Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

Received 12 December 2024
Accepted 28 February 2025
Available online 28 February 2025
Version of Record published 14 March 2025

RESEARCH

Metabolic memory in gestational diabetes enhances SARS-CoV-2 susceptibility in postpartum women: a prospective cohort study integrated with longitudinal metabolomics

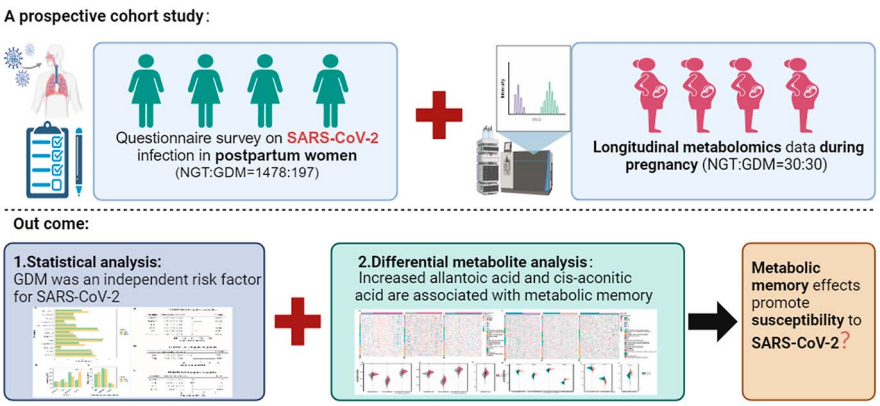
Zhangyan Li[1,*], Qiuhan Lu[2,3,*], Rui Zhang[1,*], Nuerbiya Xilifu[1,4], Yue Li[1], Xinmei Huang[1], Jiaying Zhang[2,3], Shufei Zang[1], Guozhi Jiang[2,3] and Jun Liu[1]

[1]Department of Endocrinology, Shanghai Fifth People's Hospital, Fudan University, Shanghai, China
[2]School of Public Health (Shenzhen), Shenzhen Campus of Sun Yat-sen University, Shenzhen, Guangdong, China
[3]School of Public Health (Shenzhen), Sun Yat-sen University, Guangzhou, Guangdong, China
[4]Department of Endocrinology, the Second People's Hospital of Kashgar Prefecture, Xinjiang, China

Correspondence should be addressed to S Zang: sophiazsf@fudan.edu.cn or to G Jiang: jianggzh5@mail.sysu.edu.cn or to J Liu: liu_jun@fudan.edu.cn

*(Z Li, Q Lu and R Zhang contributed equally to this work)

Graphical abstract



(Created in BioRender; https://BioRender.com/o08n003). Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine.

Abstract

Objective: Women with gestational diabetes mellitus (GDM) often develop a metabolic memory that increases the risk of future metabolic disorders, even after blood glucose levels normalize following clinical intervention. However, the impact of this metabolic memory on susceptibility to SARS-CoV-2 remains unclear. Therefore, we aim to investigate the potential association between metabolic memory in GDM and susceptibility to SARS-CoV-2 infection.

Published by Bioscientifica Ltd.
https://ec.bioscientifica.com

Methods: We conducted a prospective cohort study with 1,675 pregnant women, including 197 (11.8%) with GDM. Postpartum SARS-CoV-2 infections were tracked via telephone follow-up and categorized into negative and positive groups. Logistic regression was used to explore risk factors for SARS-CoV-2 infection. Peripheral blood samples were collected from 30 GDM and 30 normal glucose-tolerant (NGT) pregnant women in three trimesters (T1, T2 and T3) for longitudinal untargeted metabolomics to identify GDM and SARS-CoV-2-associated metabolites. Limma package was applied to find differential expressed metabolites associated with SARS-CoV-2 infection and GDM.

Results: Among 1,675 women, 1,348 (80.5%) tested positive for SARS-CoV-2. GDM postpartum women had higher SARS-CoV-2 infection rates (88.3 vs 79.4%, $P = 0.003$) than NGT women. GDM was associated with SARS-CoV-2 infection (T2: OR (95% CI): 2.17 (1.26–3.54), $P = 0.005$; T3: OR (95% CI): 1.70 (1.03–2.82), $P = 0.040$). Compared to the SARS-CoV-2 negative group, the positive group exhibited elevated levels of allantoic acid, LPE (0:0/22:6), LPC (15:0/0:0) and 1-linoleoyl-sn-glycero-3-phosphorylcholine in T1 and T2, before clinical intervention. In T3, allantoic acid remained elevated post-intervention. A similar increase as described above was observed in the GDM compared to the NGT group. The tricarboxylic acid cycle was the sole overlapping enriched pathway in the SARS-CoV-2 positive versus negative group and the GDM versus NGT group. Cis-aconitic acid, a metabolite from this pathway, was elevated in T3 in the GDM group.

Conclusion: Compared to NGT, women with GDM are at a higher risk of postnatal SARS-CoV-2 infection. Metabolic memory from GDM may heighten susceptibility to SARS-CoV-2.

Keywords: gestational diabetes mellitus; SARS-CoV-2; metabolic memory; susceptibility

Introduction

Gestational diabetes mellitus (GDM) is a maternal metabolic disorder and is characterized by any degree of hyperglycemia first identified during gestation (1). GDM results in various complications for both the mother, baby and offspring (2). GDM is associated with significantly increased risks of subsequent type 2 diabetes mellitus (T2DM) in the mother and obesity in offspring, even if the mother reverts to normal glucose following delivery. This is related to the metabolic memory effect caused by hyperglycemia during GDM pregnancy (3).

During the coronavirus disease 2019 (COVID-19) epidemic, some studies have explored the link between GDM and novel coronaviruses. A multicenter prospective observational study suggested that women who are overweight or obese and have GDM requiring insulin therapy are at increased risk of a severe course of COVID-19 infection (4). Mendez *et al.* and Zanardo *et al.* showed that the prevalence of GDM increased during the COVID-19 epidemic (5, 6). However, other studies have reached the opposite conclusion. A retrospective study in the Chinese population indicated that the prevalence of GDM decreased each year during the COVID-19 epidemic, with rates of 21.46, 19.81 and 18.48% in 2019, 2020 and 2021, respectively (7). However, the study populations have been limited to women with GDM during pregnancy, neglecting the significant population of postpartum women with a history of GDM. In addition, it remains to be explored whether the long-term adverse metabolic memory effects observed in women with GDM may influence their susceptibility to SARS-CoV-2. Women with GDM and those in the postpartum period represent a special group that requires particular attention to the infection of SARS-CoV-2.

Metabolomics enables high-throughput analysis of metabolites across a wide range of biological samples, significantly enhancing our understanding of normal bodily functions and the mechanisms underlying many diseases (8). Several studies have utilized metabolomics to investigate GDM mouse models, women with GDM and pregnant women infected with SARS-CoV-2, respectively, demonstrating significant changes in amino acid and lipid metabolites (9, 10, 11). Furthermore, metabolite pathway enrichment suggests the involvement of the tricarboxylic acid (TCA) cycle in SARS-CoV-2 infection and development (12). However, few studies have concurrently analyzed the metabolomics of GDM and SARS-CoV-2 to explore the metabolic association between these two diseases.

In light of these questions that merit further exploration, we conducted a prospective cohort study involving 1,675 pregnant women, 197 (11.8%) of whom were diagnosed with GDM. Our study recorded clinical information during pregnancy in women with GDM and normal glucose tolerance (NGT) and collected their peripheral blood throughout pregnancy for untargeted longitudinal metabolomics testing. Post-delivery, participants completed a questionnaire about SARS-CoV-2 infection. In addition, we analyzed the dynamics of metabolomics during pregnancy to identify metabolic factors that might influence postnatal susceptibility to SARS-CoV-2 infection. This study aimed to explore the association between GDM and SARS-CoV-2 infection in a prospective cohort study

and to investigate whether residual metabolic memory effects of GDM increase susceptibility to SARS-CoV-2 using a metabolomic approach.

