The effects of gestational diabetes mellitus with advanced maternal age on the metabolite profiles of serum and urine

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Abstract

Background: Gestational diabetes mellitus (GDM) is characterized by impaired glucose tolerance in pregnancy and without a history of diabetes mellitus. It can lead to adverse maternal and neonatal outcomes. The incidence of GDM is closely related to maternal age, but there are only a few pregnancy-related metabolomic studies involving advanced maternal age (AMA) in China.

Methods: 20 GDM and 20 normal pregnant participants(≥35 years old) were recruited from the Complex Lipids in Mothers and Babies (CLIMB) study. Maternal plasma and urine metabolomes collected at the first and third trimester were analyzed using gas chromatography-mass spectrometry (GC-MS).

Results: Of the metabolites identified using GC–MS, 165 metabolites and 192 metabolites were found in plasma and urine respectively. However, urine metabolomic profiles were unable to distinguish GDM from controls, while there were 14 and 39 significantly different metabolites in plasma of the two groups in first and third trimester. Especially, by combining seven metabolites including cysteine, malonic acid, stearic acid, alanine, 11,14-eicosadienoic acid, 2-methyloctadecanoic acid, and arachidic acid using multivariant receiver operating characteristic(ROC) models, we were capable of discriminating GDM from healthy pregnancies with an area under curve (AUC) of 0.928 at early gestation.

Conclusion: This study explores metabolomic profiles between GDM and normal pregnancies longitudinally. Several metabolites have the potential to be biomarkers to predict GDM with AMA. Besides, the discordant metabolome profiles between the two groups could be helpful to understand the etiology of elderly GDM.

1. Background

With the change of birth policies that came into effect in January 2016, Chinese couples are now legally allowed to have two children. However, a direct legacy of previous policies is that many couples now aiming for a second child are aged in their late 30 s or 40 s. The result is an increasing number of pregnancies with advanced maternal age (AMA), defined as maternal age ≥ 35 years at the expected date of delivery (1). The WHO estimated the global prevalence of AMA pregnancies at approximately 12.3% in 2014 (2). A 12-year cohort study indicated the incidence of pregnancies with AMA rose from 6.5–17.2% in Southwestern China in 2019 (3), and a recent observational study involving 1,260,684 births, found that the proporition of AMA increased from 8.52–15.82% between 2013 to 2017 in Zhejiang province (4). It is well known that pregnancy complications and adverse pregnancy outcomes are closely correlated with advanced maternal age. For example, a retrospective cohort study found that pregnancy complications such as preeclampsia, gestational diabetes mellitus (GDM), and thrombosis were at higher risk in pregnant women with AMA (5). Other severe adverse maternal outcomes including maternal death and maternal near-miss were also significantly elevated in AMA (6). GDM, an abnormal glucose tolerance first recognized in pregnancy has been found to be 2–3 fold higher in AMA group compared with pregnant women aged 20–30 years in China (6, 7). In addition, there is a growing recognition of the increased risk

of obesity, glucose intolerance, and cardiovascular disorders among maternal and offspring of GDM with AMA (8, 9).

Metabolomics is a promising approach to investigate low molecular weight less than 1500 Da representing the metabolic status of cells, tissues, or organisms. In recent years, it is gaining popularity as a screen tool to investigate metabolic changes and biomarker discovery for GDM. Blood is the most commonly used biospecimen in the GDM metabolomic studies due to the fact that it usually contains the highest concentration of various classes of metabolites. Zhao *et al* performed a metabolomics analysis of serum from 107 cases diagnosed with GDM compared with 107 healthy controls in the first and the second trimester. They found that six amino acids and uric acid in the case group, were significantly lower than those in the control group (10). Our study implicated both gas chromatography(GC)- and liquid chromatography-mass spectrometry(LC-MS) based metabolomics also discovered a total of 13 serum metabolites different between GDM and normal pregnancies (11). Secondly, urine is another popular specimen for metabolomics research because of its non-invasive sampling and effortless preparation (a single dilution step) prior to mass spectrometry analysis. A case-control study of maternal urine profiling used from 121 Japanese women with GDM and 121 healthy matched controls. They proposed that ethanolamine and 1,3-diphosphoglycerate in urine were capable of discriminating GDM from healthy pregnancies (12). Our group performed an untargeted metabolomics study of urine from 27 cases diagnosed with GDM compared with 34 healthy controls. The results suggested that uric acid resulting from the catabolism of purine nucleosides could accurately classify GDM patients (13).

Despite considerable previous GDM metabolomic studies, there are few exclusively investigating the metabolic changes of GDM in pregnancies of AMA. Furthermore, the ability to predict GDM in such pregnancies has not previously been assessed. Here, we explored the metabolomic profiling of maternal plasma and urine in GDM with AMA both early and late in pregnancy using a GC-MS based metabolomics approach.

2. Materials And Methods

2.1 Study participants

In this prospective case-control study, GDM (n = 20) and non-GDM (n = 20) pregnant women equal to or older than 35 years of age were recruited from Complex Lipids in Mothers and Babies (CLIMB) project (14). Maternal age, body mass index (BMI), gestational age, blood pressure (BP) were matched between the two groups. Fasted blood and urine samples were collected at the first (11–14 gestational weeks) and third trimesters (32–34 gestational weeks). During 24–28 gestational weeks, a 75 g oral glucose tolerance test (OGTT) was conducted. GDM was diagnosed according to the International Association of Diabetic Pregnancy Study Group (IADPSG) guidelines (at least fasting blood glucose ≥ 5.1 mmol/L, or 60 minutes post 75 g OGTT blood glucose level ≥ 10 mmol/L, or 120 minutes post 75 g OGTT blood glucose level ≥ 8.5 mmol/L). Multiple pregnancies or pregnancies with other complications were excluded.

