

Table S1. Unweighted and weighted participant characteristics in the discovery set and unweighted participant characteristics in the PETALS cohort

	Discovery set (n=271)		PETALS cohort (n=3,162)
	Unweighted	Weighted*	Unweighted
Age at delivery, y, mean \pm SD	32.0 \pm 4.6	30.0 \pm 0.5	30.2 \pm 5.3
Age at delivery, y, %			
<25	7.7	20.8 (12.6-28.9)	16.0
25-29	18.8	24.4 (17.7-31.2)	25.9
30-34	45.0	35.1 (28.9-41.3)	36.7
\geq 35	28.4	19.7 (15.3-24.1)	21.4
Race/ethnicity, %			
White	21.8	25.6 (19.5-31.6)	21.6
Hispanic	32.8	13.4 (6.6-20.3)	10.3
Black	9.2	15.6 (12.8-18.4)	23.1
Asian/Pacific Islander	30.3	41.3 (33.4-49.2)	41.8
Other/Unknown	5.9	4.1 (2.0-6.2)	3.2
Education, %			
High school or less	11.4	17.1 (9.6-24.6)	14.5
Some college	40.2	44.6 (35.7-53.5)	37.8
College graduate or above	48.3	38.3 (30.9-48.6)	47.6
Nulliparity, %	44.6	52.9 (44.6-61.3)	44.3
Pre-pregnancy BMI, kg/m², %[†]			
Underweight/normal weight	19.8	32.0 (24.2-39.8)	38.4
Overweight	38.5	31.9 (23.4-40.3)	31.2
Obese	41.8	36.1 (27.5-44.6)	30.4
Chronic hypertension, %	5.9	3.8 (1.8-5.8)	4.4
Family history of diabetes, %	24.4	17.8 (12.3-23.7)	23.5
History of GDM, %	6.6	1.8 (0.8-2.9)	3.1
GDM, %	33.6	9.0 (6.3-11.8)	9.8

BMI, body mass index.

* Descriptive statistics were derived by applying the sampling weight as described in the Methods section.

[†] Non-Asians were categorized as underweight (BMI <18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (\geq 30.0 kg/m²). Asians were categorized as underweight (<18.5 kg/m²), normal weight (18.5–22.9 kg/m²), overweight (23.0–27.4 kg/m²), and obese (\geq 27.5 kg/m²).

Table S2. Participant characteristics in the validation sets 1 (a random sample in the PETALS cohort) and 2 (a nested case-control study within the GLOW trial)^a

	Validation set 1				Validation set 2			
	All (n=414)	GDM (n=42)	Non-GDM (n=372)	P-value*	All (n=105)	GDM (n=35)	Non-GDM (n=70)	P-value*
Age at delivery, y, n (%)				0.59				0.28
<25	52 (12.6)	3 (7.1)	49 (13.2)		2 (1.9)	2 (5.7)	0 (0.0)	
25-29	108 (26.1)	10 (23.8)	98 (26.3)		5 (4.8)	1 (2.9)	4 (5.7)	
30-34	160 (38.6)	17 (40.5)	143 (38.4)		63 (60.0)	21 (60.0)	42 (60.0)	
≥35	94 (22.7)	12 (28.6)	82 (22.0)		35 (33.3)	11 (31.4)	24 (34.3)	
Race/ethnicity, n (%)				0.05				
White	105 (25.4)	9 (21.4)	96 (25.8)		15 (14.3)	5 (14.3)	10 (14.3)	1.00
Hispanic	105 (25.4)	8 (19.0)	97 (26.1)		27 (25.7)	9 (25.7)	18 (25.7)	
Black	99 (23.9)	7 (16.7)	92 (24.7)		3 (2.9)	1 (2.9)	2 (2.9)	
Asian/Pacific Islander	105 (25.4)	18 (42.9)	87 (23.4)		42 (40.0)	14 (40.0)	28 (40.0)	
Other/unknown					18 (17.1)	6 (17.1)	12 (17.1)	
Education, n (%)				0.80				0.08
High school or less	53 (12.8)	4 (9.5)	49 (13.2)		7 (6.7)	5 (14.3)	2 (2.9)	
Some college	151 (36.6)	16 (38.1)	135 (36.4)		17 (16.2)	6 (17.1)	11 (15.7)	
College graduate or above	209 (50.6)	22 (52.4)	187 (50.4)		81 (77.1)	24 (68.6)	57 (81.4)	
Nulliparity, n (%)	185 (44.7)	18 (42.9)	167 (44.9)	0.80	51 (48.6)	19 (54.3)	32 (45.7)	0.41
Pre-pregnancy BMI, kg/m², n (%)[†]				0.01				0.04
Underweight/normal weight	160 (38.6)	9 (21.4)	151 (40.6)		-	-	-	
Overweight	115 (27.8)	10 (23.8)	105 (28.2)		71 (67.6)	19 (54.3)	52 (74.3)	
Obese	139 (33.6)	23 (54.8)	116 (31.2)		34 (32.4)	16 (45.7)	18 (25.7)	
Chronic hypertension, n (%)	26 (6.3)	2 (4.8)	24 (6.5)	0.67	1 (1.4)	0 (0.0)	1 (2.9)	1.00
Family history of diabetes, n (%)	95 (22.9)	12 (28.6)	83 (22.3)	0.36	17 (16.2)	11 (31.4)	6 (8.6)	0.003
History of GDM, n (%)	11 (2.7)	4 (9.5)	7 (1.9)	0.004	9 (8.6)	7 (20.0)	2 (2.9)	0.01
GLOW trial arms								
Intervention	NA	NA	NA		55 (52.4)	18 (51.4)	37 (52.9)	0.89
Usual care	NA	NA	NA		50 (47.6)	17 (48.6)	33 (47.1)	