Methods

Research population

From January 2021 to January 2023, we constructed a prospective cohort of 2,918 expectant mothers who underwent prenatal checkups and experienced complication-free deliveries at the GDM Care Center of Shanghai Fifth People's Hospital affiliated with Fudan University and Shanghai Minhang Hospital of Integrative Medicine. Our study was conducted following the Declaration of Helsinki and approved by the Shanghai Fifth People's Hospital Ethics Committee (approval document number: (2020) EC (154)). The process of collecting samples is illustrated in Fig. 1. Women were excluded from the study if they had any of the following: i) any infectious diseases in the 2 weeks

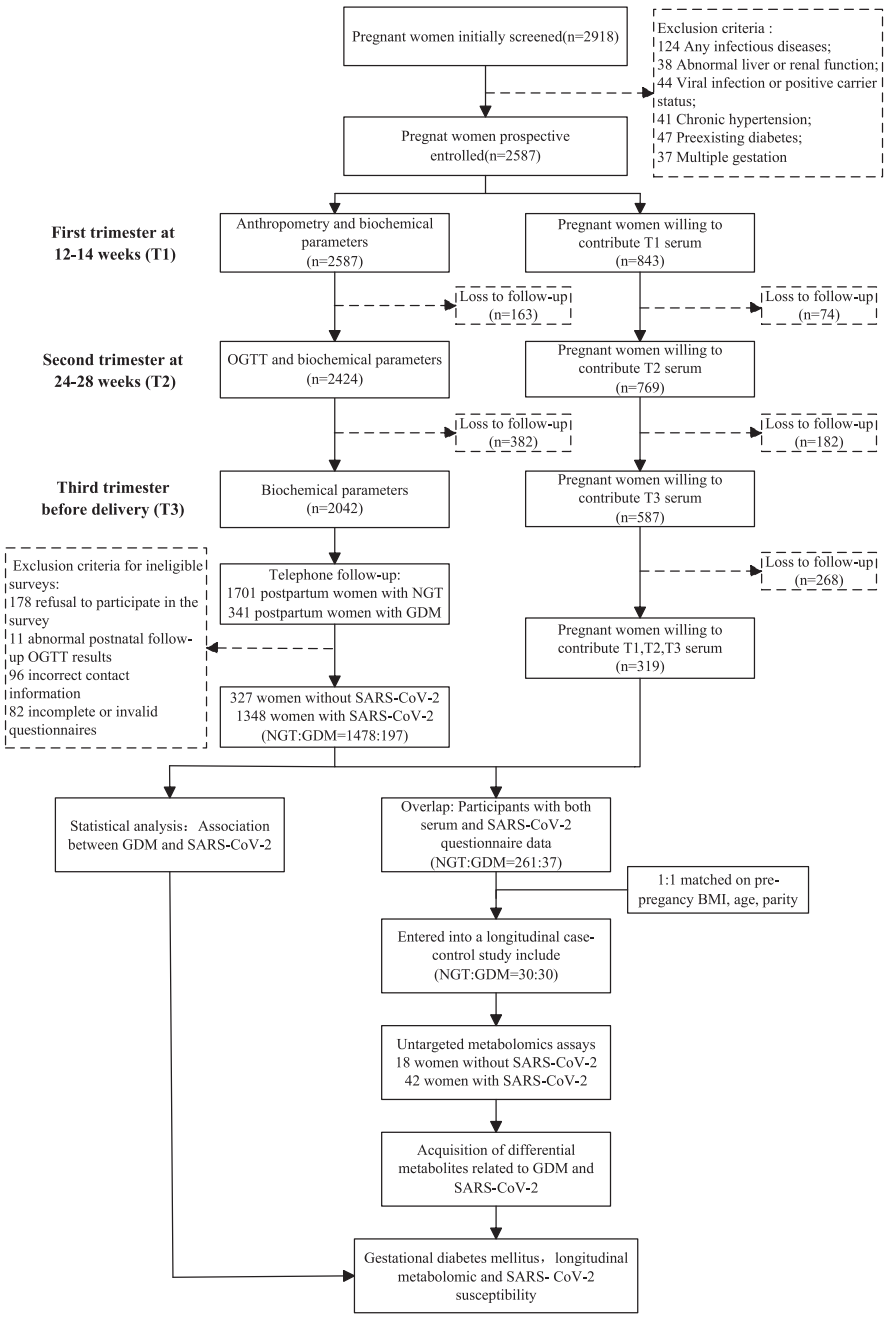


Figure 1

Flowchart of the study. Abbreviations: DM, diabetes mellitus, NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; COVID-19, coronavirus disease 2019; BMI, body mass index, T1, the first trimester; T2, the second trimester; T3, the third trimester.

before blood collection; ii) abnormal liver or renal function; iii) viral infection with or carrier of hepatitis virus, HIV, syphilis and so on; iv) history of chronic hypertension; v) history of diabetes; and vi) multiple gestations. After a rigorous screening process using inclusion-exclusion criteria, we initially included 2,587 pregnant women in the study. Clinical information was collected for each trimester of the participants, and blood samples from pregnant women who consented to contribute were cryopreserved for subsequent analysis. After delivery, a SARS-CoV-2 infection questionnaire was administered to the pregnant women. Those who met any of the following criteria were excluded from the survey as ineligible. Exclusion criteria for ineligible surveys included: i) refusal to participate in the survey; ii) abnormal postnatal follow-up oral glucose tolerance test (OGTT) results; iii) incorrect contact information; and iv) incomplete or invalid questionnaires. After completing the SARS-CoV-2 infection questionnaire, women with valid survey results who were also willing to provide blood samples for the study were selected for overlap, resulting in a total of 261 women with NGT and 37 women with GDM (clinical parameters are characterized in Supplementary Table 1 (see section on Supplementary materials given at the end of the article)), all of whom had both questionnaire results and blood samples from all three trimesters.

To minimize potential bias in the selection of metabolic group participants, matched case-control pairs were formed. Among the overlapping participants described above, 30 pregnant women with GDM and 30 pregnant women with NGT were matched based on prepregnancy body mass index (BMI) (±0.5 kg/m^2), age (±2 years) and parity. A longitudinal untargeted metabolomics study was conducted on their peripheral blood samples collected during the first trimester (T1; 14.1 (3.1) weeks), second trimester (T2; 25.5 (1.9) weeks) and third trimester (T3; 36.0 (2.1) weeks) of pregnancy.

The presence of GDM was determined by a 75-g standard OGTT, following overnight fasting at 24–28 weeks of pregnancy (the second trimester, T2), following the 2020 guidelines set by the American Diabetes Association (ADA) (13). A pregnant individual with fasting blood glucose (FBG) ≥5.1 mmol/L, 1 h blood glucose (1 h-BG) ≥10.0 mmol/L or 2 h blood glucose (2 h-BG) ≥8.5 mmol/L was considered to have GDM.

Data collection and laboratory determinations

During the initial appointment, clinical data were recorded for the expectant mother. Determination of gestational age was based on the date of the last menstrual period, with verification by gynecological ultrasound. Subsequently, the obstetric examination was performed regularly according to the week of gestation and anthropometric parameters were recorded. Participants without obvious diabetes or GDM in early pregnancy were required to fast overnight or for a minimum of 8 h before undergoing a 75 g OGTT at 24–28 weeks of gestation.

Following a period of fasting, blood samples were obtained to measure blood cell count using the Sysmex XN9000 Automatic Blood Cell Analyzer and biochemical parameters using the Roche Cobas 8000 Automatic Biochemical Analyzer. The Collaboration on the Epidemiology of Chronic Kidney Disease (CKD-EPI) equation was utilized to calculate the estimated glomerular filtration rate (eGFR). BMI was calculated as the weight in kilograms divided by the square of height in meters.

Telephone questionnaire follow-up

A maternal postpartum SARS-CoV-2 telephone questionnaire follow-up was administered from January 1 to January 31, 2023, to those of the above participants who had given birth. All participants gave written informed consent before the study commencement. Results were collected from subjects infected after the lifting of novel coronavirus restrictions in China, a positive nucleic acid or antigen test for a novel coronavirus was considered positive. During the investigation, Shanghai's main prevalent novel coronavirus strains were the BA.5 subtype of the Omicron variant and its derivatives. Before conducting the study, those responsible for designing the questionnaire, conducting the investigation and ensuring quality control (QC) received specialized training. In addition, the researchers were unaware of the respondents' subgroups. The survey was split into two sections. The initial section comprised demographic details including name, age, usual place of residence, education, occupation, SARS-CoV-2 vaccination status and nucleic acid test results. The second section focused on symptoms related to novel coronavirus infection, including the presence and intensity of fever, sore throat, rhinorrhea, otalgia, ophthalmodynia, nasal obstruction, myalgia, hyposmia, hypogeusia, encephalalgia, diarrhea, cough, asthenia and a self-assessment of symptom severity. Fever was classified as mild (37.4 to 38°C); moderate (38.1 to 39°C) or severe (39.1 to 41°C). Symptoms were based on the latest updates from the official websites of the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) regarding SARS-CoV-2 during the survey period. To minimize potential bias, surveys meeting the following conditions were excluded as invalid questionnaires from this study: i) SARS-CoV-2 infection occurs before or during pregnancy rather than postpartum; ii) no definitive nucleic acid or antigen results; and iii) questionnaires answered by relatives in place of respondents.