2.2 Sample preparations for plasma and urine

Aliquots (100 µL) of thawed urine were mixed with 80 µl 3M NaOH and 20 µL of the internal standard (IS, 2,3,3,3-d4-alanine,10 mM). The prepared liquid mixtures were stored at − 80 °C. Aliquots (100 µL) of thawed plasma were mixed with 20 µL of IS. To eliminate protein, 400 µL of cold methanol was added and tubes placed at − 20 °C for 30 minutes. A total of 350 µl of supernatant was isolated following centrifugation (4000 rpm for 20 min). Quality controls (QC) were also prepared from sub-aliquoting 20 µL of each biofluid (urine or plasma) into the collection tubes. All QC samples were prepared as the identical procedure regarding different sample types. Lastly, plasma supernatant was concentrated by in a speedvac (LABCONCO) at a speed of 4000 rpm for 8 h and 3 h respectively. All extracted biological samples were stored at – 80 °C before derivatization.

Prior to GC-MS analysis, all samples were derivatized with MCF as published by Smart *et al* (15). An Agilent GC7890B chromatograph coupled to a MSD5977A mass spectrometry with electron impact ion source set at 70 eV was used for the analysis of MCF derivatives (16).

2.3 Statistical analysis:

The GC-peaks were deconvoluted by Automated Mass Spectral Deconvolution and Identification System (AMDIS) and identified using our MCF mass spectra library (built by chemical standards) and commercial National Institute of Standards and Technology library(NIST14 library, https://www.nist.gov/nist-research-library). The metabolite identification was based on their MS spectrum > 90% similarity and within a 30 s retention time bin to its respective compound in the MS spectral library. After the removal of contamination from blank, metabolite concentration was first normalized by the level of IS, and the batch effect was minimized using median centering of QC samples (17). Lastly, the dilution effect of urine and plasma was corrected by total ion count. The metabolite levels were first transformed into Gaussian distribution using log and Pareto scalings and the differences of metabolite abundance in GDM and control groups were calculated using Student's T-test. To avoid false-positive results from multiple statistical tests, false discovery rates (FDR) for each metabolite were calculated by qvalue R package (18). The area under the receiver operating characteristic (ROC) curve was performed using the pROC R-package (19). Metabolites were linked to their corresponding metabolic pathways using Kyoto Encyclopedia of Genes and Genomes (KEGG) databased. Line plot, heatmap, and chord plot were illustrated using ggplot2 R-based packages (20).

3. Results

3.1 Characteristics of study participants:

The clinical characteristics of participants were shown in **Table 1**. There was no statistical difference in maternal age, gestational age, gravidity, systolic blood pressure(sBP), diastolic blood pressure(dBP), parity, educational level, body mass index (BMI), family income, and delivery modes between GDM and non-GDM groups, whilst fasting blood glucose, 60/120 minutes postprandial blood glucose after 75 g oral glucose differed significantly. Newborn characteristics, including birth weight and birth length were not significantly different between groups.

Table 1. Characteristics of study participants

	Controls (n=20)	GDM(n=20)	P value
Age	36(35,37.5)	36(35.25,37)	0.933[a]
Total years of schooling	16(15,16)	15.5(12,16)	0.343[a]
Tertiary Education			
Unaccepted	9(45.00)	10(50.00)	0.752[c]
Graduated	11(55.00)	10(50.00)	
House income			
less than 4000 RMB per month	1(5.00)	1(5.00)	
less than 7000 RMB per month	4(20.00)	4(20.00)	
less than 10000 RMB per month	7(35.00)	6(30.00)	0.492[c]
less than 16000 RMB per month	7(35.00)	5(25.00)	
less than 25000 RMB per month	0(0.00)	3(15.00)	
less than 70000 RMB per month	0(0.00)	1(5.00)	
More than 70000 RMB per month	1(5.00)	0(0.00)	
Gravidity	3(1.25,5)	4(3,4.75)	0.498[a]
Parity			
Nulliparous	8(40.00)	5(25.00)	0.32[c]
Primiparous	11(55.00)	15(75.00)	
Multiparous	1(5.00)	0(0.00)	
BMI	22.08±3.6	23±2.94	0.382[b]
sBP, mmHg	111.8±11.11	112±8.77	0.95[b]
dBP, mmHg	69.65±8.02	69.5±8.16	0.954[b]
Fasting blood glucose	4.55(4.43,4.8)	5.2(4.7,5.48)	0.001[a]
60-min postprandial blood glucose	7.1(6.7,8.5)	10(8.38,10.90)	<0.001[a]
120-min postprandial blood glucose	6.74±0.84	9.02±1.28	<0.001[b]
Mode of delivery			
Unassisted vaginal	5(25.00)	7(35.00)	0.478[c]
Operative vaginal	1(5.00)	0(0.00)	
Prelabour LSCS	0(0.00)	1(5.00)	
LSCS in labour	14(70.00)	12(60.00)	
Delivery gestational age (week)	39(38,39.75)	38(37,39)	0.075[a]
Birth weight (g)	3365(3172.5,3537.5)	3200(2850.5,3617)	0.304[a]
Birth length (cm)	50(49,50)	49(48.25,51)	0.484[a]

Abbreviations: sBP, systolic blood pressure; dBP, diastolic blood pressure; LSCS, lower segment cesarean section

Values are means±SD, median (IQR) or n (%)