BMI, body mass index; GDM, gestational diabetes; GLOW, Gestational Weight Gain and Optimal Wellness; NA, not applicable.

*P values for differences between women with and without GDM were obtained by student's t-test for continuous variables and Chi-square or Fisher's exact test for categorical variables.

[†]Non-Asians were categorized as underweight (BMI <18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30.0 kg/m²). Asians were categorized as underweight (<18.5 kg/m²), normal weight (18.5–22.9 kg/m²), overweight (23.0–27.4 kg/m²), and obese (≥27.5 kg/m²).

Supplemental Figure 1. Treemap showing the distribution of metabolites among super pathway (in color) and their related sub-pathways (square) for the 144 metabolites used for statistical and bioinformatics analysis.

Each number corresponds to the number of metabolites within each sub-pathway.

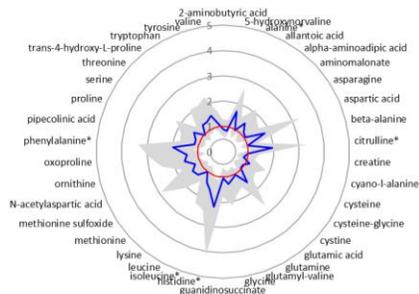


Supplemental Figure 2. Radar plots showing the univariate associations of individual metabolite within each pathway at A) 10-13 and B) 16-19 weeks of gestation with risk of subsequent gestational diabetes in the PETALS nested case-control discovery set.

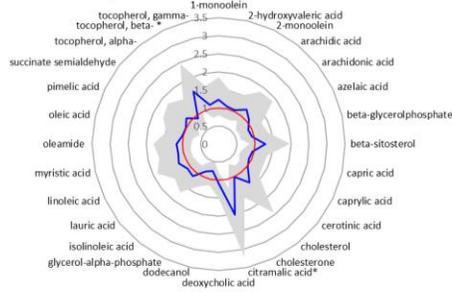
Red circle represents odds ratio (OR) of 1; blue circle represents adjusted OR of GDM risk associated with each metabolite after adjusting for covariates (age at delivery, race/ethnicity, family history of diabetes, chronic hypertension, history of gestational diabetes, pre-pregnancy body mass index, and gestational age at blood collection); and grey area represents 95% confidence interval.

*P-value after FDR adjustment <0.05.