Metabolomics processing

Peripheral blood from the above matched 30 vs 30 case-control population in T1, T2 and T3 of pregnancy were used for untargeted metabolomics testing. The techniques of metabolomics have been described in our previous study. In brief, serum samples stored at −80°C were used for lipid chromatography–tandem mass spectrometry (LC-MS/MS) analysis by pretreatment. Differentially expressed metabolites (DEMs) were filtered out if the deletion rate was >50%. Metabolic annotations are based on the Human Metabolite Database (HMDB) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (14).

Statistical analysis

Statistical Product and Service Solutions (IBM SPSS Statistics 26.0), R statistical software (version 4.3.1) and Zstats v0.90 (www.medsta.cn/software) were utilized for analyses. Student's *t*-test was used to test continuous variables that followed a normal distribution, and the Mann–Whitney U test for continuous variables that did not conform to a normal distribution. The Chi-square test/Yates' corrected Chi-square test/Fisher's exact test was employed to test classified variables. To assess whether GDM is an independent risk factor for SARS-CoV-2 infection, we conducted a logistic regression analysis, with the binary classification of SARS-CoV-2 (positive/negative) as the dependent variable. All clinical baseline data from each trimester were included in univariate logistic regression analyses, and variables with $P < 0.05$ were subsequently included in multivariate logistic regression analyses as potential risk factors. The false discovery rate (FDR) value was estimated using the 'Benjamini–Hochberg' method at a statistically significant level of $P < 0.05$. In screening for DEMs using the R language limma package, we aimed to minimize the effect of potential confounders. Based on differences in clinical baseline data, we selected monocyte percentage (MONO%), aspartate aminotransferase (AST) isoenzymes, age, gestational week, BMI and neutrophil (NEU) as covariates when using SARS-CoV-2 (positive/negative) as the grouping variable. When GDM (GDM/NGT) was the grouping variable, MONO%, NEU, age, gestational week, BMI, red blood cell (RBC) and AST isoenzymes were selected as covariates. The metabolomic data underwent a log2 transformation. The MetaboAnalyst 5.0 was utilized to perform KEGG pathway analysis of DEMs.

Results

Clinical characteristics of NGT and GDM women during pregnancy

Compared with NGT mothers, those with GDM were older, had higher BMI, white blood cell (WBC), NEU, RBC and FBG. In T1, the GDM group had a higher systolic blood pressure (SBP), hemoglobin (Hb), lymphocyte (LYMP) and platelet (PLT) count, and alanine aminotransferase (ALT), aspartate aminotransferase-isomerase (AST-isoenzyme), creatinine (Cr), triglyceride (TGs), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) level. In T2, the GDM group had a higher OGTT 1 h blood glucose (1 h-BG) and OGTT 2 h blood glucose (2 h-BG). In T3, the GDM group had higher SBP, diastolic blood pressure (DBP), ALT, AST isoenzyme, Cr, TG level and eGFR (CKD-EPI) (all $P < 0.05$) (Table 1).

Description of SARS-CoV-2 infection

Based on the recording of maternity data, we conducted a telephone survey of SARS-CoV-2 infection among 2,042 postnatal women. Finally, 1,757 questionnaires were collected, resulting in a recovery rate of 86.0%. A total of 1,675 valid questionnaires (1,478 NGT vs 197 GDM) were obtained after screening by QC staff. The validity rate of the questionnaires was 95.3%. The results of the validated questionnaire showed that 1,348 individuals had contracted SARS-CoV-2, while 327 remained uninfected, resulting in an infection rate of 80.5%. Women with GDM were more likely to have received a third novel coronavirus vaccination than those with NGT (24.9 vs 12.7%, $P < 0.001$). Crucially, there was a notable disparity in the rate of SARS-CoV-2 infection between the GDM and NGT groups (88.3 vs 79.4% respectively, $P = 0.003$) (Table 1).

Of the 1,348 patients infected with SARS-CoV-2, 174 (12.9%) had a history of GDM. In all SARS-CoV-2-positive participants, there was no statistical difference in symptoms except fever (Supplementary Table 2) (Fig. 2A). Compared with NGT women, those with a history of GDM had a higher proportion of hyperthermia after infection with SARS-CoV-2 (40.8 vs 19.4%, respectively, $P < 0.001$, Fig. 2B). No statistical significance was seen in the self-assessment of severity between the two groups (Fig. 2C).

GDM was an independent risk factor for SARS-CoV-2

In the general information, no statistical differences were observed between the SARS-CoV-2 positive and negative groups in terms of their usual place of residence, educational background or hospital. The SARS-CoV-2-positive group had a higher proportion of patients with GDM compared with the SARS-CoV-2-negative group (12.9 vs 7.0%, $P = 0.003$) (Supplementary Table 3).

Univariate logistic regression revealed that in T2 and T3, GDM was a risk factor for SARS-CoV-2 infection (Supplementary Table 4). Independent variables with $P < 0.05$ were included for further multivariate logistic regression analysis. In T1, after correcting for NEU, Hb and TC, the results suggested that MONO% (OR (95% CI): 1.28 (1.05–1.56); $P = 0.014$) and PLT (OR (95% CI): 1.01

Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

Table 1 Characteristics of women with GDM and NGT.