[a]P value from Mann–Whitney test

[b]P value from Student t-test

[c]P value from Chi square test

3.2 Metabolite profiling of plasma and urine

A total of 192 and 165 metabolites were profiled in maternal urine and plasma samples respectively. In 3rd trimester samples, one and 39 metabolites were significantly different between GDM and control groups in urine and plasma respectively (**Table 1**, Fig. 1, **Table S2**). In the urine, nicotinic acid was the only metabolite significantly lower in GDM when compared with control groups. In plasma, 25 metabolites where higher in GDM pregnancies, including the majority of amino acids, amino acid derivatives, and tricarboxylic acid (TCA) cycle intermediates, hexanoic acid and myristoleic acid, while 14 were lower in GDM pregnancies relative to controls. This included three saturated fatty acids (2-methyloctadecanoic acid, arachidic acid, stearic acid), six unsaturated fatty acids (adrenic acid, gondoic acid, bishomo-gamma-linolenic acid, 11,14-eicosadienoic acid, cis-vaccenic acid, trans-vaccenic acid), three amino acids derivatives (cysteine, N-(Carboxymethyl)-L-alanine, beta-methylamino-alanine) and two organic acids (malonic acid, benzoic acid). The greatest fold differences between the two groups in the first trimester were higher methionine and beta-alanine, and lower cysteine and arachidic acid in GDM pregnancies.

In the first trimester pregnancy samples, no differences between groups were seen in urine, while 14 metabolites were found to be significantly different in GDM plasma samples. This included 5 metabolites (lysine, N-alpha-acetyllysine, citric acid, beta-alanine, methionine) that with higher levels in GDM pregnancies relative to non-GDM, and 9 with lower levels in GDM relative to non-GDM pregnancies. The direction and magnitude of differences between GDM and non-GDM in first trimester were also seen in the 3rd trimester comparisons.

3.3 Receiver operating characteristic (ROC) curve analysis for plasma and urine

With regard to plasma ROC analysis, seven and four metabolites were shortlisted in the first and third trimester respectively with an AUC above 0.75. These included four fatty acids (arachidic acid, stearic acid, 2-methyloctadecanoic acid, and 11,14-eicosadienoic acid), two amino acid (alanine and cysteine), and one organic acid (malonic acid) in the first trimester (Fig. 2a). Meanwhile, three organic acids (2-aminobutyric acid, 2-hydroxybutyric acid, and benzoic acid) and one unsaturated fatty acid (11,14-Eicosadienoic acid) were found in the third trimester (Fig. 2b). By combining seven and four shortlisted metabolites for the first and third trimester via multivariant ROC models, we were capable of discriminating GDM from healthy pregnancies with an AUC of 0.928 and 0.898 respectively. In terms of urine profiles, only three significant metabolites (nicotinic acid, glutamic acid, and ornithine) were identified with an AUC above 0.75 in the third trimester (Fig. 3). A multivariant ROC model combining these three metabolites was established to differentiate GDM from healthy pregnancies with an AUC of 0. 838.

3.4 Longitudinal metabolite profiles of plasma and urine between first and third trimesters

To investigate how advanced pregnancy outcomes are changed over the first and third trimester periods, the interactions between GDM outcomes and time were analyzed. Figure 4a-c illustrated that 13 plasma metabolites between pregnancy outcomes were changed across trimester periods ($p < 0.05$, repeated measurement analysis of variance (ANOVA)). Among them, five metabolites displayed the opposite trend between first and third trimesters (L-alanine, 2-oxybutyric acid, hippuric acid, oleic acid and EDTA, Fig. 4a); four metabolites only displayed disparity in the first trimester (dimethyltetrdecanoic acid, glutamine,

palmitic acid, lactic acid, Fig. 4b); three metabolites only showed discrimination in the third trimester (2-aminobutyric acid, 2-hydroxybutyric acid, citraconic acid, Fig. 4C). In comparison, there were only two urine metabolites (benzoic acid and 2-hydroxycinnamic acid) were found to interact between GDM outcomes and gestation progress (Fig. 4d). An increasing difference between GDM and healthy pregnancies for benzoic acid was observed over time. Conversely, there was a reduced difference for 2-hydroxycinnamic acid across trimesters.

3.5 Metabolic pathway enrichment analysis

The identified metabolites were performed enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to further characterize into functional pathway categories. The predicated outcome demonstrated that only nine metabolic pathways appeared to be significantly altered between GDM and control groups in the first trimester (Fig. 5a). The metabolism of carbohydrate, nucleotide, and amino acid were upregulated, whilst all metabolic pathways classified into lipid metabolism were downregulated in GDM group, besides, the ATP-binding cassette (ABC) transporters pathway shown the similar trend with lipid metabolism. Especially, metabolism of amino acid and fatty acid, as well as membrane transport mainly ABC transporters involving methionine, lysine and cysteine might play an important role in the pathogenesis of GDM (Fig. 5b).

4. Discussion

In our study, we have chosen to discuss GDM with advanced maternal age, which is becoming one of the major causes of adverse maternal outcomes in China. MS-based metabolomics has been applied in diverse investigations of pregnancy-related complications, it is a reliable alternative to conventional biochemical assays, but has not been widely accepted. We implicated this technology to elucidate the pathophysiology of GDM in pregnant women more than 35 years, by conducting an untargeted metabolomics utilizing urine and plasma to characterize metabolic changes prior (11 to 14 gestational weeks) and post (32 to 34 gestational weeks) to the onset of GDM in a prospective cohort. We revealed several distinct plasma metabolic signatures in the first trimester that predicted subsequent GDM with the predictive power of AUC 0.928 (95%CI 0.806-1, $p < 0.05$). The plasma metabolome displayed much more prominent metabolic changes than urine samples, especially amino acid and lipid metabolism were significantly upregulated and downregulated in elder GDM respectively.