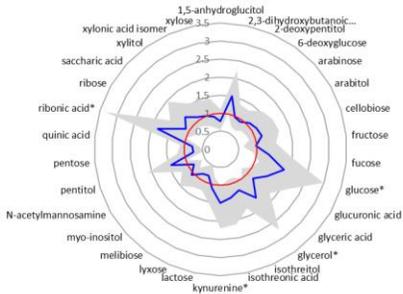
A Amino acids, peptides and analogues



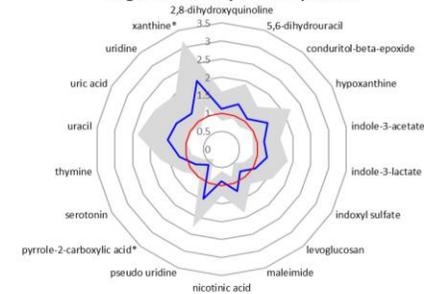
Lipids and lipid-like molecules



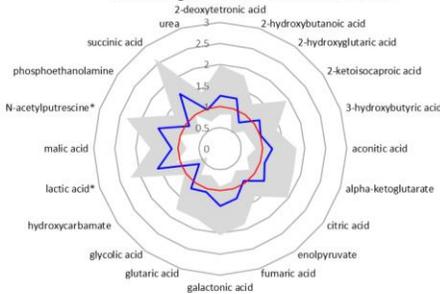
Organic oxygen compounds



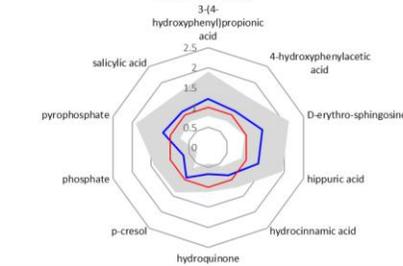
Organoheterocyclic compounds



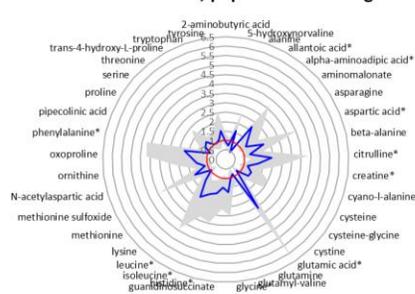
Other organic acids and derivatives



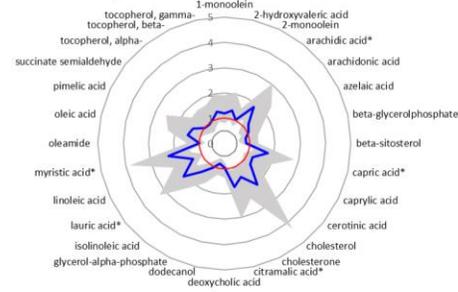
Xenobiotics



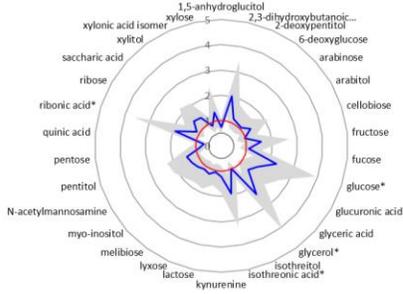
B Amino acids, peptides and analogues



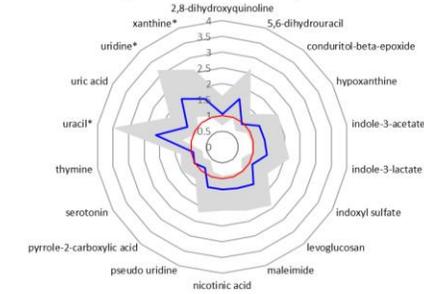
Lipids and lipid-like molecules



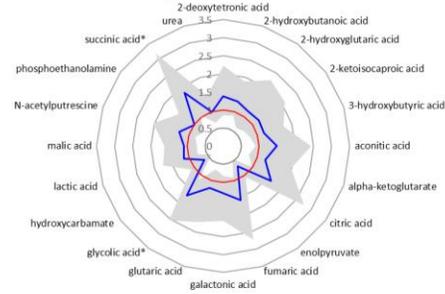