Characteristics	All (*n* = 1,675)	NGT (*n* = 1,478)	GDM (*n* = 197)	*P*
Anthropometric parameters				
Hospital				
The Fifth Hospital of Shanghai	1,257 (75.0%)	1,102 (74.6%)	155 (78.7%)	0.209
Minhang District Hospital for Integrative Medicine	418 (25.0%)	376 (25.4%)	42 (21.3%)	
Age (years)	28 (25, 32)	28 (25, 31)	32 (29, 35)	**<0.001**
Parity				
Nulliparous	624 (37.3%)	540 (36.5%)	84 (42.6%)	0.096
Parous	1,051 (62.7%)	938 (63.5%)	113 (57.3%)	
Prepregnancy BMI	21.67 (20.00, 23.88)	21.30 (19.54, 23.44)	23.31 (20.94, 25.45)	**<0.001**
Family history of DM				
No	730 (97.3%)	540 (97.5%)	190 (96.9%)	0.690
Yes	20 (2.7%)	14 (2.5%)	6 (3.1%)	
Previous history of GDM				
No	1,659 (99.0%)	1,465 (99.1%)	194 (98.5%)	0.630
Yes	16 (1.0%)	13 (0.9%)	3 (1.5%)	
Number of vaccine doses				
0	713 (42.6%)	642 (43.4%)	71 (36.0%)	**<0.001**
1	237 (14.1%)	213 (14.4%)	24 (12.2%)	
2	488 (29.1%)	436 (29.5%)	52 (26.4%)	
3	236 (14.1%)	187 (12.7%)	49 (24.9%)	
4	1 (0.1%)	0 (%)	1 (0.5%)	
SARS-CoV-2				
Negative	327 (19.5%)	304 (20.6%)	23 (11.7%)	**0.003**
Positive	1,348 (80.5%)	1,174 (79.4%)	174 (88.3%)	
T1 (first trimester)				
Gestational week (weeks)	13.4 (13.0, 15.4)	13.4 (13.0, 15.5)	13.4 (13.0, 14.4)	0.225
BMI (kg/m^2)	21.8 (20.1, 24.0)	21.6 (20.0, 23.7)	23.3 (21.0, 25.4)	**<0.001**
SBP (mmHg)	117 (109, 124)	117 (108, 124)	119 (109, 125)	**0.008**
DBP (mmHg)	70 (64, 75)	70 (64, 76)	71 (65, 76)	0.111
RBC (×10^9/L)	4.08 (3.85, 4.29)	4.06 (3.84, 4.28)	4.17 (3.93, 4.40)	**0.001**
MONO% (%)	5.20 (4.60, 6.10)	5.20 (4.50, 6.00)	5.20 (4.60, 6.20)	0.744
Hb (g/L)	122 (115, 128)	125 (118, 131)	126 (119, 131)	**<0.001**
WBC (×10^9/L)	8.50 (7.28, 9.87)	8.42 (7.27, 9.82)	9.06 (7.82, 10.69)	**<0.001**
NEU (×10^9/L)	6.14 (5.10, 7.36)	6.38 (5.31, 7.47)	6.87 (5.68, 8.08)	**<0.001**
LYMP (×10^9/L)	1.70 (1.43, 2.04)	1.53 (1.31, 1.80)	1.64 (1.40, 1.93)	**0.034**
PLT (×10^9/L)	219 (185, 253)	224 (188, 259)	235 (206, 271)	**<0.001**
FBG (mmol/L)	4.37 (4.13, 4.62)	4.35 (4.12, 4.60)	4.53 (4.20, 4.87)	**<0.001**
ALT (U/L)	13 (10, 19)	11 (8, 17)	12 (8, 20)	**0.014**
AST isoenzyme (U/L)	9 (7, 12)	10 (8, 12)	9 (6.38, 12)	**0.003**
Cr (mmol/L)	43 (38, 48)	47 (43, 52)	47 (43, 52)	**<0.001**
eGFR (CKD-EPI)	128 (123, 133)	128 (123, 133)	127 (122, 131)	0.067
TGs (mmol/L)	1.89 (1.38, 2.61)	1.38 (1.09, 1.78)	1.69 (1.34, 2.13)	**<0.001**
TC (mmol/L)	4.85 (4.24, 5.60)	4.46 (3.94, 4.99)	4.51 (4.00, 4.97)	**<0.001**
HDL-C (mmol/L)	1.67 (1.42, 1.93)	1.79 (1.57, 2.07)	1.70 (1.45, 1.93)	0.105
LDL-C (mmol/L)	2.70 (2.22, 3.24)	2.44 (2.01, 2.86)	2.57 (2.04, 2.93)	**<0.001**
T2 (second trimester)				
Gestational week (weeks)	25.9 (25.0, 26.4)	25.7 (25.0, 26.4)	26 (25.1, 26.7)	0.072
BMI (kg/m^2)	24.72 (22.76, 27.22)	24.42 (22.58, 26.72)	25.90 (23.99, 28.37)	**<0.001**
SBP (mmHg)	116 (34)	116 (38)	116 (10)	0.887
DBP (mmHg)	66 (61, 71)	65 (60, 70)	65 (59, 71)	0.724
Hb (g/L)	113 (106, 119)	114 (108, 119)	114 (109, 121)	0.040
WBC (×10^9/L)	9.34 (8.00, 10.82)	9.39 (7.97, 10.72)	9.95 (8.35, 11.67)	**<0.001**
NEU (×10^9/L)	6.70 (5.56, 8.00)	6.86 (5.71, 7.98)	7.40 (6.12, 9.01)	**<0.001**
LYMP (×10^9/L)	1.80 (1.50, 2.15)	1.75 (1.50, 2.08)	1.74 (1.49, 2.10)	0.110
PLT (×10^9/L)	207 (173, 243)	209 (173, 245)	210 (181, 253)	0.273
RBC (×10^9/L)	3.63 (3.45, 3.85)	3.61 (3.44, 3.81)	3.68 (3.50, 3.95)	**0.001**
MONO% (%)	6.10 (5.30, 7.10)	6.10 (5.30, 7.10)	6.10 (5.40, 7.05)	0.919
FBG (mmol/L)	4.07 (3.74, 4.44)	4.33 (4.10, 4.55)	4.76 (4.32, 5.24)	**<0.001**
1 h-BG (mmol/L)	6.94 (5.77, 8.16)	7.12 (5.99, 8.01)	10.16 (8.96, 10.85)	**<0.001**
2 h-BG (mmol/L)	6.12 (5.41, 7.07)	5.99 (5.41, 6.78)	8.64 (7.46, 9.33)	**<0.001**

(Continued)

Table 1 Continued.

Characteristics	All (*n* = 1,675)	NGT (*n* = 1,478)	GDM (*n* = 197)	*P*
T3 (third trimester)				
Gestational week (weeks)	36.1 (35.4, 36.7)	36.1 (35.9, 36.9)	36.4 (36.0, 37.0)	0.295
BMI (kg/m^2)	27.24 (25.22, 29.55)	27.12 (24.78, 29.69)	28.04 (25.71, 31.13)	**0.011**
SBP (mmHg)	120 (10)	119 (10)	122 (10)	**0.002**
DBP (mmHg)	73 (8)	73 (8)	75 (8)	**<0.001**
Hb (g/L)	111.75 (12.26)	110.92 (12.34)	118.00 (9.66)	**<0.001**
WBC (×10^9/L)	9.10 (7.70, 10.80)	8.50 (7.19, 10.18)	8.26 (7.24, 9.44)	**<0.001**
NEU (×10^9/L)	6.73 (5.40, 8.30)	6.03 (5.16, 7.67)	5.90 (4.86, 6.90)	**<0.001**
LYMP (×10^9/L)	1.60 (1.30, 1.92)	1.58 (1.35, 1.96)	1.68 (1.35, 1.97)	0.180
PLT (×10^9/L)	204 (171, 241)	209 (163, 239)	200 (164, 246)	0.526
RBC (×10^9/L)	3.86 (0.35)	3.84 (0.35)	3.93 (0.33)	**0.002**
MONO% (%)	7.05 (6.1, 8.1)	7.5 (6.3, 8.58)	7.1 (6.1, 8.0)	0.574
FBG (mmol/L)	4.36 (0.49)	4.27 (0.41)	4.52 (0.57)	**<0.001**
ALT (U/L)	10 (7, 14)	9 (7, 12)	8 (6, 11)	**<0.001**
AST-isoenzyme (U/L)	11 (8, 12)	11 (9, 12)	9 (7, 11)	**<0.001**
Cr (mmol/L)	47.80 (9.73)	47.09 (9.49)	53.38 (9.80)	**<0.001**
eGFR (CKD-EPI)	123 (118, 129)	121.5 (117, 129)	121 (114, 126)	**0.002**
TGs (mmol/L)	3.1 (2.43, 3.92)	2.86 (2.25, 3.36)	3.24 (2.58, 4.23)	**<0.001**
TC (mmol/L)	5.91 (1.21)	5.91 (1.17)	5.91 (1.30)	0.947
HDL-C (mmol/L)	1.80 (0.36)	1.81 (0.36)	1.78 (0.37)	0.348
LDL-C (mmol/L)	3.29 (1.16)	3.34 (1.11)	3.16 (1.24)	0.111

Values are given as the mean (SD), median (interquartile range) or *n* (%). Bold indicates statistical significance. Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; BMI, body mass index; DM, diabetes mellitus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; T1, first trimester; T2, second trimester; T3, third trimester;

SBP, systolic blood pressure; DBP, diastolic blood pressure; RBC, red blood cell; MONO%, monocyte percentage; Hb, hemoglobin; WBC, white blood cell; NEU, neutrophil; LYMP, lymphocyte; PLT, platelet; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST isomerase, aspartate aminotransferase isomerase; eGFR, estimated glomerular filtration rate; CKD-EPI, collaboration on the epidemiology of chronic kidney disease; Cr, creatinine; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; 1 h-BG, OGTT 1 h blood glucose; 2 h-BG, OGTT 2 h blood glucose.

(1.01–1.01); P = 0.042) were significantly associated with SARS-CoV-2 infection (Fig. 3A). In T2, when corrected for RBC, GDM (OR (95% CI): 2.17 (1.26–3.54), P = 0.005) was the risk factor for infection (Fig. 3B). In T3, the inclusion of MONO% and NEU for analysis revealed that PLT (OR (95% CI): 1.01 (1.00–1.01), P = 0.001) and GDM (OR (95% CI): 1.70 (1.03–2.82), P = 0.040) remained risk factors for infection (Fig. 3C).

