

Supplementary Table S1. Differentially abundant genera and species between GDM and non GDM groups identified by *LEfSe* analysis.

Variable	GDM group (n=98)	Non-GDM group (n=538)	<i>p</i> -Value
Age	31.28 ± 4.36	29.84 ± 3.92	0.064
Ethnicity			0.772
Han	96 (98.00)	523 (97.20)	
Other	2 (2.00)	15 (2.80)	
Height (cm)	160.85 ± 4.37	160.52 ± 4.32	0.583
Weight (kg)	61.12 ± 7.15	56.33 ± 6.52	<0.001
BMI (kg/m ²)	23.56 ± 2.83	21.88 ± 2.69	<0.001
Systolic blood pressure (mmHg)	112.42 ± 9.52	110.63 ± 9.11	0.179
Diastolic blood pressure (mmHg)	71.45 ± 6.92	70.33 ± 6.81	0.242
Fasting glucose (mmol/L,)	4.85 ± 0.42	4.61 ± 0.38	<0.001
Triglycerides (mmol/L)	1.69 ± 0.49	1.54 ± 0.42	0.024
Total cholesterol (mmol/L,)	5.14 ± 0.91	5.02 ± 0.86	0.327
HDL-C (mmol/L,)	1.58 ± 0.27	1.62 ± 0.28	0.214
LDL-C (mmol/L)	2.92 ± 0.69	2.84 ± 0.65	0.412
Albumin (g/L)	43.72 ± 2.85	43.54 ± 2.96	0.642
ALT (U/L)	18.21 ± 7.62	17.68 ± 8.01	0.504
AST (U/L)	21.35 ± 6.93	20.81 ± 7.24	0.568
Creatinine (μmol/L,)	47.21 ± 5.34	46.84 ± 5.26	0.653
Uric acid (μmol/L)	193.42 ± 35.11	185.97 ± 31.44	0.071
Urea (mmol/L)	2.51 ± 0.48	2.49 ± 0.46	0.737
Obstetric history			
Gravidity			0.212
1	43 (43.90)	278 (51.70)	
≥2	55 (56.10)	260 (48.30)	
Parity			0.164
0	66 (67.30)	402 (74.70)	
≥1	32 (32.70)	136 (25.30)	
Education level			0.684
≤High school	41 (41.80)	211 (39.20)	
College	35 (35.70)	183 (34.00)	
≥Undergraduate	22 (22.50)	144 (26.80)	
Occupation			0.892
Worker	26 (26.50)	148 (27.50)	
Technician	22 (22.40)	111 (20.60)	

Farmer	18 (18.40)	93 (17.30)	
Other	32 (32.70)	186 (34.60)	
Monthly household income(CNY)			0.516
<5000	5 (10.60)	7 (7.40)	
5000–10000	19 (40.40)	50 (53.20)	
10001–20000	19 (40.40)	31 (33.00)	
>20000	4 (8.50)	6 (6.40)	
Alcohol use			0.988
Yes	3 (3.10)	17 (3.20)	
No	95 (96.90)	521 (96.80)	
Passive smoking			0.646
Yes	21 (21.40)	127 (23.60)	
No	77 (78.60)	411 (76.40)	
Sleep quality			0.574
Good	9 (19.10)	21 (22.30)	
Fair	28 (59.60)	46 (48.90)	
Poor	9 (19.10)	26 (27.70)	
Bad	1 (2.10)	1 (1.10)	

Note: Continuous variables are presented as mean $\pm$ standard deviation and categorical variables as number (percentage). p-values were calculated using t-tests or chi-square tests, as appropriate. GDM refers to gestational diabetes mellitus.

Supplementary Table S2. Differentially abundant genera and **species** between GDM and non GDM groups identified by *LEfSe* analysis.