Organic oxygen compounds



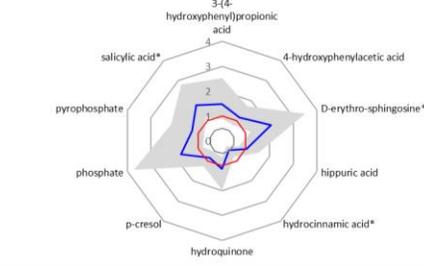
Organoheterocyclic compounds



Other organic acids and derivatives



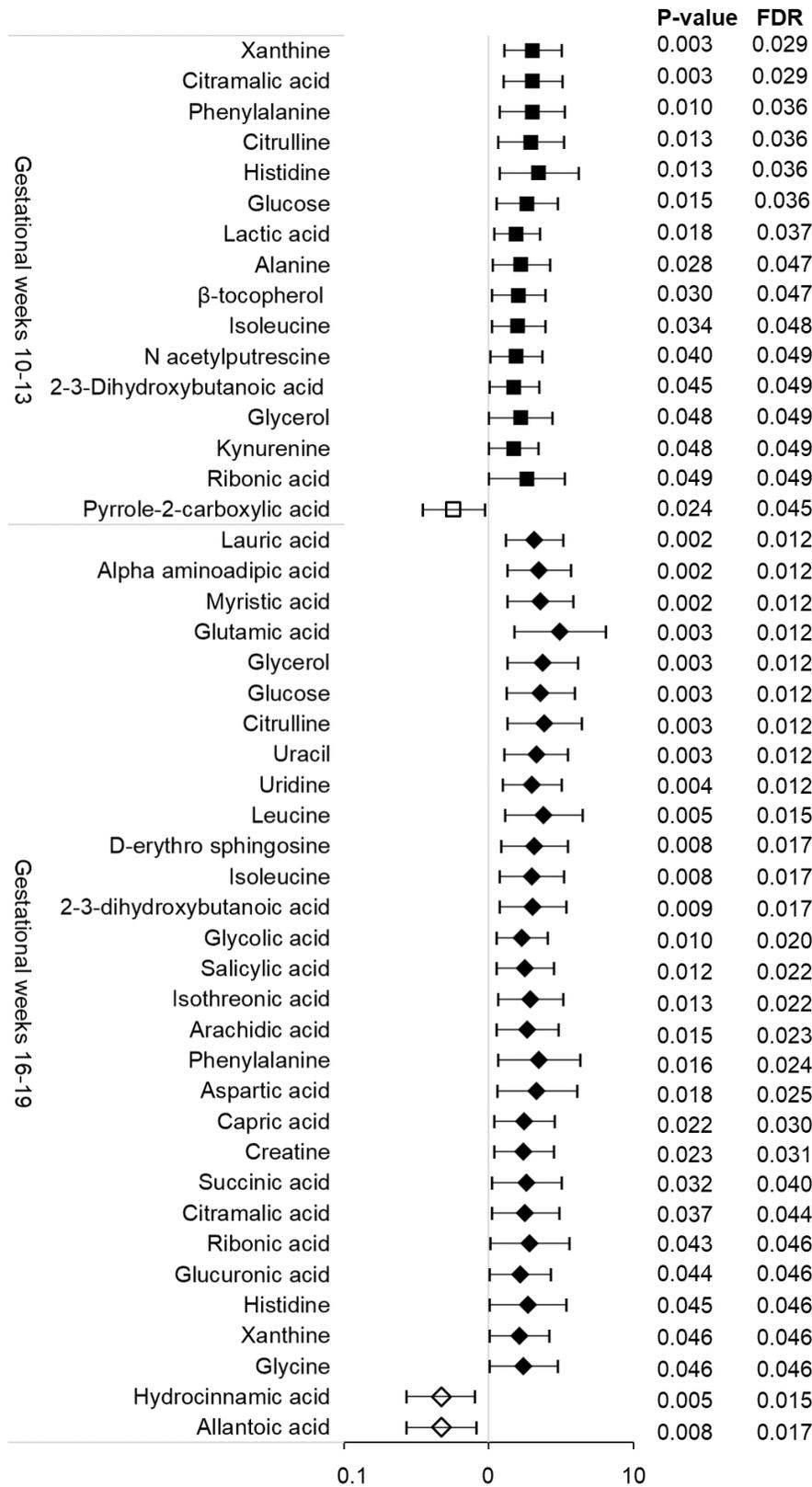
Xenobiotics



Supplemental Figure 3. Univariate analysis: Individual metabolites at gestational weeks 10-13 and 16-19 significantly associated with risk of gestational diabetes in the PETALS nested case-control discovery set*

*Adjusted for maternal age at delivery, race/ethnicity, family history of diabetes, chronic hypertension, history of gestational diabetes, pre-pregnancy body mass index, and gestational age at blood collection.