Screening for differential metabolites in metabolomics

Screening for differential metabolites associated with SARS-CoV-2 infection

The clinical characteristics of the matched 30 pregnant women with NGT and GDM during pregnancy are shown in Supplementary Table 5. Based on the SARS-CoV-2 infection outcomes in these pregnant women, we categorized them into SARS-CoV-2 negative and SARS-CoV-2 positive groups. Metabolite assays were performed on peripheral blood obtained from these women during T1, T2 and T3. Metabolomic processing identified 2,257 metabolites. A significance threshold of P < 0.05 was adopted to identify DEMs linked to SARS-CoV-2 infection. When performing differential analysis using the R language limma package, we adjusted for MONO%, AST isoenzyme, age, gestational weeks and BMI in T1 to minimize the impact of various confounding factors. Similarly, in T2, adjustment was made for NEU, age, gestational weeks and BMI. In T3, adjustment was made for covariates including MONO%, age, gestational weeks and BMI. In T1, T2 and T3, 93, 125 and 100 metabolites were respectively identified as DEMs. The DEMs in T1 and T2 were predominantly glycerophospholipids, amino acids and their derivatives. In T3, they were predominantly glycerophosphates, organic acids and their derivatives (Fig. 4A). Abnormalities in glycerophospholipids were a major metabolic change in the metabolic profile of pregnancy associated with SARS-CoV-2 infection (Supplementary Table 6). Notably, LPE (0:0/22:6), LPC (15:0/0:0) and 1-linoleoyl-sn-glycero-3-phosphorylcholine of the DEMs remained consistently elevated during both T1 and T2 (Fig. 4B and C). Meanwhile, in T3, allantoic acid was elevated (Fig. 4D).

Screening for differential metabolites associated with GDM

Conducting differential analysis between the 30 women with NGT and the 30 women with GDM described above, with GDM as the outcome, FDR <0.05 as a threshold value. After adjusting for MONO%, NEU, age, gestational weeks

Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

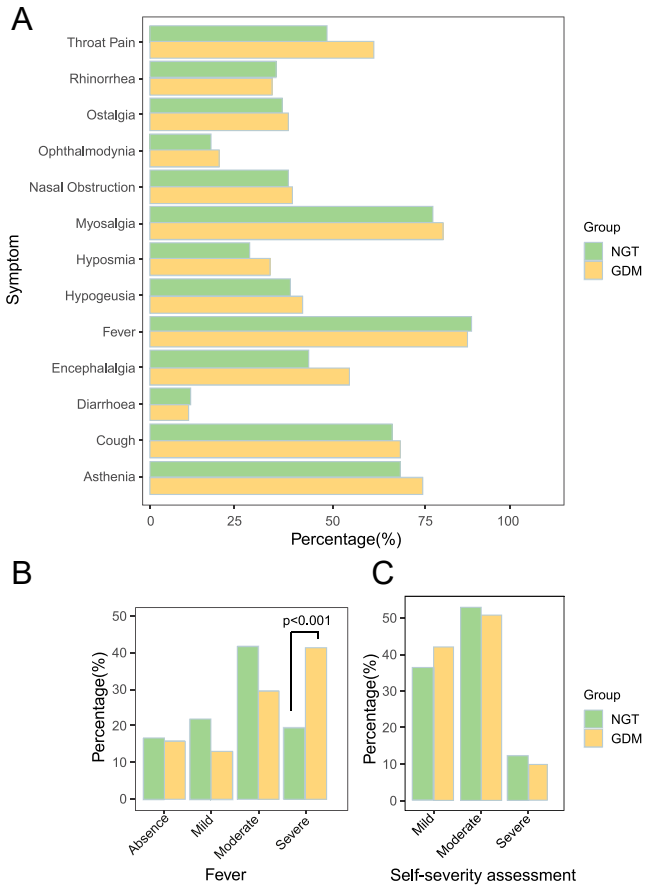


Figure 2

Symptom statistics of SARS-CoV-2 infection. (A) Bar graph of symptoms. The X-axis represents the percentage, Y-axis represents symptom categories. (B) Bar graph of fever level. The X-axis represents fever classification, Y-axis represents the percentage. (C) Bar graph of patient self-assessment of severity. The X-axis represents severity classification, Y-axis represents the percentage.

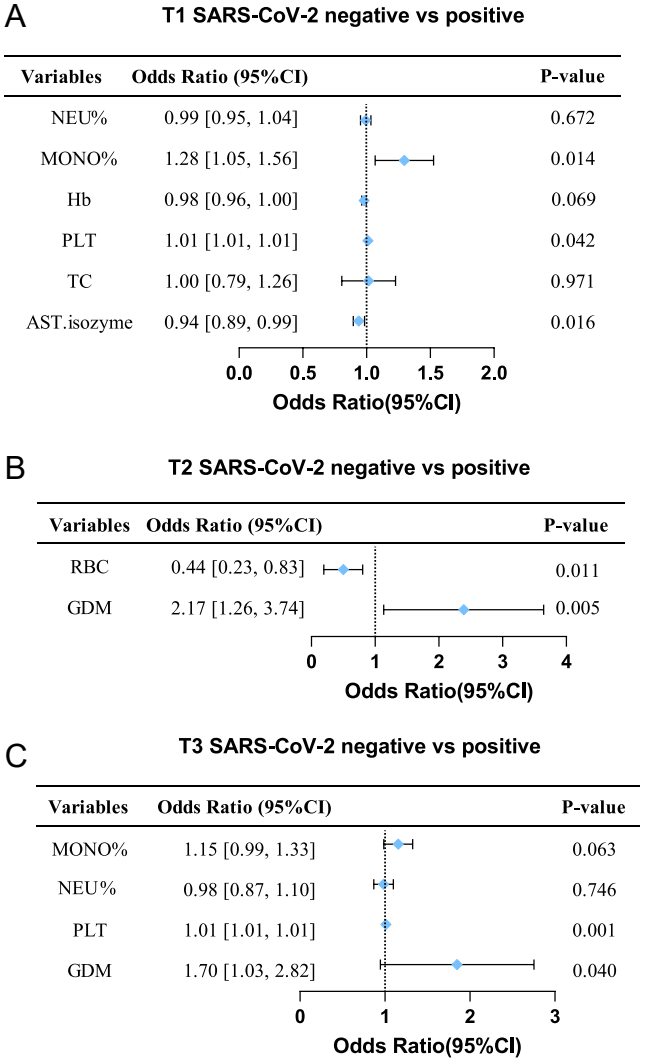


Figure 3

Forest plots for multifactor logistic regression. (A), (B), (C) represent the results of multifactorial logistic regression of SARS-CoV-2 in T1, T2 and T3 trimesters, respectively. Abbreviations: NEU, neutrophil; NEU%, neutrophil percentage; MONO%, percentage monocytes; Hb, hemoglobin; PLT, platelet; TC, total cholesterol; AST-isoenzyme, aspartate aminotransferase isomerase; RBC, red blood cell.

and BMI, in T1, we identified 219 DEMs comprised principally of glycerophospholipids, amino acids and their derivatives. In T2, 200 DEMs were identified (adjusted for NEU, age, gestational weeks and BMI). The main categories of DEMs in T2 were ketones, esters and glycerophospholipids. In T3, after adjusting for RBC, AST isoenzymes, age, gestational weeks and BMI, 86 DEMs were revealed, comprised mainly of organic acids and their derivatives, along with amino acids and their derivatives (Fig. 5A) (Supplementary Table 7). Consistent with the SARS-positive group in the GDM group, the three glycerophospholipids, LPE (0:0/22:6), LPC (15:0/0:0) and 1-linoleoyl-sn-glycero-3-phosphorylcholine were also elevated in T1 (Fig. 5B), with LPE (0:0/22:6) continuing the trend in T2 (Fig. 5C). Interestingly, allantoic acid, which was elevated in SARS-positive T3 DEMs, remained consistently elevated during T1, T2 and T3 in the GDM group (Fig. 5D).

