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Plasma Amino Acids in Early Pregnancy and Midpregnancy and Their Interplay With Phospholipid Fatty Acids in Association With the Risk of Gestational Diabetes Mellitus: Results From a Longitudinal Prospective Cohort

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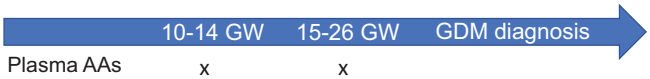
Main Aims and Objectives: To prospectively examine the associations of plasma amino acids (AAs) and the joint associations of AAs and phospholipid fatty acids (FAs) with the risk of gestational diabetes mellitus (GDM)
Study Population and Design:
Nested matched case-control study of 107 GDM and 214 non-GDM control subjects from *the Eunice Kennedy Shriver National Institute of Child Health and Human Development Fetal Growth Studies – Singleton Cohorts*
Exposures: Maternal plasma AAs and phospholipid FAs assessed at 10-14 and 15-26 gestational weeks (GW)
Outcome: GDM according to *the Carpenter-Coustan criteria*
Statistical Analysis: Odds ratio and 95% confidence interval (OR, 95% CI) adjusted for age, prepregnancy BMI, family history of diabetes, gestational age at blood draw, and parity

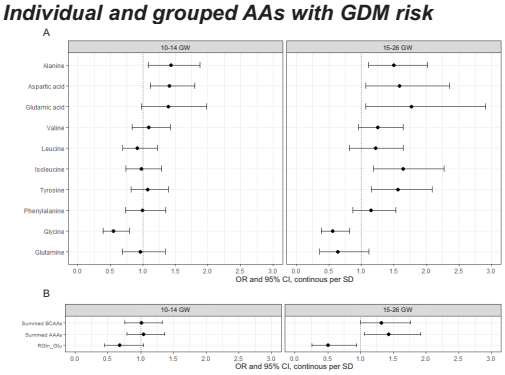


	10-14 GW	15-26 GW
Plasma AAs	x	x
Phospholipid FAs	x	x

Conclusions:
Plasma glucogenic AAs, branched-chain AAs, and aromatic AAs were significantly and prospectively associated with the risk of GDM. Several AAs and phospholipid FAs were significantly and jointly associated with the risk of GDM. These findings suggested potential roles of these AAs in the development of GDM starting in early pregnancy.

Results

Individual and grouped AAs with GDM risk



Joint AA and FA with GDM risk

AA	Phospholipid FA	OR (95% CI) at 10-14 GW
Glycine	Summed even-chain saturated FAs	
Low (<14.2 μmol/dL)	Low (<39.9%)	0.38 (0.16, 0.89)
Low (<14.2 μmol/dL)	Low (<39.9%)	REF (OR=1.00)
High (≥14.2 μmol/dL)	High (≥39.9%)	0.15 (0.06, 0.37)
High (≥14.2 μmol/dL)	High (≥39.9%)	0.50 (0.23, 1.12)

ARTICLE HIGHLIGHTS

- Examining plasma amino acids (AAs) in the context of other metabolites may inform the roles of AAs and their interplay in gestational diabetes mellitus (GDM) development.
- We investigated the associations of AAs and the joint associations of AAs and phospholipid fatty acids (FAs) with GDM.
- AAs were differentially associated with GDM risk starting from early pregnancy. Some associations were enhanced by phospholipid FA profile.
- Findings support distinct roles of AAs in GDM development.

ORIGINAL ARTICLE

Plasma Amino Acids in Early Pregnancy and Midpregnancy and Their Interplay With Phospholipid Fatty Acids in Association With the Risk of Gestational Diabetes Mellitus: Results From a Longitudinal Prospective Cohort

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OBJECTIVE

We prospectively evaluated plasma amino acids (AAs) in early pregnancy and midpregnancy and their interplay with phospholipid fatty acids (FAs) in association with gestational diabetes mellitus (GDM) risk.

RESEARCH DESIGN AND METHODS

From a longitudinal pregnancy cohort of 2,802 individuals, concentrations of 24 plasma AAs at 10–14 and 15–26 gestational weeks (GW) were assessed among 107 GDM case subjects and 214 non-GDM control subjects. We estimated adjusted odds ratios (OR) and 95% CI for the associations of plasma AAs and the joint associations of plasma AAs and phospholipid FAs with GDM risk, adjusting for risk factors including age, prepregnancy BMI, and family history of diabetes.