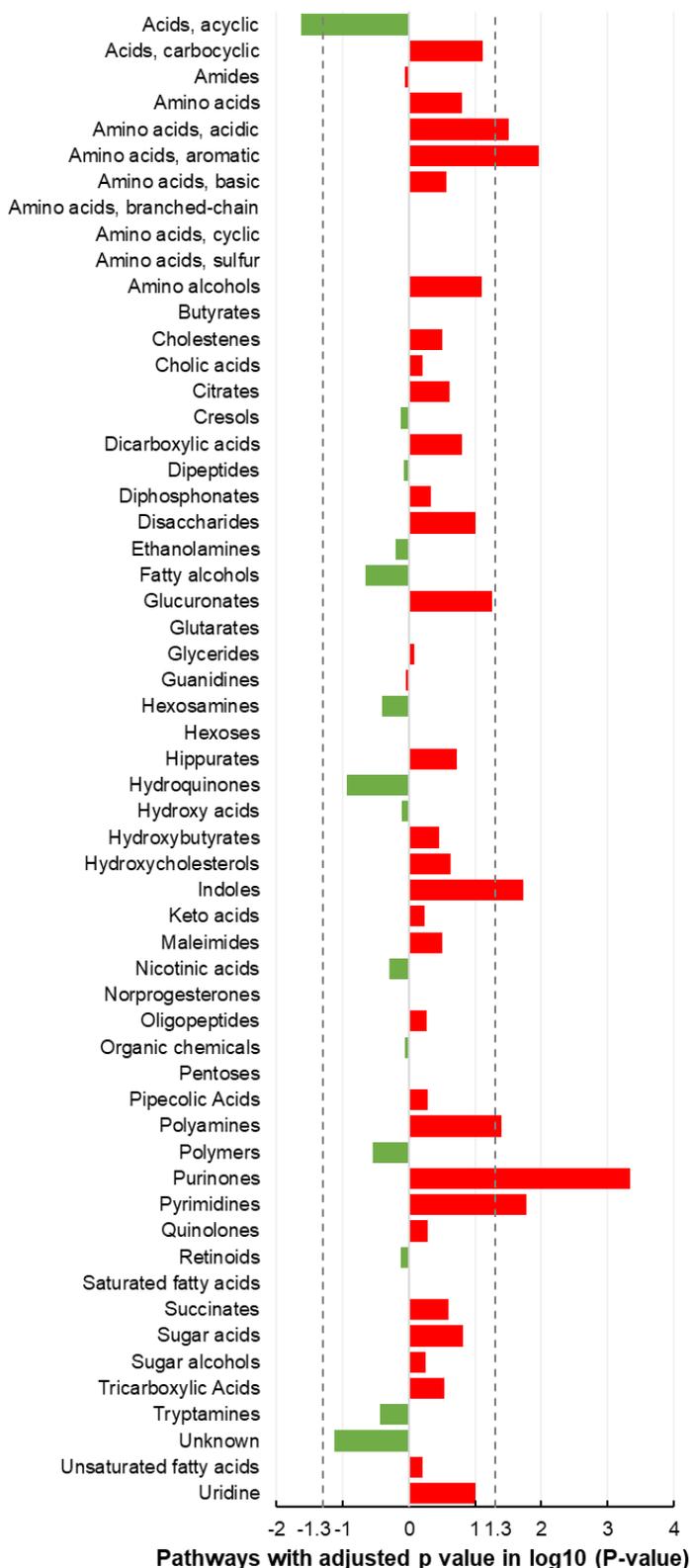
Metabolites in the plot were ordered based on the significance level of P-value for positive and inverse associations at each time window, respectively.



Supplemental Figure 4. Multivariate ChemRICH enrichment plots depicting all pathways identified at A) 10-13 weeks and 2) 16-19 weeks of gestation in the PETALS nested case-control discovery set

Red bars, upregulation; green bars, downregulation. Dashed lines: $P < 0.05$ corresponds to $-\log_{10}$ of P-value > 1.3 for upregulated pathways and \log_{10} of P-value < -1.3 for downregulated pathways.