KEGG pathway enrichment analysis of differential metabolites

We performed pathway analyses of DEMs for every trimester to better understand the biological pathways linked to metabolic alterations during pregnancy. In T1, metabolites associated with SARS-CoV-2 infection exhibited enrichment in pathways such as the biosynthesis of valine, leucine and isoleucine, metabolism of phosphonate and phosphinate and taurine and hypotaurine metabolism. In T2, DEMs showed enrichment in the tricarboxylic acid (TCA) cycle. In T3, pathways related to glycine, serine and

Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

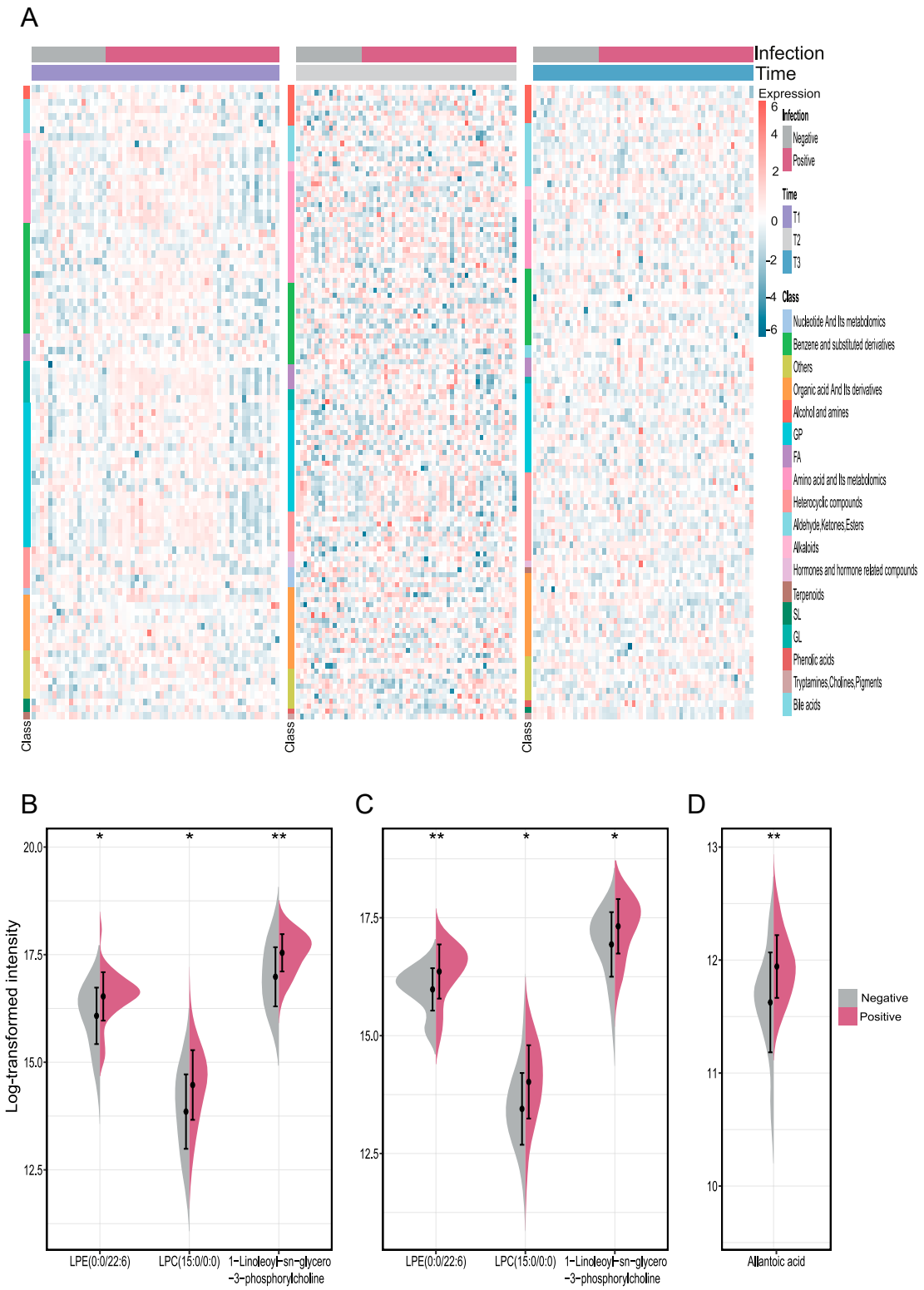


Figure 4

Screening for differential metabolites associated with SARS-CoV-2 infection. (A) Heat map of differential metabolites (DEMs) in T1, T2 and T3 in SARS-CoV-2 positive vs SARS-CoV-2 negative group. Columns represent the trimesters and groups of the samples, while rows correspond to categories of metabolites. Differential metabolite expression has been transformed using log2. (B), (C), (D) Violin plot distribution of LPE (0:0/22:6), LPC (15:0/0:0), 1-linoleoyl-sn-glycero-3-phosphorylcholine and allantoic acid in SARS-CoV-2-positive and -negative groups in T1, T2 and T3, respectively. The Y-axis represents log2 transformed metabolite intensities. X-axis labels differential metabolites. Violin plot data were analyzed by *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Abbreviations: GP, glycerophospholipid; FA, fatty acids; SL, sphingolipid; GL, glycerolipid; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine.

Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

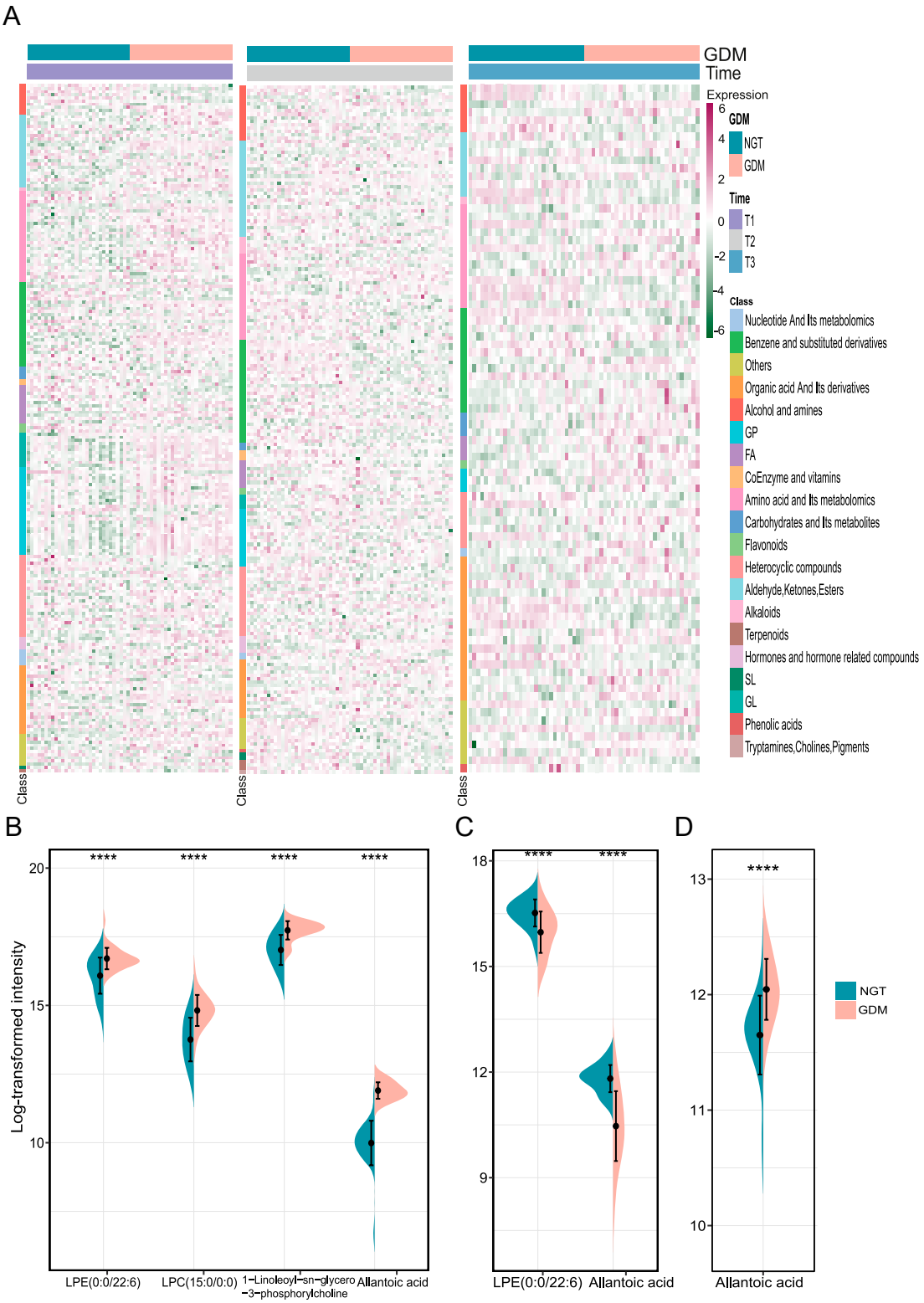


Figure 5

Screening for differential metabolites associated with GDM. (A) Heat map of differential expressed metabolites (DEMs) in T1, T2 and T3 in NGT vs GDM group. Columns represent the trimesters and groups of the samples, while rows correspond to categories of metabolites. Differential metabolite expression has been transformed using log2. (B), (C), (D) Violin plot: distribution of LPE (0:0/22:6), LPC (15:0/0:0), 1-linoleoyl-sn-glycero-3-phosphorylcholine and allantoic acid in NGT vs GDM group in T1, T2 and T3, respectively. The Y-axis represents log2 transformed metabolite intensities. X-axis labels differential metabolites. Violin plot data were analyzed by *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Abbreviations: GP, glycerophospholipid; FA, fatty acids; SL, sphingolipid; GL, glycerolipid; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine.