Our study indicates that plasma is a more promising biofluid than urine to discriminate GDM from normal pregnancies in elderly women, especially in the third trimester. Cross-sectional and longitudinal analyses revealed that 39 and 13 plasma metabolites were significantly fluctuated throughout the GDM pregnancy accordingly, while merely one and two metabolites were significantly altered in urine samples (Fig. 1, 4). This is in line with several other studies related to GDM. Our group conducted a metabolomic study involving 49 GDM cases and 44 healthy controls using gas and liquid chromatography–mass spectrometry. The results demonstrated 2-aminobutyric acid in plasma was capable of differentiating GDM cases from normal controls, while none of urine metabolites was significant to distinguish between these two groups in the second trimester (11). Besides, Lorenzo-Almoros *et al* suggested that plasma sample can be implicated as predictive and diagnostic biomarkers for GDM because the plasma can

provide extensive information on dysfunctional adipose tissue and placental-derived factors, those may contribute to low-grade inflammation and insulin resistance (21). On the contrary, there are limited metabolomic biomarkers from urine to predict GDM and associate with metabolic diseases (21, 22). This may be owing to the different metabolic nature between plasma and urine as all organs and tissues are bathed in blood, and it can represent as a reasonable metabolic proxy for an organism, which is defined as “metabolic fingerprinting” (23). Thus, the blood sample is more ideal for studying cellular physiology and cellular processes. On the other hand, urine is the result of blood filtered by the glomerulus and reabsorbed by the renal tubules. It is considered as “metabolic footprinting”, an excreted biofluid that mainly reflects important physiology of kidney function (24). Since all recruited participants had no kidney dysfunction in our study, little difference between elder GDM and non-GDM groups was indeed observed.

Metabolic profiling unveiled that dysregulated ABC transporters and abnormal amino acid compositions may be associated with the development of GDM among advanced maternal age (AMA) women. Firstly, KEGG pathway enrichment analysis displayed GDM related amino acids, including methionine, lysine, and cysteine, were participated in the ABC transporters (Fig. 5b). Previous studies have shown that the disruption of amino acid transportation might be related to age-specific diseases (25, 26). A human study on elderly population indicated that there was an inability of essential amino acid absorption by skeletal muscle after resistance exercise due to lack of expression on ABC transporters in older adults (27). Additionally, Samantha *et al* revealed that compromised amino acid transportation in placentae was observed in AMA mice (28, 29). Secondly, our findings on the elevated plasma methionine and decreased cysteine in elder GDM group may shed light on specific dietary amino acid composition for elderly pregnancy. Previous aged animal study revealed methionine restriction and cysteine supplementation diet could ameliorate insulin resistance (30, 31). Virginia L *et al* indicated methionine restriction could reduce insulin and homeostasis model assessment in leptin-deficient obese mice fed with methionine restriction diet intervention between 10–24 weeks of age (32). Wanders *et al* shown that methionine restriction could significantly improve insulin sensitivity that were impaired by aging using microarray analysis of white adipose tissue and liver in rat (33). Blouet *et al* demonstrated insulin resistant rats induced by high sucrose diet could be rescued by cysteine supplementation (34). Interestingly, literatures suggested that the therapeutic intervention of methionine restriction and cysteine supplementation are attributable to their antioxidant capability against oxidative stress during aging. Rats fed with methionine restriction diet showed a reduction in reactive oxygen species (ROS) generation and protected mitochondrial DNA from ROS damage in liver, brain, heart, and kidney (35). Cysteine supplementation could ameliorate insulin resistance and age-related degenerations by improving skeletal muscle functions and reducing oxidative stress (31, 34). These outcomes highlight the importance of clinical consideration of specific nutrient composition for diet instructions to GDM women more than 35 years old.

Fatty acids are reported to negatively associate with insulin resistance (36). In line with previous results, a reduction of fatty acid was found in plasma of elder GDM women in our study. These changes were persisted throughout pregnancy and could likely to be associated with onset of GDM. The concentrations of saturated fatty acids, including stearic acid, arachidic acid, bishomo-gamma-linolenic acid (dihomo-

gamma-linolenic acid, DGLA), and adrenic acid, in maternal plasma were lower than those in control participants in the third trimester (Fig. 1). The similar findings were observed by a recent study, which involved 84 GDM cases and 90 healthy controls. The authors demonstrated a significantly lower level of stearic acid, palmitic acid, DGLA in maternal and cord serum using GC-MS (25). Furthermore, disturbances of lipid metabolism have been associated with inflammation occurring in an elderly GDM population. On one hand, as early in 1966, a total downregulation of fatty acid biosynthesis was found in the adipose tissue of 23 months old rats (37). On the other hand, Marseille-Tremblay *et al* have demonstrated a lower concentration of fatty acids in placenta accompanied by elevated inflammatory markers such as IL-1β and TNF-α in GDM (27). Moreover, Chen *et al* demonstrated that maternal individual free fatty acids such as stearic acid, palmitic acid and DGLA, uniquely affected insulin resistance and secretion by modulating the inflammatory response in a prospective cohort involving 81 GDM and 1287 normal controls (38). Chronic low-grade inflammation has been demonstrated in the elderly population (39), therefore inflammatory cytokines might play a role in the reduction of fatty acids in our study. However, the specific signaling pathway that may link lipid metabolism with glucose homeostasis in GDM remain unclear. Future work is warranted to elucidate the exact metabolic mechanism for age-related aberrant lipid metabolism.

