

Supplementary Table 1 Mean and its 95% confidence interval of plasma amino acids among GDM cases and non-GDM controls at 10-14 and 15-26 weeks of gestation, the NICHD Fetal Growth Studies-Singleton Cohort

	Mead (95% confidence interval), umol/dL					
	10-14 gestational weeks			15-26 gestational weeks		
Amino acids	Controls (n=214)	GDM (n=104)	p-value*	Controls (n=214)	GDM (n=94)	p-value*
Glycine	14.6 (14.2, 15.1)	12.8 (12.2, 13.4)	<0.0001	14.6 (14.2, 15.2)	13.1 (12.4, 13.7)	0.0009
Alanine	28.6 (27.9, 29.4)	30.4 (29.2, 31.5)	0.01	28.5 (27.7, 29.3)	30.0 (28.8, 31.1)	0.03
Aspartic acid	0.69 (0.63, 0.74)	0.84 (0.76, 0.91)	0.002	0.97 (0.87, 1.07)	1.13 (1.01, 1.25)	0.004
Asparagine	5.18 (4.98, 5.39)	4.88 (4.60, 5.16)	0.07	4.33 (4.14, 4.51)	4.11 (3.88, 4.34)	0.05
Arginine	5.45 (5.13, 5.78)	5.71 (5.28, 6.14)	0.29	6.47 (5.95, 6.99)	6.97 (6.30, 7.63)	0.13
Glutamine	32.8 (31.4, 34.2)	32.4 (30.6, 34.1)	0.61	27.5 (25.3, 29.7)	25.9 (23.5, 28.3)	0.05
Glutamic acid	11.8 (10.6, 13.0)	12.9 (11.5, 14.4)	0.07	15.8 (13.9, 17.8)	18.1 (16.0, 20.3)	0.004
Glutamine-to-glutamic acid-ratio	4.48 (3.94, 5.02)	3.77 (3.13, 4.42)	0.02	3.98 (3.25, 4.70)	3.01 (2.15, 3.87)	0.009
Threonine	12.1 (11.7, 12.5)	12.9 (12.3, 13.4)	0.02	15.0 (14.7, 15.4)	15.4 (14.9, 16.0)	0.26
Serine	7.77 (7.53, 8.01)	7.37 (7.05, 7.70)	0.04	8.30 (7.91, 8.49)	8.22 (7.83, 8.61)	0.92
Methionine	1.85 (1.78, 1.92)	1.81 (1.71, 1.91)	0.49	1.80 (1.74, 1.85)	1.88 (1.81, 1.96)	0.03
Histidine	6.99 (6.84, 7.14)	6.77 (6.55, 6.99)	0.10	6.97 (6.80, 7.14)	6.82 (6.57, 7.06)	0.28
Lysine	15.2 (14.7, 15.7)	15.6 (14.9, 16.2)	0.34	14.8 (14.5, 15.1)	15.3 (14.8, 15.8)	0.11