Endocrine Connections (2025) **14** e240681
https://doi.org/10.1530/EC-24-0681

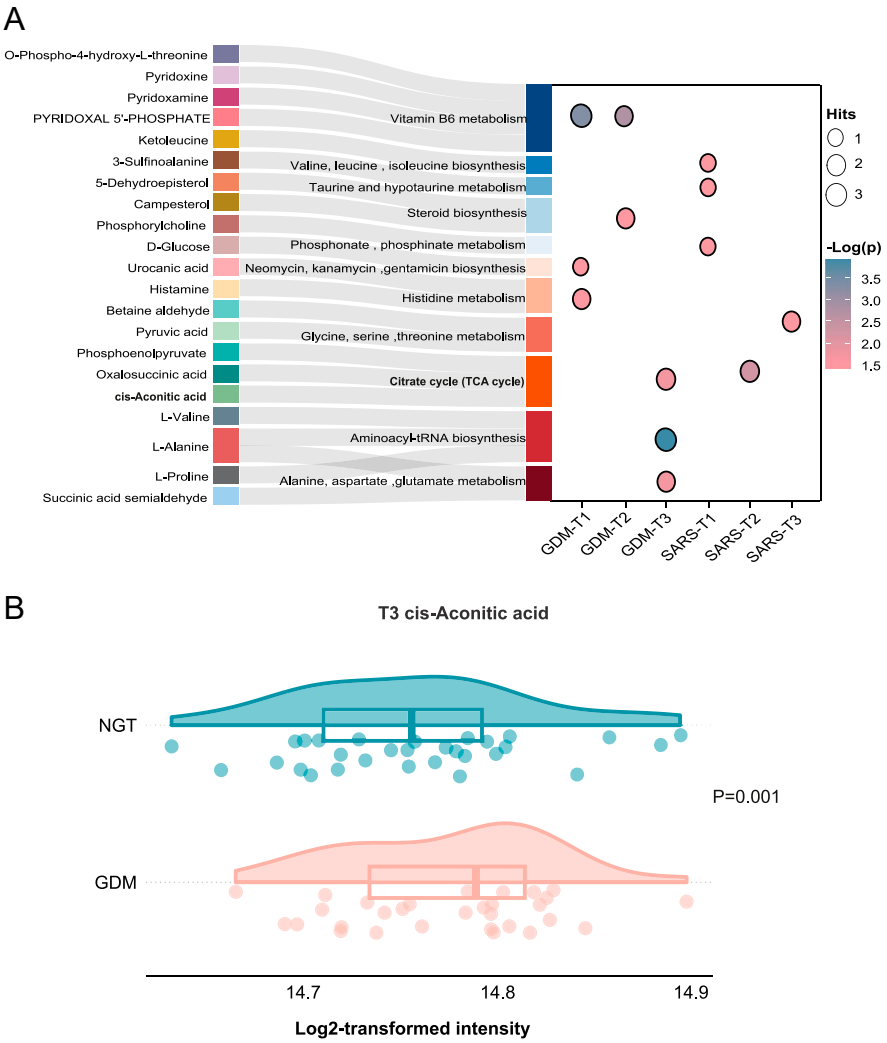


Figure 6

KEGG pathway enrichment analysis of differential metabolites. (A) Sankey diagram illustrating pathways and metabolites significantly enriched in T1, T2 and T3 in the GDM and SARS-CoV-2 groups. (B) Raincloud plot: the distribution of cis-aconitic acid in NGT vs GDM groups in T3. The horizontal coordinates represent the log2-transformed intensity of differential metabolites.

threonine metabolism were enriched (Fig. 6A, Supplementary Table 8).

Regarding the DEMs of GDM, pathways such as vitamin B6 metabolism, neomycin, kanamycin, gentamicin biosynthesis and histidine metabolism were enriched in T1. In T2, the steroid biosynthesis pathway showed enrichment of DEMs. In T3, there was an abundance of DEMs in the TCA cycle, aminoacyl tRNA biosynthesis and alanine, aspartate and glutamate metabolism. Both SARS-CoV-2 infection and GDM exhibited dysregulation of the TCA cycle (Fig. 6A, Supplementary Table 8). Cis-aconitic acid was enriched in the TCA cycle (Fig. 6B).

Discussion

To our best knowledge, this is the first to explore the metabolic impact of a history of GDM on SARS-CoV-2 infection and the association of these two diseases from a metabolomic perspective. Women with a history of GDM were more susceptible to SARS-CoV-2 infection, even though they had received more COVID-19 vaccinations. Among all SARS-CoV-2-positive patients, those with a history of GDM were more likely to have hyperthermia than NGT. We revealed that GDM was a standalone determinant of SARS-CoV-2 infection.

Another important finding of this study was the discovery of similar metabolic changes in both the SARS-CoV-2 positive and GDM groups across all three trimesters through longitudinal metabolomics analysis. In T1, LPE (0:0/22:6), LPC (15:0/0:0) and 1-linoleoyl-sn-glycero-3-phosphorylcholine levels were elevated in both groups. In T2, LPE (0:0/22:6) remained elevated, and in T3, allantoic acid was increased. This similarity suggests a metabolic association between SARS-CoV-2 and GDM, indicating that these altered metabolites may underlie the mechanism of this association.

We hypothesized that the increased susceptibility to SARS-CoV-2 in women with a history of GDM may be related to this metabolic association. So, is this metabolic association the metabolic memory effect we are interested in?

Metabolic association and memory effect

In our study, allantoic acid was consistently elevated in T1, T2 and T3 in the GDM group. In addition, KEGG pathway enrichment analysis revealed that the TCA cycle was enriched in both the SARS-CoV-2 positive and GDM groups, with cis-aconitic acid, a component of this pathway, elevated in T3 of the GDM group. Given that GDM is often managed through clinical interventions such as dietary control or insulin to normalize blood glucose levels after diagnosis, the T1 and T2 data reflect a natural state without intervention. In contrast, the T3 data reflect the state post-intervention.

Allantoic acid remained consistently elevated throughout all three trimesters, and cis-aconitic acid was elevated in T3, with both unaffected by clinical intervention. The 'metabolic memory effect' of GDM is characterized by its long-term persistence and does not disappear even when blood glucose is normalized by clinical intervention. Allantoic acid and cis-aconitic acid trends align with this metabolic memory effect. Therefore, we hypothesize that these metabolites may be part of the mechanism behind the adverse metabolic memory effect.

The metabolic memory effect has not been conclusively explained in current research, with oxidative stress, inflammation and epigenetics suggested as key factors (15). Allantoic and cis-aconitic acids, which were identified in our metabolomics data, have been previously shown to be involved in oxidative stress and inflammation. This further supports our hypothesis, which we will explore in more detail below.

Allantoic acid, oxidative stress

In our study, allantoic acid was increased throughout the T1, T2 and T3 phases of GDM and T3 of the SARS-CoV-2 positive group. Allantoic acid is produced from uric acid in the body by redox reaction with nicotinamide adenine dinucleotide (NAD^+) (16) and an increase is associated with excessive oxidative stress (17, 18). Previous studies have shown that both GDM and SARS-CoV-2 are closely related to oxidative stress. Physiologically, pregnancy and hyperglycemia increase the body's reactive oxygen species (ROS) level, so patients with GDM are prone to oxidative stress (19). SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE-2) in host cells through the receptor-binding domain of its spike glycoprotein, leading to an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This leads to the production of $NADP^+$ and the release of ROS (20). A study of hyperglycemia and COVID-19 showed that glycosylated ACE-2 induced by oxidative stress and hyperglycemia was the true receptor that binds to SARS-CoV-2, not total ACE-2 (21). We hypothesized that GDM-mediated oxidative stress and hyperglycemia result in the production of more glycosylated ACE-2 that may contribute to the metabolic memory effect and increase susceptibility to SARS-CoV-2 infection.