There were some limitations worth noting in our study. First, we have only analyzed the maternal plasma and urine metabolome using GC-MS. Although this technique has optimized the detection of low molecular metabolites, our work could be complemented by utilizing multiple analytical platforms to acquire an overall picture of the metabolome. Second, we assessed maternal metabolites at 11–14 and 32–34 gestational weeks, which precludes analysis of shifts in metabolite levels across pregnancy. Third, the recruited pregnant women from CLIMB were 35–40 years old. Future investigations involving women with more advanced maternal age (≥ 40 years) are warranted to verify the metabolic dysregulation of GDM in elderly population.

5. Conclusions

In conclusion, we observed that GDM women over 35 years have a subclinical dysmetabolism in early gestation. The potential mechanism may include defects in the metabolism of amino acid and fatty acid, and further worsening insulin resistance. We found that combination of several long-chain fatty acid and amino acids could predict GDM with AMA in the first trimester. Confirming these metabolite markers is essential for further application of MS-based metabolomics in the clinical field to accept and adopt the results from biomarker discovery studies. Additionally, the metabolomic profiling provides hint for clinical obstetricians to instruct GDM women over 35 years old for specific diet nutrient composition. Those changes in amino acid and lipid metabolism from the early gestational period are unlikely the consequence of GDM development but may be associated with onset and progression of the disease.

List Of Abbreviations

GDM, gestational diabetes mellitus; AMA, advanced maternal age; CLIMB, Complex Lipids in Mothers and Babies; GC–MS, gas chromatography-mass spectrometry; ROC,receiver operating characteristic; AUC,area under curve; LC-MS, liquid chromatography-mass spectrometry; BMI, body mass index; OGTT, oral glucose tolerance test; IADPSG, International Association of Diabetic Pregnancy Study Group; QC, quality controls; MS, mass spectrometry; IS, internal standard; FDR, false discovery rates; CI, confidence interval; KEGG, Kyoto Encyclopedia of Genes and Genomes; TCA cycle, tricarboxylic acid cycle; ABC transporters,ATP-binding cassette transporters; ROS, reactive oxygen species; DGLA,bishomo-gamma-linolenic acid (dihomo-gamma-linolenic acid); ANOVA, analysis of variance.

Declarations

Ethics approval and consent to participate. This research was approved by the Ethics Committee of Chongqing Medical University. The participants all consent to participate and written form were preserved.

Availability of data and materials. The datasets generated during this study are available from the corresponding author on reasonable request.

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Conflicts of Interest The authors declare that they have no competing interests.

Authors' Contributions X.L.H. contributed to data collection and analysis, interpreted the results, and wrote the manuscript. X.J.H contributed to data analysis and wrote the manuscript. B.Y.L. contributed to data collection and interpreted the results. X.L.H., X.Z. and T.L.H. performed the statistical analysis. J.D.S, Y.Y.X, P.B and H.B.Q revised the manuscript, remarked on the design, and interpreted the findings. T.L.H. and H.Z. managed the original clinical study, interpreted the results, helped the writing of the manuscript, and guided the project. All authors have read, commented, and consented the final version of the manuscript. T.L.H. and H.Z. are the guarantor of this study and, therefore, had full access to all the data of this work and are responsible for the integrity of the data and the validity of the data analysis. All authors read and approved the final manuscript.