	Mead (95% confidence interval), umol/dL					
	10-14 gestational weeks			15-26 gestational weeks		
Amino acids	Controls (n=214)	GDM (n=104)	p-value*	Controls (n=214)	GDM (n=94)	p-value*
Proline	13.3 (!2.8, 13.8)	13.0 (12.3, 13.7)	0.48	12.1 (11.7,12.6)	11.8 (11.1, 12.4)	0.34
BCAAs						
Valine	18.6 (18.0, 19.3)	19.1 (18.2, 20.0)	0.40	16.1 (15.8, 16.5)	16.9 (16.5, 17.4)	0.007
Leucine	9.59 (9.16, 10.0)	9.42 (8.83, 10.0)	0.64	8.24 (8.07, 8.41)	8.49 (8.23, 8.74)	0.11
Isoleucine	5.37 (5.10,5.64)	5.37 (5.00, 5.74)	0.99	4.80 (4.68, 4.92)	5.11 (4.94, 5.29)	0.002
Summed BCAAs†	33.6 (32.4, 34.8)	33.9 (32.1, 35.6)	0.79	29.2 (28.6, 29.7)	30.5 (29.7, 31.4)	0.008
Aromatic amino acids						
Phenylalanine	5.31 (5.16, 5.47)	5.29 (5.07, 5.50)	0.83	4.63 (4.52, 4.73)	4.70 (4.55, 4.86)	0.39
Tyrosine	4.70 (4.50, 4.89)	4.90 (4.62, 5.18)	0.24	3.94 (3.85, 4.03)	4.25 (4.13, 4.40)	<0.0001
Summed aromatic amino acids‡	10.0 (9.69, 10.3)	10.2 (9.73, 10.6)	0.54	8.57 (8.40, 8.73)	8.97 (8.73, 9.20)	0.004
Others						
Taurine	9.16 (8.48, 9.84)	10.6 (9.62, 11.6)	0.02	7.88 (7.29, 8.48)	8.58 (7.68, 9.48)	0.20
Citrulline	1.77 (1.71, 1.83)	1.77 (1.69, 1.85)	0.08	1.67 (1.62, 1.73)	1.69 (1.62, 1.76)	0.70
Ornithine	3.01 (2.87, 3.15)	3.04 (2.85, 3.23)	0.81	2.28 (2.18, 2.38)	2.39 (2.25, 2.53)	0.17

	Mead (95% confidence interval), umol/dL					
	10-14 gestational weeks			15-26 gestational weeks		
Amino acids	Controls (n=214)	GDM (n=104)	p-value*	Controls (n=214)	GDM (n=94)	p-value*
Hydroxyproline	1.14 (1.03, 1.24)	1.17 (1.03, 1.32)	0.69	1.16 (1.07, 1.24)	1.15 (1.02, 1.28)	0.90
Alpha-aminobutyric acid	1.43 (1.37, 1.49)	1.44 (1.37, 1.52)	0.76	1.31 (1.23, 1.39)	1.31 (1.21, 1.42)	0.97
Cystine	1.36 (1.08, 1.63)	1.58 (1.27, 1.89)	0.08	0.87 (0.60, 1.14)	1.10 (0.80, 1.40)	0.04

Abbreviations: BCAA: branched chain amino acid, FDR: false-discovery rate, GDM: gestational diabetes mellitus

* P-values for differences between case and control participants were obtained by linear mixed models with associated likelihood ratio tests, accounting for matched case-control pairs.

† Summed BCAs: summed valine, leucine, and isoleucine.

‡ Summed aromatic amino acids: summed tyrosine and phenylalanine.

Supplementary Table 2 Median and interquartile range of plasma amino acids among GDM cases and non-GDM controls at 10-14 and 15-26 weeks of gestation, the NICHD Fetal Growth Studies-Singleton Cohort