Cis-aconitic acid, oxidative stress, inflammation

In addition to allantoic acid, cis-aconitic acid screened in KEGG enrichment analysis is also of interest. Following KEGG analysis, the TCA cycle was revealed to be enriched in both the GDM group and the SARS-CoV-2 positive group. The TCA cycle, also known as the Krebs cycle, oxidizes nutrients such as sugars, proteins and lipids to provide energy (22). Cis-aconitic acid is an intermediate in the TCA cycle that decarboxylates to produce itaconic acid (23). It has been documented that itaconic acid plays a role in reducing inflammation and combating oxidative stress (24). Imbalanced conversion of cis-aconitic acid to itaconic acid may promote oxidative stress and inflammation. Our results showed that cis-aconitic acid was increased in the GDM group, suggesting an imbalance between oxidative and antioxidant, inflammation and anti-inflammation. Thus, the oxidative stress and inflammation that accompany the imbalance in energy metabolism during GDM may also serve as metabolic memory leading to susceptibility to SARS-CoV-2.

Our study is the first to confirm with a large sample size that GDM history is an independent risk for SARS-CoV-2 infection and to explore the association of these two diseases from a metabolomic perspective. Nonetheless, there are some limitations: first, the sample size for metabolomics was small and may have affected statistical efficiency. Second, metabolites identified in our study require validation in further study.

Conclusions

Our survey data indicate that women with a history of GDM are more vulnerable to SARS-CoV-2 infection and experience more intense fever following infection compared to those without a history of GDM. Screening of DEMs suggests that the adverse metabolic memory effects of GDM, as evidenced by elevated levels of allantoic and cis-aconitic acids, may play a role in increasing susceptibility to SARS-CoV-2.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-24-0681.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

Funding

The research was supported by the National Natural Science Foundation of China (82270852), the Science and Technology Innovation Action Plan 'Biomedical Technology Support Special Project' of Shanghai Municipal Science and Technology Commission (21S31901800), Scientific Research

Project funded by Shanghai Municipal Science and Technology Commission (21ZR1450500), Program of Science and Technology Cooperation with Hong Kong, Macao and Taiwan (22410760500), the Shenzhen Science and Technology Program (JCYJ20220530145210024) and the Guangdong Basic and Applied Basic Research Foundation (2023A1515012624, 2024A1515013260).

Author contribution statement

ZYL helped in conceptualization, data curation, writing of the original draft and visualization. QHL helped in conceptualization, formal analysis, visualization, writing of the review and editing. RZ helped in conceptualization, methodology, writing of the review and editing and project administration. NEBY.XLF and YL helped in investigation and validation. XMH and JYZ helped with resources and software. SFZ, GZJ and JL helped with conceptualization, funding acquisition, project administration and supervision. ZYL, QHL and RZ should be considered joint first authors. SFZ, GZJ and JL should be considered joint corresponding authors. All authors read and approved the final manuscript.

Data availability

Relevant data from this study can be obtained from the corresponding author upon reasonable request.

Acknowledgments

We thank the mothers, and all clinical and research personnel who participated in this research!

References

1 McIntyre HDCP, Zhang C, Desoye G, *et al.* Gestational diabetes mellitus. *Nat Rev Dis Primers* 2019 **5** 47. (https://doi.org/10.1038/s41572-019-0098-8)

2 Johns EC, Denison FC, Norman JE, *et al.* Gestational diabetes mellitus: mechanisms, treatment, and complications. *Trends Endocrinology Metabolism* 2018 **29** 743–754. (https://doi.org/10.1016/j.tem.2018.09.004)

3 Dong H, Sun Y, Nie L, *et al.* Metabolic memory: mechanisms and diseases. *Signal Transduct Targeted Ther* 2024 **9** 38. (https://doi.org/10.1038/s41392-024-01755-x)

4 Kleinwechter HJ, Weber KS, Mingers N, *et al.* Gestational diabetes mellitus and COVID-19: results from the COVID-19-related obstetric and neonatal outcome study (CRONOS). *Am J Obstet Gynecol* 2022 **227** 631.e1–631.e19. (https://doi.org/10.1016/j.ajog.2022.05.027)

5 Mendez Y, Alpuing Radilla LA, Delgadillo Chabolla LE, *et al.* Gestational diabetes mellitus and COVID-19. The epidemic during the pandemic. *World J Diabetes* 2023 **14** 1178–1193. (https://doi.org/10.4239/wjd.v14.i8.1178)

6 Zanardo V, Tortora D, Sandri A, *et al.* COVID-19 pandemic: impact on gestational diabetes mellitus prevalence. *Diabetes Res Clin Pract* 2022 **183** 109149. (https://doi.org/10.1016/j.diabres.2021.109149)

7 Yin B, Wu K, Hu L, *et al.* Gestational diabetes mellitus in the COVID-19 pandemic: a retrospective study from Hangzhou, China. *J Diabetes* 2022 **14** 711–720. (https://doi.org/10.1111/1753-0407.13324)

8 Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. *Physiol Rev* 2019 **99** 1819–1875. (https://doi.org/10.1152/physrev.00035.2018)

9 Zhou Y, Zhao R, Lyu 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 **13** 3644. (https://doi.org/10.3390/nu13103644)

10 Ye D, Huang J, Wu J, *et al.* Integrative metagenomic and metabolomic analyses reveal gut microbiota-derived multiple hits connected to development of gestational diabetes mellitus in humans. *Gut Microbes* 2023 **15** 2154552. (https://doi.org/10.1080/19490976.2022.2154552)

11 Hora S, Pahwa P, Siddiqui H, *et al.* Metabolic alterations unravel the maternofetal immune responses with disease severity in pregnant women infected with SARS-CoV-2. *J Med Virol* 2023 **95** e29257. (https://doi.org/10.1002/jmv.29257)

12 Wang K, Khoramjoo M, Srinivasan K, *et al.* Sequential multi-omics analysis identifies clinical phenotypes and predictive biomarkers for long COVID. *Cell Rep Med* 2023 **4** 101254. (https://doi.org/10.1016/j.xcrm.2023.101254)

13 American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020. *Diabetes Care* 2020 **43** S14–S31. (https://doi.org/10.2337/dc20-S002)

14 Lu Q, Li Y, Ye D, *et al.* Longitudinal metabolomics integrated with machine learning identifies novel biomarkers of gestational diabetes mellitus. *Free Radic Biol Med* 2023 **209** 9–17. (https://doi.org/10.1016/j.freeradbiomed.2023.10.014)

15 Berezin A. Metabolic memory phenomenon in diabetes mellitus: achieving and perspectives. *Diabetes Metabol Syndr* 2016 **10** S176–S183. (https://doi.org/10.1016/j.dsx.2016.03.016)

16 Triplett EW, Blevins DG & Randall DD. Allantoic acid synthesis in soybean root nodule cytosol via xanthine dehydrogenase. *Plant Physiol* 1980 **65** 1203–1206. (https://doi.org/10.1104/pp.65.6.1203)

17 Feng L, Wu HW, Song GQ, *et al.* Chronical sleep interruption-induced cognitive decline assessed by a metabolomics method. *Behav Brain Res* 2016 **302** 60–68. (https://doi.org/10.1016/j.bbr.2015.12.039)

18 Chen S, Zhang M, Bo L, *et al.* Metabolomic analysis of the toxic effect of chronic exposure of cadmium on rat urine. *Environ Sci Pollut Res Int* 2018 **25** 3765–3774. (https://doi.org/10.1007/s11356-017-0774-8)

19 Saucedo R, Ortega-Camarillo C, Ferreira-Hermosillo A, *et al.* Role of oxidative stress and inflammation in gestational diabetes mellitus. *Antioxidants* 2023 **12** 1812. (https://doi.org/10.3390/antiox12101812)

20 Paul AK, Hossain MK, Mahboob T, *et al.* Does oxidative stress management help alleviation of COVID-19 symptoms in patients experiencing diabetes? *Nutrients* 2022 **14** 321. (https://doi.org/10.3390/nu14020321)

21 Brufsky A. Hyperglycemia, hydroxychloroquine, and the COVID-19 pandemic. *J Med Virol* 2020 **92** 770–775. (https://doi.org/10.1002/jmv.25887)

22 Arnold PK & Finley LWS. Regulation and function of the mammalian tricarboxylic acid cycle. *J Biol Chem* 2023 **299** 102838. (https://doi.org/10.1016/j.jbc.2022.102838)

23 Michelucci A, Cordes T, Ghelfi J, *et al.* Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc Natl Acad Sci U S A* 2013 **110** 7820–7825. (https://doi.org/10.1073/pnas.1218599110)

24 Shi X, Zhou H, Wei J, *et al.* The signaling pathways and therapeutic potential of itaconate to alleviate inflammation and oxidative stress in inflammatory diseases. *Redox Biol* 2022 **58** 102553. (https://doi.org/10.1016/j.redox.2022.102553)