	Maximum Mean Abundance (log ₁₀)	LDA Score	p-Value	Enriched group
Genera				
<i>Collinsella</i>	3.64	3.07	0.040	GDM group
<i>Peptostreptococcaceae</i>	3.25	2.72	0.020	Control group
<i>Oscillibacter</i>	3.81	3.03	0.040	Control group
<i>Bacteroides</i>	5.36	4.65	0.002	Control group
Species				
<i>Collinsella aerofaciens</i>	3.58	3.02	0.006	GDM group
<i>Clostridium bartlettii</i>	3.64	2.10	0.040	GDM group
<i>Anaerotruncus colihominis</i>	2.93	2.49	0.005	Control group
<i>Catenibacterium mitsuokai</i>	2.99	2.69	0.030	Control group
<i>Alistipes finegoldii</i>	3.31	2.71	0.030	Control group
<i>Bacteroidales bacterium ph8</i>	3.55	2.97	0.030	Control group
<i>Bacteroides xylanisolvens</i>	3.64	3.10	0.050	Control group
<i>Streptococcus salivarius</i>	3.74	3.14	0.040	Control group
<i>Lactobacillus casei</i>	3.51	3.24	0.030	Control group
<i>paracasei</i>				
<i>Alistipes putredinis</i>	4.57	3.94	0.030	Control group
<i>Eubacterium eligens</i>	4.57	3.99	0.005	Control group

Note: Differentially abundant genera and species were identified using LEfSe analysis. The significance threshold was set at $p < 0.05$ and LDA score > 2.0 . “Enriched group” indicates the group in which the taxon was relatively more abundant. GDM, gestational diabetes mellitus; LDA, linear discriminant analysis.

Supplementary Table S3. Differentially abundant metabolic pathways between GDM and non GDM groups identified by *LEfSe* analysis.

Metabolic pathways	Maximum Mean Abundance (log ₁₀)	LDA Score	p-Value	Enriched/ Depleted in GDM
D_galactarate degradation I	2.99	2.57	0.040	enriched
D_galacturonate degradation I	3.72	2.88	<0.001	enriched
Fatty acid salvage	2.6	2.31	<0.001	enriched
Superpathway of beta_D_glucuronide and D_glucuronate degradation	3.65	2.67	0.040	enriched
acetyl_CoA fermentation to butanoate II	3.02	2.23	0.001	depleted
Biotin biosynthesis II	2.89	2.09	0.030	depleted
Colonic acid building blocks biosynthesis	3.04	2.26	0.001	depleted
D_galactose degradation V_Leloir pathway	3.89	2.96	0.020	depleted
dTDP_L_rhamnose biosynthesis I	3.97	2.94	0.040	depleted
Fatty acid elongation_saturated	2.84	2.00	<0.001	depleted
Gluconogenesis I	3.07	2.36	<0.001	depleted
Gluconogenesis III	3.10	2.32	0.004	depleted
L_histidine degradation I	3.09	2.26	0.003	depleted
L_histidine degradation III	2.98	2.18	0.008	depleted
preQ0 biosynthesis	3.89	2.83	0.020	depleted
stachyose degradation	3.84	2.56	0.030	depleted
Super pathway of histidine_purine_and pyrimidine biosynthesis	3.46	2.45	0.003	depleted
Super pathway of L_methionine biosynthesis by sulphydrylation	3.10	2.42	0.001	depleted
Super pathway of pyridoxal5_phosphate biosynthesis and salvage	3.54	2.60	0.030	depleted
Super pathway of pyrimidine deoxyribonucleotides de novo biosynthesis	3.44	2.52	0.009	depleted

Note: Differential metabolic pathways were identified using LEfSe with an LDA score > 2.0 and p < 0.05. “Enriched” or “Depleted” in GDM indicates whether the pathway abundance was higher or lower in the GDM group compared with controls. GDM, gestational diabetes mellitus; LDA, linear discriminant analysis.

Supplementary Table S4. Differential Metabolites Identified Among Comparison Groups under the OPLS-DA Model