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Figures

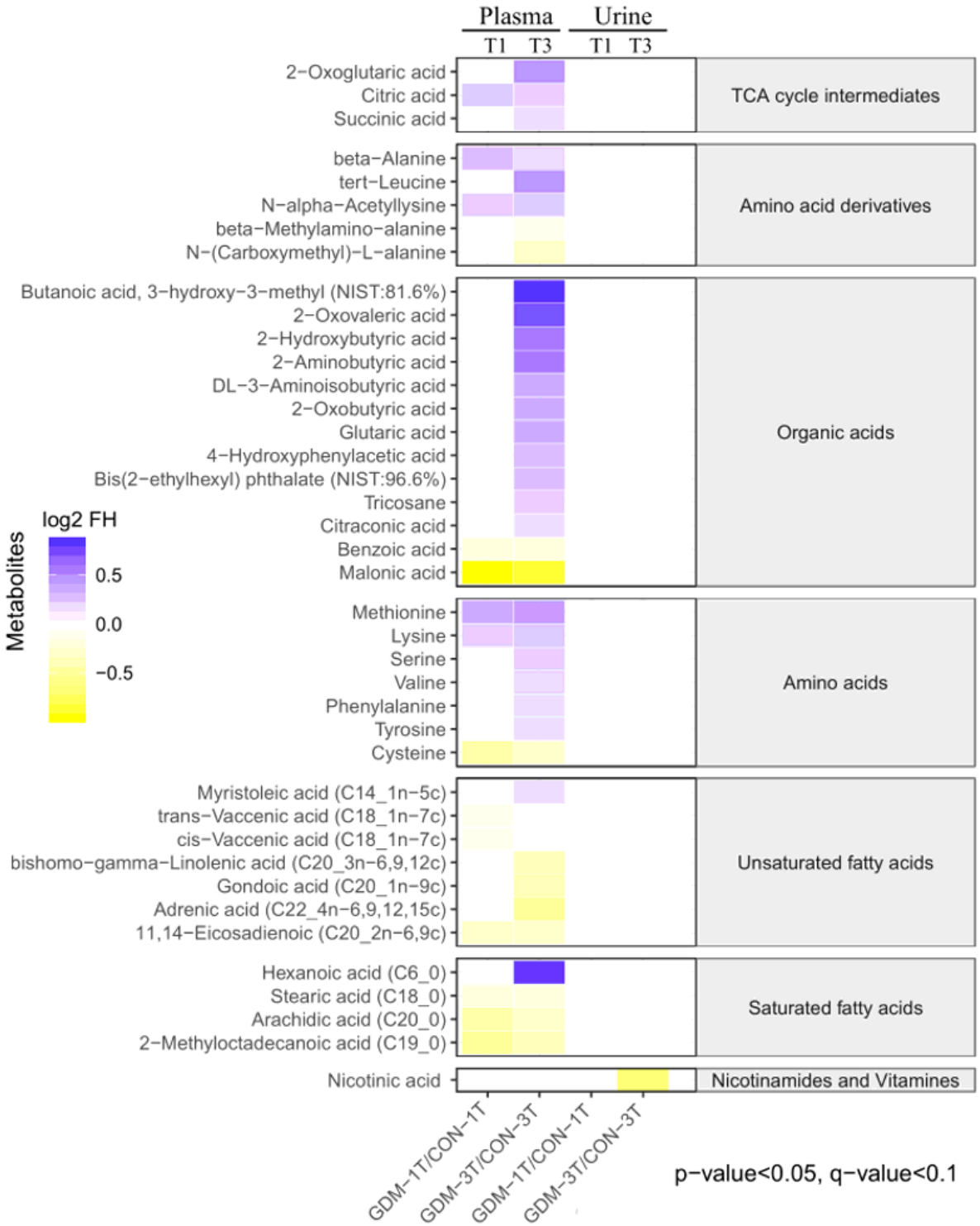

Figure 1

Heatmap the metabolome profiles of first and third trimester plasma and urine in GDM relative to non-GDM pregnancies of advanced maternal age. The relative abundance of metabolites are illustrated on a log2 scale. Fold difference of metabolite concentrations compared with their corresponding controls are plotted as shades of purple (increasing levels) or yellow (decreasing levels). Only metabolites with a significant p-value (Tukey's HSD: $p < 0.05$), q-value (FDR: $q < 0.1$) are shown.

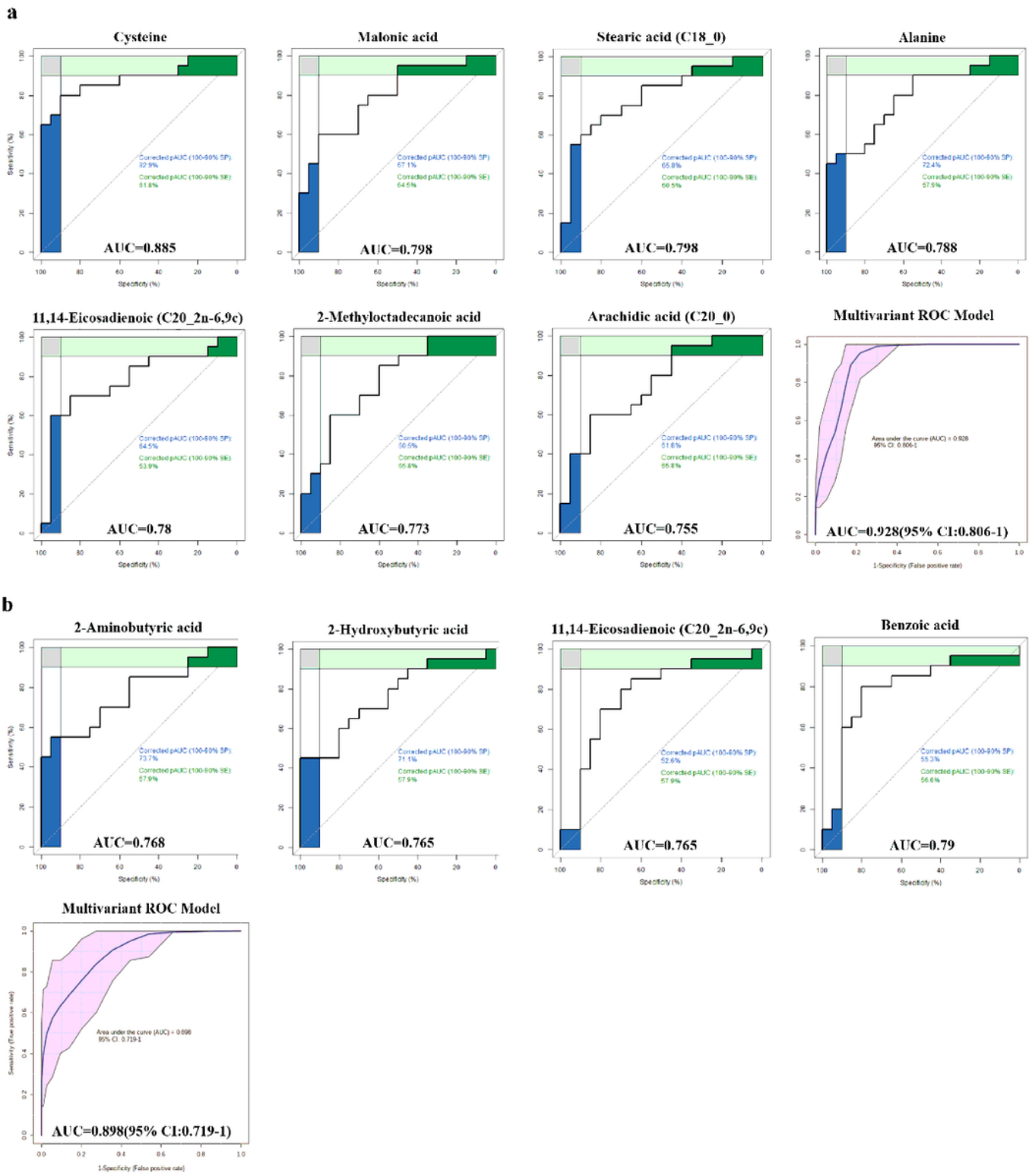

Figure 2

ROC curve of seven and four metabolites with AUC above 0.75 in the first (a) and third (b) trimester respectively using plasma samples between GDM and normal pregnancies. A multivariant ROC model and corresponding 95% Confidence Interval (CI) combining the seven and four metabolites are shown in the last plot.