A Pathways at Gestational Weeks 10-13



B Pathways at Gestational Weeks 16-19

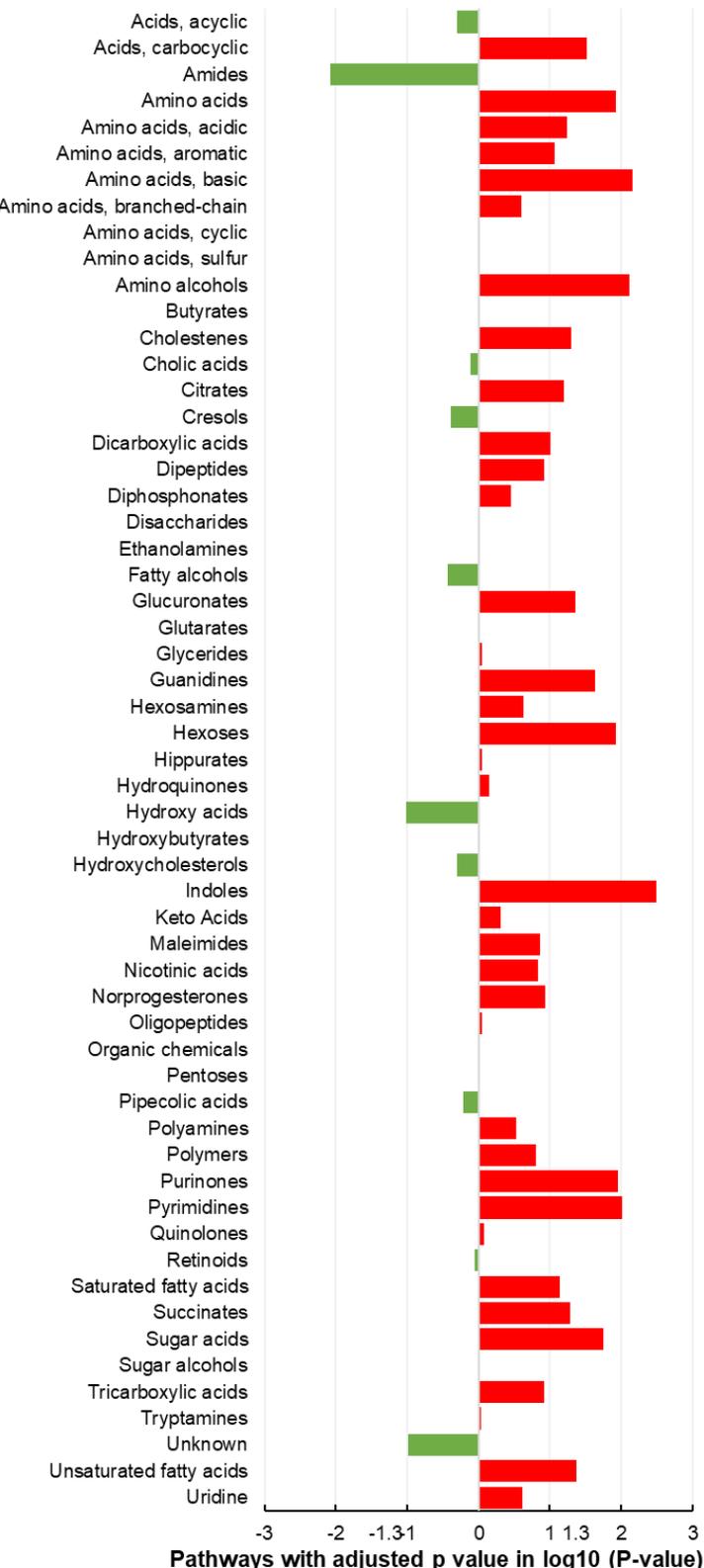


Table S3. Univariate analysis: Changes in individual metabolites from gestational weeks 10-13 to 16-19 significantly associated with risk of gestational diabetes in the PETALS nested case-control discovery set*

Metabolites	Adjusted OR (95% CI)[†]	P-value	FDR
Positive associations			
Creatine	1.63 (1.06, 2.49)	0.025	0.695
Fructose	1.61(1.04, 2.49)	0.034	0.695
N-acetylmannosamine	1.63(1.03, 2.56)	0.037	0.695
Myristic acid	1.54 (1.02, 2.31)	0.038	0.695
Phosphate	1.62 (1.02, 2.57)	0.042	0.695
Uracil	1.64 (1.01, 2.66)	0.045	0.695
Inverse associations			
Enolpyruvate	0.64 (0.43, 0.96)	0.030	0.695

*Changes in metabolite concentrations were assessed by the ratio of metabolite concentrations at study clinic visit 2 (gestational weeks 16-19) divided by the concentrations at study clinic visit 1 (gestational weeks 10-13).

[†]Adjusted for maternal age at delivery, race/ethnicity, family history of diabetes, pre-existing hypertension, history of gestational diabetes, pre-pregnancy body mass index, and the difference in gestational age at blood collection between study clinical visits 1 and 2.

Table S4. The putative pathways linking metabolites at gestational weeks 10-13 and 16-19 to risk of gestational diabetes using the multivariate ChemRICH analysis in the PETALS nested case-control discovery set.

Time window	Pathway	P-value*	FDR	Hits	Metabolite hits	Key metabolite†
Gestational weeks 10-13	Inverse association					
	Acids, acyclic	0.024	0.118	1	Pyrrole-2-carboxylic acid	Pyrrole-2-carboxylic acid
	Positive association					
	Amino acids, acidic	0.031	0.129	3	Aspartic acid, glutamic acid, N-acetylaspartic acid	Glutamic acid
	Amino acids, aromatic	0.011	0.118	3	Phenylalanine, tryptophan, tyrosine	Phenylalanine
	Indoles	0.019	0.118	3	Indole-3-acetate, indole-3-lactate, indoxyl sulfate	Indole-3-acetate
	Polyamines	0.040	0.143	1	N-acetylputrescine	N-acetylputrescine
	Purinones	0.0005	0.011	3	Hypoxanthine, uric acid, xanthine	Xanthine
Pyrimidines	0.017	0.118	4	5,6-dihydrouracil, thymine, uracil, uridine	Uracil	
Gestational weeks 16-19	Inverse association					
	Amides	0.008	0.041	1	Allantoic acid	Allantoic acid
	Positive association					
	Acids, carbocyclic	0.030	0.074	4	3-(4-hydroxyphenyl)propionic acid, 4-hydroxyphenylacetic acid, hydrocinnamic acid, quinic acid	Hydrocinnamic acid
	Amino acids, basic	0.007	0.041	4	Asparagine, glutamine, lysine, ornithine	Glutamine
	Amino acids, other	0.012	0.041	8	5-hydroxynorvaline, alanine, beta-alanine, citrulline, glycine, kynurenine, serine, threonine	Citrulline
	Amino alcohols	0.008	0.041	1	D-erythro-sphingosine	D-erythro-sphingosine
	Glucuronates	0.044	0.092	1	Glucuronic acid	Glucuronic acid
	Guanidines	0.023	0.063	1	Creatine	Creatine
	Hexoses	0.012	0.041	6	6-deoxyglucose, fructose, fucose, glucose, levoglucosan	Levoglucosan
	Indoles	0.003	0.041	3	Indole-3-acetate, indole-3-lactate, indoxyl sulfate	Indole-3-acetate
	Purinones	0.011	0.041	3	Hypoxanthine, uric acid, xanthine	Xanthine
	Pyrimidines	0.010	0.041	4	5,6-dihydrouracil, thymine, uracil, uridine	Uracil
	Sugar acids	0.018	0.054	4	2,3-dihydroxybutanoic acid, galactonic acid, glyceric acid, saccharic acid	2,3-dihydroxybutanoic acid
	Unsaturated fatty acids	0.043	0.092	4	Arachidonic acid, isolinoleic acid, linoleic acid, oleic acid	Isolinoleic acid