Metabolites	VIP	Mean (GDM)	Variance (GDM)	Mean (CTR)	Variance (CTR)	log ₂ (FC)	Increased/D eased in GDM	p-Value
<i>Methyluric.acid</i>	2.6	3265.89	4678.34	183.86	263.33	4.151	increased	0.001
<i>CDP</i>	1.5	34979.59	72196.7	4211.96	23127	3.054	increased	0.038
<i>Glycerol.3.phosphate</i>	1.7	8198.58	16301.76	1341.52	6014.68	2.612	increased	0.042
<i>Sulfapyridine</i>	1.76	6691.71	10611.41	1338.03	4908.31	2.322	increased	0.014
<i>Testolactone</i>	1.75	4855.35	7635.66	1032.02	2758.83	2.234	increased	0.013
<i>Levetiracetam</i>	2.02	8609.89	12940.52	1837.22	4620.32	2.228	increased	0.009
<i>S_.3.4.Dihydroxybutyric.acid</i>	2.24	892208.85	1010177.31	202145.43	541431.97	2.142	increased	0.001
<i>Polystyrene.sulfonate</i>	2.33	10725.02	10159.68	2451.23	6284.63	2.129	increased	<0.001
<i>trans.4.Hydroxycyclohexylacetic .acid</i>	2.04	9675.45	13321.74	2253.78	5581.29	2.102	increased	0.006
<i>Barium</i>	2.03	4473.1	5213.87	1247.34	2526.8	1.842	increased	0.002
<i>Chlorotyrosine</i>	1.6	2887.4	4581.6	807.54	1900.02	1.838	increased	0.03
<i>Levonordefrin</i>	2.14	7150.18	6126.7	2442.23	4632.79	1.55	increased	<0.001
<i>N.Desmethyldiltiazem</i>	1.36	7768.19	9807.86	2992.92	7511.64	1.376	increased	0.03
<i>Hydroxypicolinic.acid</i>	1.68	8082.19	7330.89	3462.87	6950.41	1.223	increased	0.006
<i>Iodine</i>	1.24	8605.2	11407.6	3788.06	5852.07	1.184	increased	0.048
<i>Secobarbital</i>	1.3	57874.01	56885.15	29014.3	47335.42	0.996	increased	0.027
<i>Guanidinosuccinic.acid</i>	2.15	29390.74	16787.31	15736.7	11765.94	0.901	increased	<0.001
<i>Glycerophosphocholine</i>	2.41	32976.41	18588.75	18721.16	13223.26	0.817	increased	<0.001
<i>L.Phenylalanine</i>	1.79	292656.23	243258.36	168838.32	132768.45	0.794	increased	0.015
<i>p.Hydroxyl.ethotoin</i>	1.13	5716.73	5083.22	3421.19	4053.04	0.741	increased	0.048
<i>Aminoethoxy acetic acid</i>	1.45	134173.59	102933.47	84250.76	67723.36	0.671	increased	0.027
<i>Nitroxoline</i>	1.67	14007.97	6229.77	8998.03	6701.6	0.639	increased	0.001
<i>L.Tyrosine</i>	1.39	43517.39	24113.48	29005.09	22178.16	0.585	increased	0.008
<i>Indoxyl.sulfate</i>	1	6308.09	5435.12	9680.35	8606.15	-0.618	decreased	0.036

Metabolites	VIP	Mean (GDM)	Variance (GDM)	Mean (CTR)	Variance (CTR)	log ₂ (FC)	Increased/ Decreased in GDM	p-Value
<i>Desacetylvinblastine</i>	1.35	545.41	361.23	856.87	887.51	-0.652	decreased	0.028
<i>Gamma.Butyrolactone</i>	1.67	133556.99	120357.4	215256.94	115442.39	-0.689	decreased	0.003
<i>Diaminosalicylic.acid</i>	1.18	3299.66	4406.85	5772.3	5396.35	-0.807	decreased	0.03
<i>Phenylalanylphenylalanine</i>	1.12	75900.33	80701.49	133081.67	122048.75	-0.81	decreased	0.011
<i>Pentosidine</i>	1.42	3128.74	3727.55	5487.07	4423.51	-0.81	decreased	0.011
<i>Methylmercaptapurine</i>	1.29	9945.54	9423.84	17866.48	16753.9	-0.845	decreased	0.005
<i>Desacetylcefotaxime</i>	1.34	418.72	399.97	762.43	706.52	-0.865	decreased	0.004
<i>Clavulanate</i>	1.25	177541.77	313840.01	332926.92	310697.95	-0.907	decreased	0.039
<i>Stearic.acid</i>	1.59	6665.37	7397.12	12559.45	6664.86	-0.914	decreased	<0.001
<i>Trifluoroacetic.acid</i>	1.53	275405.65	477363.52	524654.29	332237.24	-0.93	decreased	0.016
<i>Pyridoxamine</i>	1.07	363.3	260.06	766.57	986.88	-1.077	decreased	0.004
<i>Sulfamerazine</i>	1.08	1118.38	1346.39	2559.56	3566.76	-1.194	decreased	0.008
<i>Fluorouracil</i>	1.71	45414.94	64739.68	106605.36	81379.01	-1.231	decreased	<0.001
<i>Sulfite</i>	1.05	2925.15	7288.33	7230.29	10061.67	-1.306	decreased	0.03
<i>Hydroxy.deoxyguanosine</i>	1.22	1397.98	3827.8	3883.3	7001.01	-1.474	decreased	0.043
<i>Torasemide</i>	1.72	277.48	296.26	811.41	769.92	-1.548	decreased	<0.001
<i>L.Tryptophan</i>	1.51	34797.78	83197.06	102962.59	119341.39	-1.565	decreased	0.002
<i>Sevoflurane</i>	1.48	2719.52	6118.44	8090.48	9395.91	-1.573	decreased	0.001
<i>Selenium.Sulfide</i>	1.33	781.38	2645.01	2638.02	4129.7	-1.755	decreased	0.013
<i>Sulfametopyrazine</i>	1.39	593.54	909.06	2120.16	3214.35	-1.837	decreased	0.001
<i>L.Cystine</i>	1.35	736.83	1872.06	2758.9	3491.36	-1.905	decreased	<0.001
<i>Ezetimibe</i>	2.11	121	152.3	482.89	502.49	-1.997	decreased	<0.001
<i>Methoxybenzenepropanoic.acid</i>	1.34	3166.17	3773.39	13409.53	21649.86	-2.082	decreased	<0.001
<i>Nalidixic.Acid</i>	1.43	328.89	242.51	1607.2	3430.23	-2.289	decreased	0.006
<i>Eplerenone</i>	1.3	2692.34	9127.08	14289.25	28221.53	-2.408	decreased	0.005
<i>Myristic.acid</i>	1.59	49509.34	101104.49	269427.46	465802.76	-2.444	decreased	0.001