RESULTS

Glycine at 10–14 GW was inversely associated with GDM (adjusted OR [95% CI] per SD increment: 0.55 [0.39–0.79]). Alanine, aspartic acid, and glutamic acid at 10–14 GW were positively associated with GDM (1.43 [1.08–1.88], 1.41 [1.11–1.80], and 1.39 [0.98–1.98]). At 15–26 GW, findings for glycine, alanine, aspartic acid, and the glutamine–to–glutamic acid ratio were consistent with the directions observed at 10–14 GW. Isoleucine, phenylalanine, and tyrosine were positively associated with GDM (1.64 [1.19–2.27], 1.15 [0.87–1.53], and 1.56 [1.16–2.09]). All *P* values for linear trend were <0.05. Several AAs and phospholipid FAs were significantly and jointly associated with GDM. For instance, the lowest risk was observed among women with higher glycine and lower even-chain saturated FAs at 10–14 GW (adjusted OR [95% CI] 0.15 [0.06, 0.37]).

CONCLUSIONS

Plasma AAs may be implicated in GDM development starting in early pregnancy. Associations of AAs with GDM may be enhanced in the copresence of phospholipid FA profile.

Gestational diabetes mellitus (GDM) is a common pregnancy complication affecting 7–9% of pregnancies in the U.S. and up to 25% worldwide (1). An understanding of

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the underlying mechanisms and identification of potentially modifiable risk factors are critical for GDM prevention (2,3). Studies of potentially modifiable maternal biomarkers of GDM are one potential avenue for such research.

Growing evidence suggests that circulating amino acids (AAs) contribute to the development of GDM (4,5). Among AAs, branched-chain AAs (BCAAs), aromatic AAs, and several glucogenic AAs (e.g., alanine, glycine, and glutamic acid) are of particular interest for their proposed mechanisms related to insulin resistance (6) and their associations with diabetes in the general population (7,8). To date, several studies have prospectively examined some maternal circulating AAs with risk of GDM; they present equivocal results (9–16). Of note, it is important to evaluate AAs at multiple gestational windows to capture and reflect the dynamic changes in maternal physiology, including changes in AA concentrations during pregnancy, and to investigate the interplay of AAs with key metabolism-related pathways in the development of GDM (17). However, most of the studies examined one-time measurements of AAs with risk of GDM. In addition to AAs, emerging evidence has shown that circulating phospholipid fatty acids (FAs), including saturated FAs (SFAs) and polyunsaturated FAs (PUFAs), may be implicated in the development of GDM (18–20). It is biologically plausible that AAs may be differentially associated with GDM risk depending on the profiles of phospholipid FAs, given the interrelated pathways of protein and lipid metabolisms and the shared roles of AAs and FAs on insulin resistance and β-cell function (14,21). However, studies evaluating the associations of AAs in the context of phospholipid FAs are lacking.

Therefore, we prospectively examined the associations of plasma AAs at 10–14 and 15–26 gestational weeks (GW) with risk of GDM in a case-control study nested within a large multiracial longitudinal U.S. pregnancy cohort. We examined the joint associations of AAs and phospholipid FAs with the risk of GDM. To gain mechanistic insights, we prospectively examined the correlations between AAs and markers of glucose homeostasis and cardiometabolism prior to GDM diagnosis.

RESEARCH DESIGN AND METHODS

Study Population and Design

The study population consisted of participants from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies–Singleton Cohort, a multicenter, multiracial, longitudinal cohort of low-risk singleton pregnant women conducted between 2009 and 2013. To examine the etiology of GDM, we conducted a case-control study of 107 GDM case subjects and 214 non-GDM control subjects nested within the original cohort. Details of the original source cohort can be found elsewhere (18,19). Briefly, 2,802 pregnant women aged 18–40 years without preexisting major chronic diseases were recruited between 8 and 13 weeks of gestation from 12 clinical centers across the U.S. The NICHD Fetal Growth Studies–Singleton Cohort was approved by the institutional review boards of all participating institutions. All participants provided written informed consent.

GDM Ascertainment

GDM status was ascertained via review of medical records. Diagnosis of GDM was made based on the 100-g, 3-h oral glucose tolerance test (OGTT) according to the Carpenter and Coustan criteria endorsed by the American Diabetes Association and the American College of Obstetricians and Gynecologists (22). The OGTT was performed at (mean ± SD) 27 ± 4 weeks of gestation among GDM cases. For the 107 GDM case subjects, 214 non-GDM control subjects were randomly selected from the same source cohort and individually matched at a 2:1 ratio to the case subjects on age (±2 years), race and ethnicity (i.e., non-Hispanic White, non-Hispanic Black, Hispanic, and Asian/Pacific Islander), and gestational age at blood collection (±2 weeks).