	Median (interquartile range), umol/dL			
	10-14 gestational weeks		15-26 gestational weeks	
Amino acids	Controls (n=214)	GDM (n=104)	Controls (n=214)	GDM (n=94)
Glycine	14.2 (12.0, 16.5)	12.2 (11.0, 14.4)	14.0 (12.3, 16.6)	12.5 (11.1, 14.6)
Alanine	27.6 (24.5, 32.6)	30.5 (26.8, 33.9)	27.9 (24.4, 31.8)	30.3 (25.7, 33.7)
Aspartic acid	0.62 (0.44, 0.86)	0.78 (0.52, 1.02)	0.88 (0.51, 1.25)	0.93 (0.60, 1.58)
Asparagine	5.11 (4.25, 5.93)	4.73 (4.03, 5.47)	1.36 (3.58, 4.94)	4.22 (3.39, 4.83)
Arginine	4.82 (4.14, 6.04)	5.05 (4.30, 6.41)	5.44 (4.20, 7.35)	5.44 (4.58, 7.99)
Glutamine	35.0 (27.3, 39.0)	34.4 (27.4, 38.6)	27.3 (18.0, 38.4)	29.2 (15.8, 36.3)
Glutamic acid	9.62 (6.3, 15.6)	11.7 (7.2, 17.9)	13.0 (6.85, 24.2)	13.8 (8.42, 25.6)
Glutamine-to-glutamic acid-ratio	3.50 (1.84, 6.07)	3.28 (1.61, 5.71)	2.05 (0.74, 5.56)	2.24 (0.59, 4.24)
Threonine	12.1 (10.1, 13.7)	12.8 (10.6, 14.9)	14.9 (12.9, 16.9)	15.3 (13.2, 17.2)
Serine	7.69 (6.65, 8.81)	7.16 (6.07, 8.43)	7.81 (6.90, 9.13)	7.82 (6.84, 9.39)
Methionine	1.76 (1.47, 2.13)	1.76 (1.49, 2.08)	1.78 (1.58, 2.02)	1.85 (1.68, 2.14)
Histidine	6.62 (6.16, 7.60)	6.74 (6.00, 7.32)	6.98 (6.35, 7.73)	6.86 (6.36, 7.39)
Lysine	14.9 (13.1, 16.8)	15.3 (12.8, 17.3)	14.5 (13.3, 16.1)	15.0 (13.5, 16.9)
Proline	12.7 (10.8, 15.2)	12.0 (10.7, 15.3)	11.4 (9.73, 14.3)	11.3 (9.62, 13.5)
BCAAs				
Valine	18.0 (15.6, 21.0)	18.4 (15.7, 21.6)	16.1 (14.7, 17.5)	16.8 (15.2, 18.0)
Leucine	9.08 (7.53, 10.9)	8.99 (7.66, 10.9)	8.17 (7.65, 8.99)	8.56 (7.69, 9.46)
Isoleucine	5.01 (4.07, 6.03)	5.29 (4.30, 6.37)	4.78 (4.26, 5.24)	5.10 (4.44, 5.72)
Summed BCAAs*	32.2 (27.3, 38.5)	32.7 (27.9, 39.0)	29.3 (26.9, 31.1)	30.5 (27.4, 33.3)
Aromatic amino acids				
Phenylalanine	5.01 (4.49, 5.93)	5.10 (4.69, 5.82)	4.54 (4.26, 4.91)	4.75 (4.40, 5.18)

	Median (interquartile range), umol/dL			
	10-14 gestational weeks		15-26 gestational weeks	
Amino acids	Controls (n=214)	GDM (n=104)	Controls (n=214)	GDM (n=94)
Tyrosine	4.40 (3.69, 5.45)	4.73 (4.10, 5.65)	3.85 (3.53, 4.27)	4.28 (3.79, 4.65)
Summed aromatic amino acids†	9.54 (8.21, 11.4)	10.0 (8.72, 11.3)	8.37 (7.83, 9.22)	9.09 (8.32, 9.62)
Others				
Taurine	7.61 (5.48, 12.7)	10.16 (5.58, 15.6)	6.82 (4.44, 10.52)	7.78 (5.21, 11.8)
Citrulline	1.72 (1.47, 2.02)	1.76 (1.40, 2.13)	1.66 (1.47, 1.88)	1.64 (1.41, 1.96)
Ornithine	2.81 (2.32, 3.45)	2.88 (2.35, 3.55)	2.11 (1.82, 2.66)	2.23 (1.87, 2.68)
Hydroxyproline	0.99 (0.72, 1.35)	1.07 (0.73, 1.48)	1.09 (0.78, 1.54)	1.11 (0.78, 1.45)
Alpha-aminobutyric acid	1.37 (1.16, 1.64)	1.36 (1.10, 1.78)	1.30 (1.04, 1.58)	1.34 (1.12, 1.57)
Cystine	0.66 (0.07, 2.40)	0.98 (0.06, 2.74)	0.42 (0.08, 0.92)	0.57 (0.06, 1.41)

Abbreviations: BCAA: branched chain amino acid, GDM: gestational diabetes mellitus

* Summed BCAAs: summed valine, leucine, and isoleucine.

† Summed aromatic amino acids: summed tyrosine and phenylalanine.