Metabolites	VIP	Mean (GDM)	Variance (GDM)	Mean (CTR)	Variance (CTR)	log ₂ (FC)	Increased/ Decreased in GDM	p-Value
<i>N.isopropylterephthalamide</i>	1.47	312.5	919.54	1712.07	2451.53	-2.454	decreased	<0.001
<i>Hexachlorophene</i>	1.49	15889.05	50286.5	87966.8	117463.34	-2.469	decreased	<0.001
<i>Acetylglycine</i>	1.99	216.87	292.06	1236.75	1519.65	-2.512	decreased	<0.001
<i>Niacinamide</i>	1.57	7674.63	27819.98	48117.75	89869.56	-2.648	decreased	0.002
<i>desmethyldomipramine</i>	1.71	742.76	2189.64	4922.6	7604.8	-2.728	decreased	<0.001
<i>3,7-Trimethyluric acid</i>	1.47	872.5	482.49	5788.01	10364.22	-2.73	decreased	<0.001
<i>Oxoadipic acid</i>	1.61	3692.41	16181.92	26139.48	35912.75	-2.824	decreased	<0.001
<i>O.Desmethylnaproxen</i>	1.74	893.83	2714.64	6565.99	9208.52	-2.877	decreased	<0.001
<i>3,4-Hydroxy-3-methoxyphenyl-2-methylpropionic acid</i>	1.55	115.72	162.63	851.1	1410.24	-2.879	decreased	<0.001
<i>Telbivudine</i>	1.36	211	677.64	1556.84	3110.54	-2.883	decreased	0.002
<i>Hydroxybenzaldehyde</i>	1.73	8973.38	32167.46	72840.4	100693.13	-3.021	decreased	<0.001
<i>Tamsulosin</i>	2.7	531.49	1194.77	4876.19	4086.42	-3.198	decreased	<0.001
<i>Sorbitol-6-phosphate</i>	2.43	195.74	221.56	3219.72	3477.9	-4.04	decreased	<0.001
<i>Hydroxydodecanedioic acid</i>	1.6	249.02	475.44	8764.15	16760.89	-5.137	decreased	<0.001

Note: Differential metabolites were identified using orthogonal partial least squares-discriminant analysis (OPLS-DA). Selection criteria were $|\log_2(\text{FC})| > 0.415$, $\text{VIP} \geq 1.0$, and Benjamini–Hochberg adjusted p-value < 0.05 . “Increased” or “Decreased” in GDM indicates higher or lower metabolite levels in the GDM group relative to controls. GDM, gestational diabetes mellitus; CTR, control; VIP, variable importance in projection; FC, fold change.