Biomarker Assessment

Maternal blood specimens were longitudinally collected at four time points during pregnancy: 10–14, 15–26, 23–31, and 33–39 GW. The second blood specimen, at 15–26 GW, was requested after an overnight fast of 8–14 h for both case and control subjects, whereas random blood specimens were collected at the other three visits with no differences in fasting duration between case and control subjects (19). Fasting status at each visit was confirmed via a biospecimen collection form filled out by the participants at the time of blood collection. For the current investigation, plasma samples collected at the two visits prior to GDM diagnosis (i.e., 10–14 and 15–26 GW) were assayed among all case and control subjects. To ensure study temporality, we excluded one case at 10–14 GW and five cases at 15–26 GW from the analytic sample whose blood specimens were taken after GDM diagnosis (19).

Based on the processed plasma samples, concentrations of 24 targeted AAs (μmol/dL) were quantified on an AA analyzer (Hitachi L-8900) as described previously (17). The interassay coefficients of variation for assessed AAs all were $<5.0\%$, with the case-control GDM status blinded to investigators. The 24 AAs assessed in the study included 18 standard AAs, namely, glycine, alanine, aspartic acid, asparagine, arginine, glutamine, glutamic acid, threonine, serine, valine, leucine, isoleucine, phenylalanine, tyrosine, methionine, histidine, lysine, and proline, and 6 nonstandard AAs, namely, taurine, citrulline, ornithine, hydroxyproline, α-aminobutyric acid, and cystine. Considering the underlying physiological pathways and current literature reports, we derived a glutamine–to–glutamic acid ratio (i.e., glutamine/glutamic acid) (23), grouped BCAAs by summing valine, leucine, and isoleucine, and grouped aromatic AAs by summing phenylalanine and tyrosine.

Consistent with AA assessment, concentrations of individual plasma phospholipid even- and odd-chain SFAs and PUFAs at the two visits prior to GDM diagnosis were assessed as described previously (19). The content of individual phospholipid FAs was expressed as a weight percentage of the total phospholipid FAs.

We measured a panel of biomarkers related to glucose homeostasis and cardiometabolism using blood samples collected at 10–14 and 15–26 GW, including glucose, insulin, HOMA of insulin resistance, hemoglobin A_{1c} (HbA_{1c}), C-peptide, hs-CRP, high-molecular-weight adiponectin, HDL cholesterol, LDL cholesterol, and triglycerides. Biomarker assessments and quality control have been described elsewhere (19).

Covariate Assessment

Maternal demographic, health-related, and clinical information were collected

through a structured questionnaire at the time of enrollment or extracted from medical records. Maternal age, race and ethnicity, family history of diabetes, parity, education status, marital status, smoking status, and alcohol consumption were assessed. Prepregnancy BMI (kg/m^2) was calculated based on self-reported prepregnancy body weight and staff-measured height. Gestational age at each visit was estimated based on date of the visit and self-reported date of the last menstrual period confirmed by ultrasound measurement at time of enrollment.

Statistical Analysis

The main associations of interest were maternal plasma AAs at 10–14 and 15–26 GW with subsequent risk of GDM. Differences in baseline population characteristics according to GDM status were assessed by linear mixed-effects models with associated likelihood ratio tests for continuous variables and by logistic regressions with generalized estimating equations for categorical variables; matched case-control pairs were accounted for by including a matched pair-specific random intercept. We calculated group-specific means and 95% CIs of AA concentrations at 10–14 and 15–26 GW; statistical differences in AAs according to status of the GDM event were assessed by the linear mixed-effects models as described above.

In the main analysis, we first examined the associations of AAs at 10–14 and 15–26 GW, respectively, with the risk of GDM. Conditional logistic regressions were used to estimate the crude or adjusted odds ratios (ORs) and corresponding 95% CIs. Individual AAs were categorized into quartiles based on the distribution among the control subjects; the lowest quartile was set as the reference group. Quartile-specific median values were assigned and entered into the models as a continuous variable to estimate *P* value for trend; continuous values were fitted in the models to obtain ORs and 95% CIs of one unit of increment per SD. A priori selected covariates adjusted in the models included maternal age, gestational age at blood draw, family history of diabetes, parity, and prepregnancy BMI. Matching factors assessed as continuous variables (i.e., maternal age and gestational age at blood draw) were included in the models to improve precision of the risk estimates.

We next examined the joint associations of plasma AAs and phospholipid FAs with the risk of GDM. We considered glycine and individual BCAAs as potential AAs based on their proposed interrelated mechanisms with FAs (24,25). Our group previously examined plasma phospholipid SFAs and PUFAs in the same GDM cohort (18,19); based on the findings, we considered individual or grouped SFAs and PUFAs that were significantly associated with the risk of GDM at 10–14 and/or 15–26 GW. For SFAs, summed even-chain SFAs (positive association with GDM) and summed odd-chain SFAs (inverse association with GDM) were considered (18). For PUFAs, we considered three individual n-6 PUFAs that were identified to be differentially associated with GDM risk: 18:3n-6 (γ-linolenic acid [GLA]; positive with GDM), 20:3n-6 (dihomo-γ-linolenic acid [DGLA]; positive with GDM), and 22:4n-6 (docosatetraenoic acid [DTA]; inverse with GDM) (19). Binary variables of AA or FA were categorized by median value among the controls, and four joint categories were defined for all the possible combinations (low AA and low FA, low AA and high FA, high AA and low FA, and high AA and high FA). We used the conditional logistic regression models as described earlier to estimate the adjusted ORs and 95% CIs of the joint categories with GDM risk.