Supplementary Table 3 Adjusted odds ratios of gestational diabetes mellitus risk associated with individual and grouped plasma amino acids with additional adjustment of glucose or HbA1c at 10-14 weeks of gestation, the NICHD Fetal Growth Studies-Singleton Cohort

Adjusted OR (95% CI) for risk of GDM by quartiles of plasma amino acids*						
Amino acids	Further adjusting for glucose					
	Q1	Q2	Q3	Q4	p-trend†	Continuous (per SD)‡
Glycine	1.00 (REF)	0.43 (0.19, 0.96)	0.40 (0.16, 0.98)	0.25 (0.09, 0.66)	0.008	0.57 (0.39, 0.83)
Alanine	1.00 (REF)	1.92 (0.78, 4.74)	2.07 (0.87, 4.95)	2.69 (1.09, 6.65)	0.04	1.30 (0.95, 1.77)
Glutamic acid	1.00 (REF)	0.94 (0.41, 2.18)	1.57 (0.60, 4.09)	2.54 (0.86, 7.52)	0.05	1.48 (1.02, 2.15)
Further adjusting for HbA1c						
Amino acids	Q1	Q2	Q3	Q4	p-trend	Continuous (per SD)
Glycine	1.00 (REF)	0.34 (0.14, 0.79)	0.33 (0.13, 0.85)	0.25 (0.10, 0.64)	0.01	0.55 (0.38, 0.80)
Alanine	1.00 (REF)	1.80 (0.73, 4.46)	2.97 (1.23, 7.21)	2.94 (1.19, 7.26)	0.007	1.39 (1.03, 1.87)
Glutamic acid	1.00 (REF)	0.77 (0.34, 1.76)	1.27 (0.50, 3.19)	1.90 (0.66, 5.53)	0.12	1.38 (0.94, 2.01)

Abbreviations: CI: confidence interval, GDM: gestational diabetes mellitus, OR: odds ratio, SD: standard deviation

* Models were adjusted for family history of diabetes (yes, no), nulliparity (yes/no), pre-pregnancy body mass index (<25.0, 25.0–29.9, 30.0–34.9, 35.0–44.9 kg/m²), maternal age (years), and gestational week at the time of blood draw.

† Tests of linear trend were conducted by using the median value for each quartile and fitted as a continuous variable in the conditional logistic regression models. P-value<0.05 after FDR correction was marked with **; none of the amino acids at 10-14 weeks was statistically significant after false-discovery rate correction.

‡ ORs (95% CIs) for continuous amino acids (per standard deviation) were presented for those amino acids whose p-trend was significant (i.e., p-value<0.05).

Supplementary Table 4 Adjusted odds ratios of gestational diabetes mellitus risk associated with individual and grouped plasma amino acids with additional adjustment of fasting glucose or HbA1c at 15-26 weeks of gestation, the NICHD Fetal Growth Studies-Singleton Cohort

Adjusted odds ratios (95% CI) for risk of GDM by quartiles of plasma amino acids*						
Amino acids	Further adjusting for glucose					
	Q1	Q2	Q3	Q4	p-trend†	Continuous (per SD)‡
Glycine	1.00 (REF)	0.73 (0.30, 1.73)	0.32 (0.12, 0.83)	0.13 (0.04, 0.40)	0.0002**	0.82 (0.72, 0.93)
Alanine	1.00 (REF)	0.67 (0.25, 1.80)	0.80 (0.27, 2.35)	1.44 (0.52, 4.01)	0.24	
Isoleucine	1.00 (REF)	2.23 (0.79, 6.29)	0.81 (0.29, 2.30)	2.13 (0.80, 5.68)	0.27	
Phenylalanine	1.00 (REF)	1.01 (0.38, 2.68)	1.24 (0.48, 3.20)	1.49 (0.60, 3.68)	0.31	