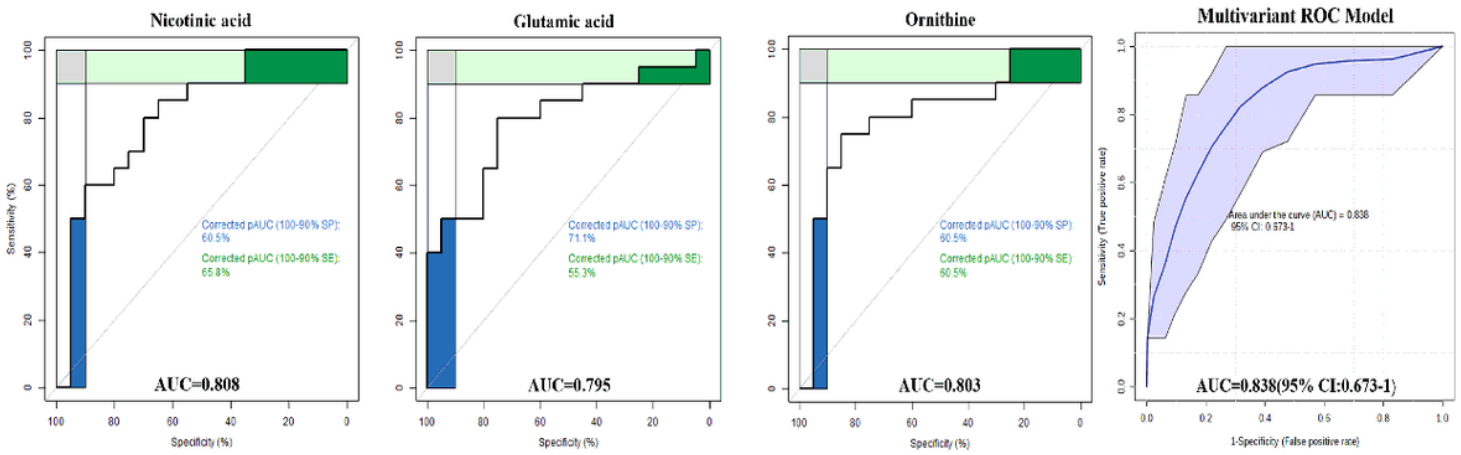

Figure 3

ROC curve of three metabolites with AUC above 0.75 using urine samples in the third trimester between GDM and normal pregnancies. A multivariant ROC model and corresponding 95% CI combining all of the three metabolites are plotted.