*The P value of each metabolite pathway was obtained by the Kolmogorov-Smirnov test after false discovery rate adjustment.

†The most significant metabolite within that pathway.

Supplemental Figure 5. Model optimization of LASSO regression models for the selection of multi-metabolite panels at gestational weeks 10-13 (A) and 16-19 (B) in the PETALS nested case-control discovery set.

Dotted lines on the left highlighted (A) 23 features (17 metabolites* and 6 conventional risk factors†) and (B) 19 features (13 metabolites‡ and 6 conventional risk factors†) that generated the highest area under the curve (AUC) statistics. Dotted lines on the right indicated the marker for AUC within one standard error of the highest AUC.

*The 17-metabolite panel included: 1-5 anhydroglucitol, 1-monoolein, 2-3-dihydroxybutanoic acid, 2-hydroxyglutaric acid, 5-6-dihydrouracil, alanine, alpha amino adipic acid, beta alanine, beta-sitosterol, cellobiose, citramalic acid, citric acid, lactic acid, N-acetylputrescine, β -tocopherol, uric acid, and urea.

†Included age at delivery, family history of diabetes, chronic hypertension, history of gestational diabetes, pre-pregnancy body mass index, and fasting serum glucose.

‡The 13-metabolite panel included: 1-5 anhydroglucitol, 2-3-dihydroxybutanoic acid, 2 aminobutyric acid, alpha amino adipic acid, arachidic acid, aspartic acid, citric acid, hydrocinnamic acid, lauric acid, oleic acid, quinic acid, uracil, uridine.