Further adjusting for fasting HbA1c						
Amino acids	Further adjusting for fasting HbA1c					
	Q1	Q2	Q3	Q4	p-trend	Continuous (per SD)
Glycine	1.00 (REF)	0.76 (0.34, 1.69)	0.31 (0.12, 0.78)	0.15 (0.05, 0.43)	0.0002**	0.50 (0.34, 0.76)
Alanine	1.00 (REF)	1.00 (0.39, 2.57)	1.28 (0.48, 3.44)	2.94 (1.16, 7.45)	0.008	1.43 (1.03, 1.97)
Isoleucine	1.00 (REF)	1.75 (0.65, 4.76)	1.32 (0.49, 3.54)	3.02 (1.17, 7.82)	0.03	1.59 (1.14, 2.21)
Phenylalanine	1.00 (REF)	0.95 (0.38, 2.41)	1.38 (0.56, 3.40)	1.85 (0.76, 4.48)	0.10	1.13 (0.84, 1.52)

Abbreviations: CI: confidence interval, GDM: gestational diabetes mellitus, OR: odds ratio, SD: standard deviation

* Models were adjusted for family history of diabetes (yes, no), nulliparity (yes/no), (<25.0, 25.0–29.9, 30.0–34.9, 35.0–44.9 kg/m²), maternal age (years), and gestational week at the time of blood draw.

† Tests of linear trend were conducted by using the median value for each quartile and fitted as a continuous variable in the conditional logistic regression models. P-value<0.05 after FDR correction was marked with **.

‡ ORs (95% CIs) for continuous amino acids (per standard deviation) were presented for those amino acids whose p-trend was significant (i.e., p-value<0.05).

Supplementary Table 5 Adjusted odds ratios of gestational diabetes mellitus associated with continuous per standard deviation increment of plasma amino acids at 10-14 weeks of gestation stratified by major risk factors, the NICHD Fetal Growth Studies-Singleton Cohort

Adjusted odds ratios (95% CI) for risk of GDM by per SD increment of plasma amino acids*				
Covariate	Glycine	Alanine	Aspartic acid	Glutamic acid
Race/ethnicity				
White	0.51 (0.26, 1.01)	1.59 (0.81, 3.09)	1.50 (0.94, 2.40)	1.30 (0.76, 2.22)
Black	0.75 (0.28, 2.04)	1.91 (0.86, 4.26)	1.79 (0.67, 4.79)	1.21 (0.52, 2.80)
Hispanic	0.50 (0.28, 0.92)	0.99 (0.63, 1.55)	1.26 (0.85, 1.86)	1.21 (0.80, 1.84)
Asian	0.53 (0.25, 1.10)	2.02 (1.09, 3.73)	2.17 (1.07, 4.43)	1.16 (0.65, 2.07)
Family history of diabetes				
No	0.49 (0.31, 0.76)	1.40 (1.02, 1.90)	1.57 (1.18, 2.08)	1.23 (0.89, 1.80)
Yes	0.70 (0.42, 1.18)	1.28 (0.73, 2.24)	1.10 (0.65, 1.87)	1.07 (0.69, 1.65)
Nulliparity				
No	0.59 (0.38, 0.90)	1.03 (0.71, 1.49)	1.28 (0.96, 1.70)	1.12 (0.81, 1.55)
Yes	0.56 (0.33, 0.95)	1.90 (1.26, 2.87)	1.87 (1.17, 2.96)	1.28 (0.85, 1.93)
Pre-pregnancy BMI, kg/m ²				
<30.0	0.56 (0.38, 0.81)	1.43 (1.06, 1.91)	1.58 (1.19, 2.11)	1.13 (0.86, 1.48)
≥30.0	0.64 (0.30, 1.38)	1.30 (0.66, 2.55)	1.18 (0.75, 1.88)	1.52 (0.72, 3.21)

Abbreviations: CI: confidence interval, GDM: gestational diabetes mellitus, SD: standard deviation

* Models were adjusted for family history of diabetes (yes, no), nulliparity (yes/no), pre-pregnancy body mass index (<25.0, 25.0–29.9, 30.0–34.9, 35.0–44.9 kg/m²), maternal age (years), and gestational week at the time of blood draw.

