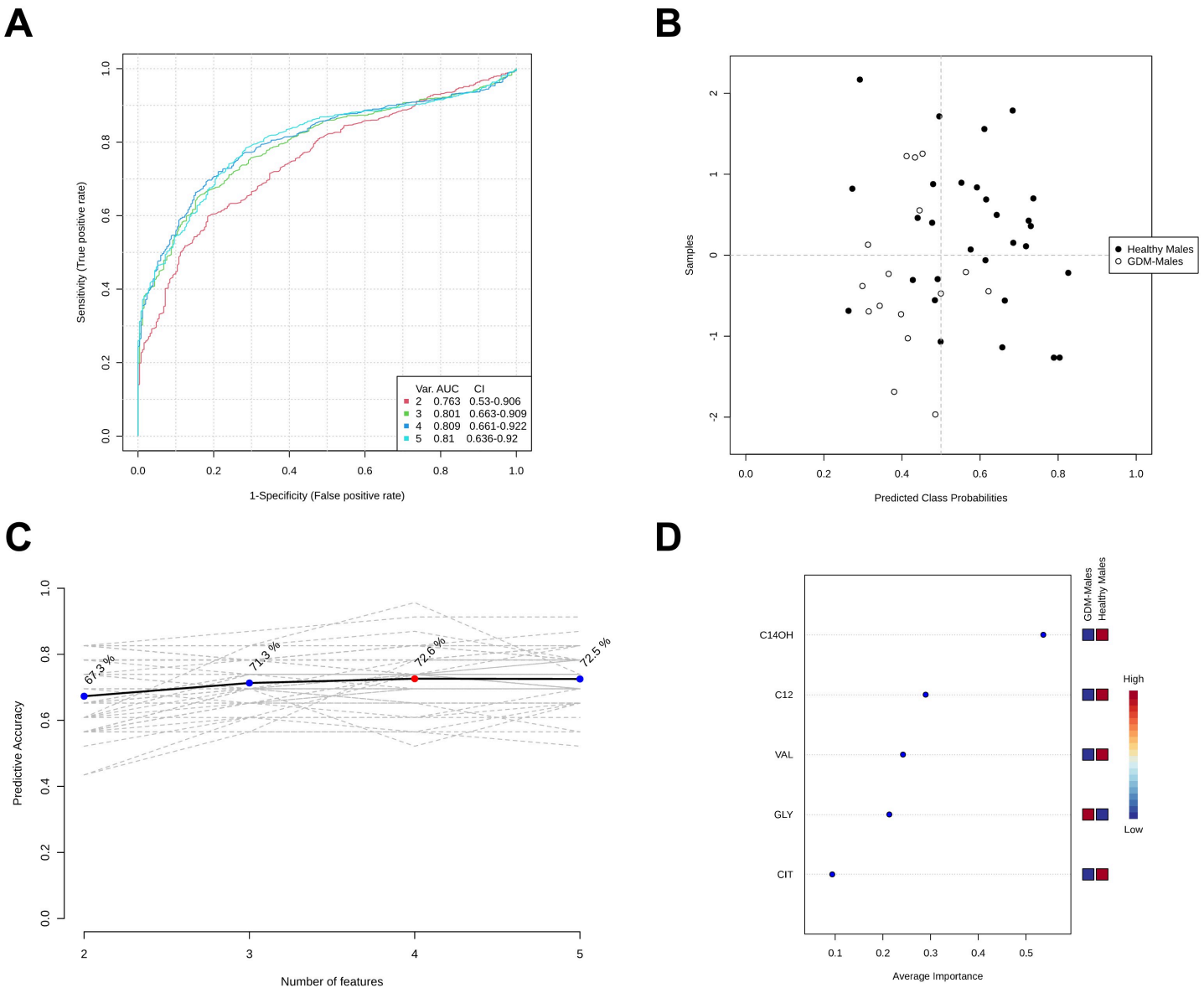


Figure 4

Multivariate ROC Curve based exploratory analysis results in males. **A)** Shows the AUC results for all possible combinations within the five metabolites with the 5 metabolite one having the highest AUC with 0.81. **B)** Predicted class probabilities for all samples with GDM males in white and healthy males in black. **C)** Predictive accuracy graph through Random Forest analysis with the 4-metabolite model having the highest accuracy with 72.6%. **D)** The ranking of the 5 metabolites through average mean importance.

Supplementary Files

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

- Rawdata.xlsx