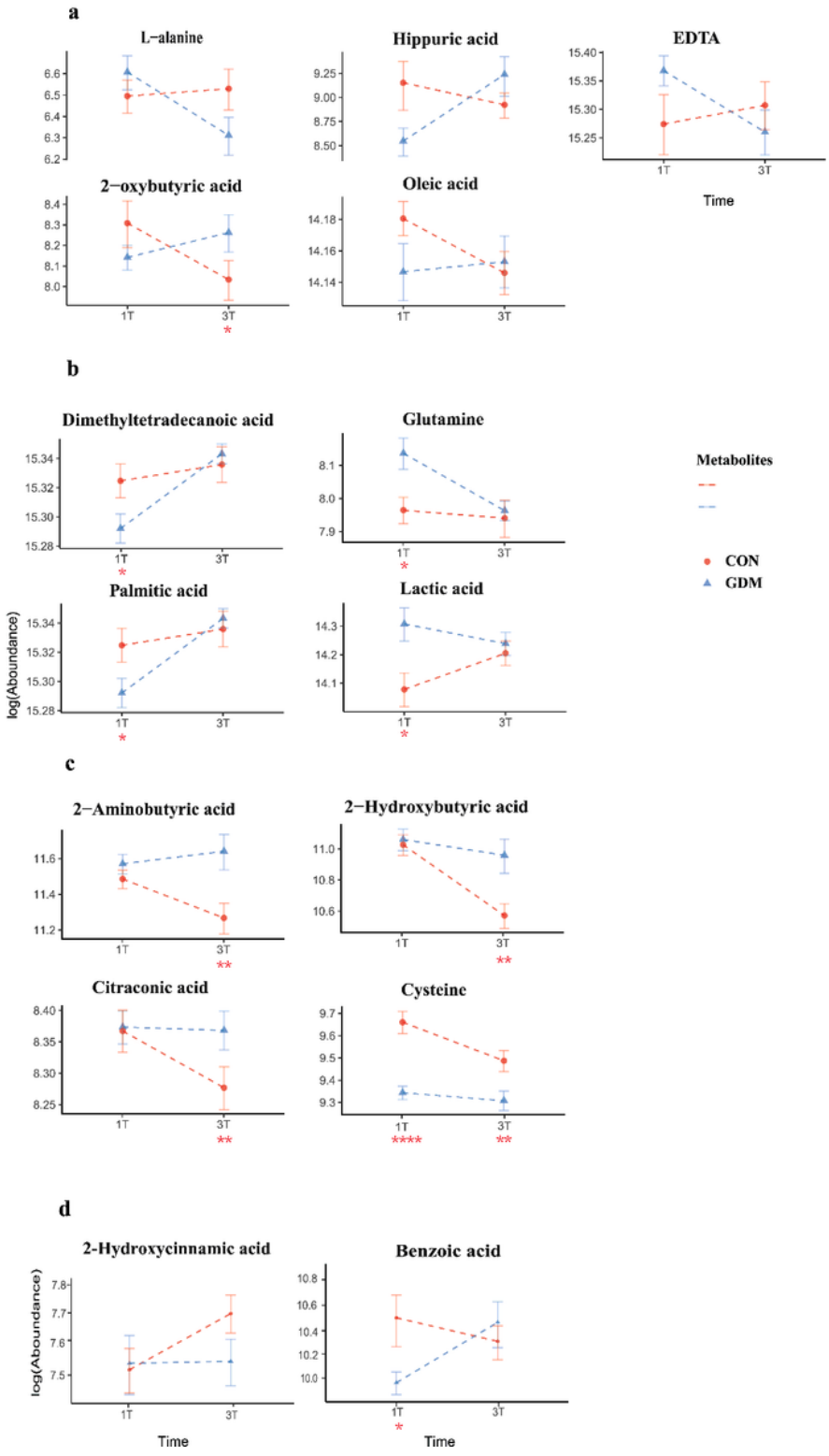

Figure 4

The relative abundance of significant plasma (a-c) and urine (d) metabolites in the first and third trimesters collected from GDM and normal pregnancies. a) Opposite trend between first and third trimesters. b) Only displayed disparity in the first trimester.

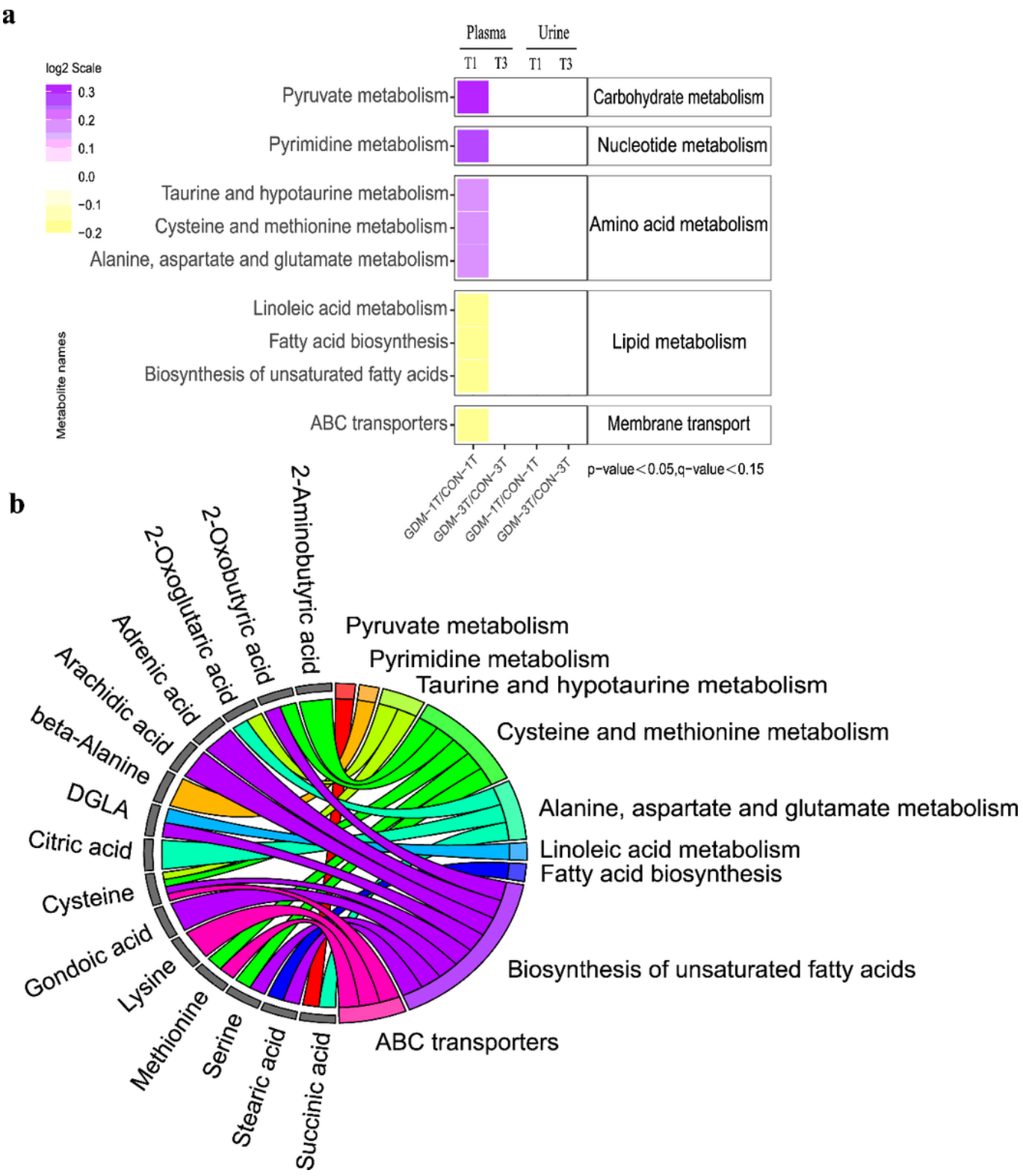

Figure 5

The heatmap shows the metabolic pathways of plasma and urine from GDM compared with normal controls in the first and third trimester. The relative abundances of metabolites were illustrated with log2 scale (Figure 5a). Fold changes of metabolite concentrations compared with their corresponding controls are plotted by purple color (increasing levels) and yellow color (decreasing levels). Only metabolites with a significant p-value (Tukey's HSD: $p<0.05$), q-value (FDR: $q<0.15$) are shown. Metabolism of amino acid

and fatty acid, as well as membrane transport mainly ABC transporters might play an important role in the pathogenesis of GDM (Figure 5b).

Supplementary Files

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

- SupplementaryTable.docx