Supplementary Table 6 Adjusted odds ratios of gestational diabetes mellitus associated with continuous per standard deviation increment of plasma amino acids at 15-26 weeks of gestation stratified by major risk factors, the NICHD Fetal Growth Studies-Singleton Cohort

Covariate	Adjusted odds ratios (95% CI) for risk of GDM by per SD increment of plasma amino acids*							
	Glycine	Alanine	Aspartic acid	Glutamine-glutamic acid ratio	Isoleucine	Phenylalanine	Tyrosine	
Race/ethnicity								
White	0.61 (0.31, 1.22)	1.11 (0.63, 1.95)	1.38 (0.77, 2.44)	0.95 (0.50, 1.78)	1.26 (0.57, 2.80)	2.30 (0.88, 6.00)	1.96 (0.96, 4.02)	
Black	0.89 (0.31, 2.55)	2.18 (0.94, 5.07)	1.57 (0.71, 2.44)	0.52 (0.15, 1.84)	1.01 (0.45, 2.26)	0.87 (0.46, 1.62)	1.79 (0.83, 3.84)	
Hispanic	0.52 (0.28, 0.97)	1.30 (0.81, 2.08)	1.48 (0.93, 2.36)	0.79 (0.43, 1.46)	1.39 (0.93, 2.07)	0.97 (0.65, 1.44)	1.54 (0.99, 2.38)	
Asian	0.39 (0.16, 0.95)	1.44 (0.78, 2.65)	1.01 (0.56, 1.85)	0.73 (0.29, 1.85)	3.19 (1.39, 7.28)	1.13 (0.75, 1.69)	1.41 (0.67, 2.97)	
Family history of diabetes								
No	0.56 (0.36, 0.86)	1.38 (1.01, 1.90)	1.57 (1.16, 2.13)	0.61 (0.38, 1.01)	1.92 (1.36, 2.72)	1.27 (0.96, 1.69)	1.74 (1.25, 2.43)	
Yes	0.75 (0.41, 1.35)	1.40 (0.81, 2.41)	0.83 (0.47, 1.46)	1.00 (0.60, 1.69)	0.86 (0.51, 1.44)	0.79 (0.47, 1.33)	1.23 (0.73, 2.05)	
Nulliparity								
No	0.70 (0.47, 1.04)	1.34 (0.94, 1.91)	1.40 (1.00, 1.95)	0.73 (0.40, 1.33)	1.48 (1.03, 2.14)	1.16 (0.87, 1.55)	1.66 (1.15, 2.41)	
Yes	0.48 (0.26, 0.90)	1.48 (0.98, 2.25)	1.25 (0.82, 1.92)	0.80 (0.53, 1.21)	1.47 (0.96, 2.25)	1.00 (0.67, 1.48)	1.39 (0.91, 2.11)	
Pre-pregnancy BMI, kg/m²								
<30.0	0.56 (0.39, 0.83)	1.36 (1.00, 1.85)	1.23 (0.92, 1.65)	0.81 (0.56, 1.17)	1.79 (1.29, 2.49)	1.12 (0.86, 1.47)	1.50 (1.10, 2.04)	
≥30.0	0.86 (0.37, 2.00)	1.52 (0.83, 2.79)	1.68 (0.88, 3.23)	0.64 (0.27, 1.53)	0.85 (0.45, 1.48)	1.03 (0.62, 1.72)	1.61 (0.83, 3.12)	

Abbreviations: CI: confidence interval, GDM: gestational diabetes mellitus, SD: standard deviation

* Models were adjusted for family history of diabetes (yes, no), nulliparity (yes/no), pre-pregnancy body mass index (<25.0, 25.0–29.9, 30.0–34.9, 35.0–44.9 kg/m²), maternal age (years), and gestational week at the time of blood draw.

Supplementary Figure 1 Heatmap of partial Spearman's correlation coefficients between plasma amino acids at 10-14 weeks of gestation and fasting plasma cardiometabolic biomarkers at 15-26 weeks of gestation among non-GDM controls, the NICHD Fetal Growth Studies-Singleton Cohort. Partial Spearman correlation coefficient was calculated with adjustment of family history of diabetes (yes, no), nulliparity (yes/no pre-pregnancy body mass index (<25.0, 25.0–29.9, 30.0–34.9, 35.0–44.9 kg/m²), maternal age, and gestational week at the time of blood draw.