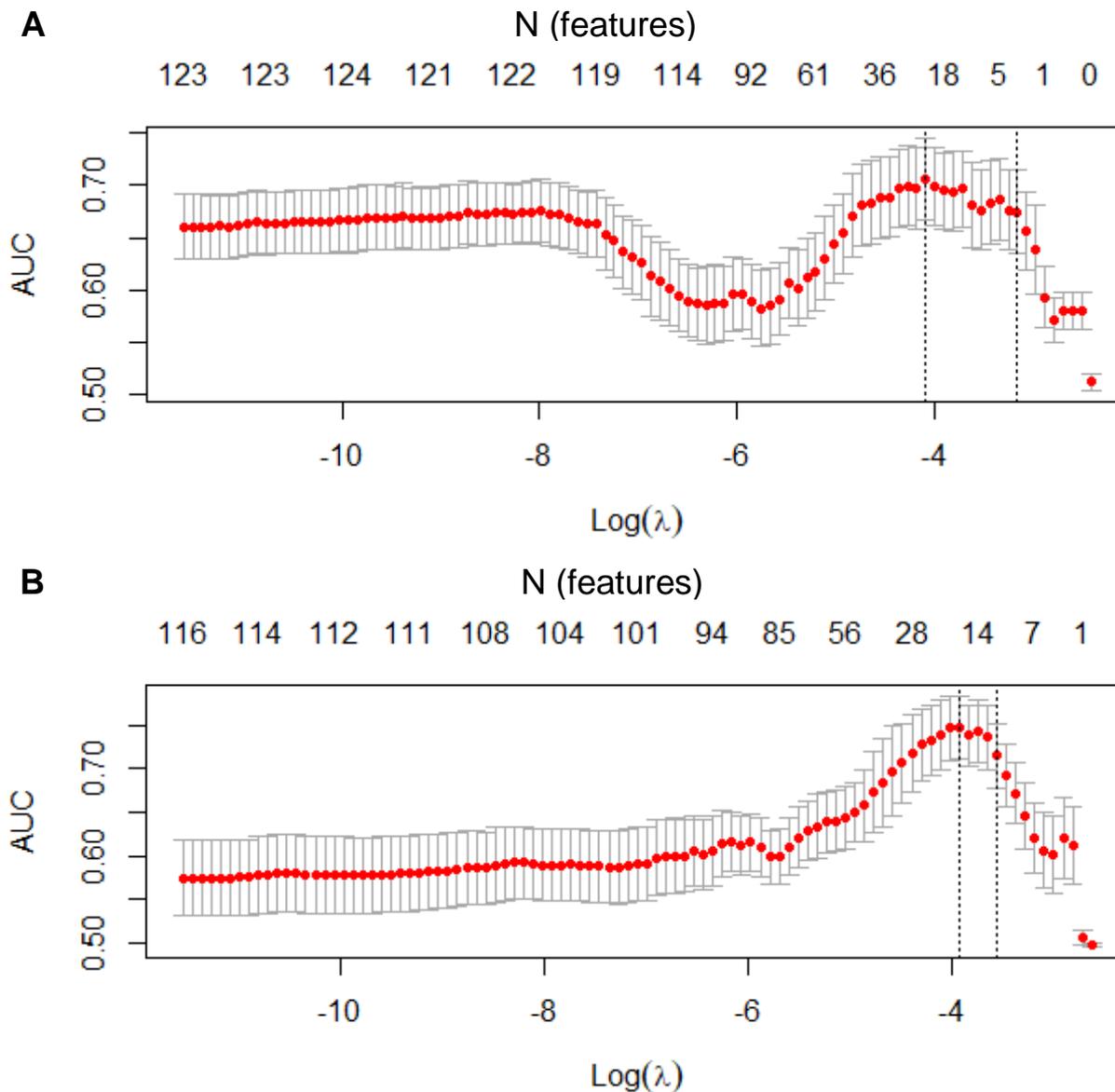


Table S5. Predictive performance of multi-metabolite panels at gestational weeks 10-13 and 16-19 beyond conventional risk factors for gestational diabetes using LASSO regression algorithms

	Discovery Set ¹		Validation Set 1 ²		Validation Set 2 ³	
	PPV	NPV	PPV	NPV	PPV	NPV
Gestational weeks 10-13						
Model 1 (conventional factors) ⁴	0.700	0.774	0.479	0.853	0.655	0.789
Model 2 (metabolites) ⁵	0.677	0.855	0.721	0.952	0.794	0.887
Model 3 (conventional + metabolites) ⁶	0.720	0.913	0.706	0.890	1.000	0.959
Gestational weeks 16-19						
Model 1 (conventional factors) ⁴	0.457	0.855	0.500	0.917	NA	NA
Model 2 (metabolites) ⁷	0.947	0.799	0.879	0.938	NA	NA
Model 3 (conventional + metabolites) ⁶	0.810	0.844	0.857	0.886	NA	NA

LASSO, least absolute shrinkage and selection operator; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

¹Discovery set was a matched case-control study of 91 GDM cases and 180 non-GDM controls in the Pregnancy Environment and Lifestyle Study (PETALS) cohort.

²Validation set 1 was a random sample of 42 GDM and 372 non-GDM women in the PETALS cohort.

³Validation set 2 was a case-control study of 30 GDM cases and 60 non-GDM controls in the Gestational Weight Gain and Optimal Wellness (GLOW) randomized controlled trial.

⁴Model 1 included conventional risk factors: age, race/ethnicity, family history of diabetes, pre-existing hypertension, history of gestational diabetes, pre-pregnancy body mass index, and gestational age at blood collection, and serum glucose levels.

⁵Model 2 included a 17-metabolite panel selected by LASSO regression at gestational weeks 10-13 (1-5 anhydroglucitol, 1-monoolein, 2-3-dihydroxybutanoic acid, 2-hydroxyglutaric acid, 5-6-dihydrouracil, alanine, alpha aminoadipic acid, beta alanine, beta-sitosterol, cellobiose, citramalic acid, citric acid, lactic acid, N-acetylputrescine, β -tocopherol, uric acid, and urea).

⁶Model 3 included conventional risk factors in Model 1 and metabolites in Model 2.

⁷Model 2 included a 13-metabolite panel selected by LASSO regression at gestational weeks 16-19 (1-5 anhydroglucitol, 2-3-dihydroxybutanoic acid, 2 aminobutyric acid, alpha aminoadipic acid, arachidic acid, aspartic acid, citric acid, hydrocinnamic acid, lauric acid, oleic acid, quinic acid, uracil, uridine).