To gain mechanistic insights while preserving temporality, we calculated partial Spearman's correlation coefficients of individual AAs at 10–14 GW with markers of glucose homeostasis and cardiometabolism at 15–26 GW among the non-GDM control subjects.

We performed several sensitivity analyses. We additionally adjusted for fasting status (<8 h and ≥8 h) for the associations of AAs at 10–14 GW. We adjusted the level of statistical significance using the Benjamini-Hochberg false discovery rate (FDR)-controlling method to account for multiple comparisons (26). We additionally adjusted for glucose and HbA_{1c}, one at a time, in the main models of AAs with GDM risk to explore potential pathways in addition to glucose metabolism. We conducted exploratory analyses to evaluate potential heterogeneity in the associations of AAs with GDM by major risk factors, including race and ethnicity, family history of diabetes, nulliparity, and prepregnancy BMI; adjusted OR and 95% CI was estimated for individual AAs (continuous per SD) from the same conditional logistic regression models as those used in the main analysis, stratified by the covariate of interest. All analyses were performed with SAS software (version 9.4; SAS Institute Inc.). A two-sided *P* value <0.05 was considered statistically significant unless otherwise stated.

RESULTS

Compared with non-GDM control subjects, GDM case subjects were more likely to have a family history of diabetes and a higher prepregnancy BMI (Table 1). Overall, mean maternal age was 30.5 years (SD 5.7). We determined 23.4%, 14.0%, 38.3%, and 24.3% of the study population was non-Hispanic White, non-Hispanic Black, Hispanic, and Asian/Pacific Islander, respectively.

Plasma concentrations of individual AAs varied by AA type at 10–14 GW and 15–26 GW (Supplementary Tables 1 and 2). At 10–14 GW, concentrations of several glucogenic AAs (glycine, alanine, aspartic acid, glutamic acid, and glutamine) as well as threonine, serine, and taurine differed between the case and control subjects ($P < 0.05$). At 15–26 GW, in addition to those glucogenic AAs, concentrations of methionine, individual BCAAs, aromatic AAs, and cystine also differed by the case-control status ($P < 0.05$).

Plasma AAs in Early Pregnancy to Midpregnancy in Association With Subsequent GDM Risk

After adjusting for major risk factors, at 10–14 GW, alanine, aspartic acid, and glutamic acid were significantly and positively associated with the risk of GDM (*P* value for trend [*P*-trend] <0.05). The per-SD increment (adjusted OR, 95% CI) in alanine, aspartic acid, and glutamic acid was associated with 43% (1.43, 1.08–1.88), 41% (1.41, 1.11–1.80), and 39% (1.39, 0.98–1.98), respectively, higher risk of GDM (Table 2 and Fig. 1). Conversely, glycine was inversely associated with the risk of GDM (*P*-trend = 0.004). The per-SD increment (adjusted OR, 95% CI) in glycine was related to 45% (0.55, 0.39–0.79) lower risk of GDM.

At 15–26 GW, associations for glycine, alanine, and aspartic acid persisted as observed at 10–14 GW. Significant associations with GDM risk were additionally observed for glutamine–to–glutamic acid

Table 1—Population characteristics according to status of GDM (i.e., case and control subjects) from the NICHD Fetal Growth Studies–Singleton Cohort

	GDM case subjects (*n* = 107)	Non-GDM control subjects (*n* = 214)	*P* value*
Age (years), mean (SD)	30.5 (5.7)	30.4 (5.4)	NA
Race and ethnicity, *n* (%)			NA
Non-Hispanic White	25 (23.4)	50 (23.4)	
Non-Hispanic Black	15 (14.0)	30 (14.0)	
Hispanic	41 (38.3)	82 (38.3)	
Asian/Pacific Islander	26 (24.3)	52 (24.3)	
Education, *n* (%)			0.18
Less than high school	17 (15.9)	26 (12.1)	
High school graduate or equivalent	15 (14.0)	23 (10.7)	
More than high school	75 (70.1)	165 (77.1)	
Insurance, *n* (%)			0.43
Private or managed care	68 (63.5)	143 (66.8)	
Medicaid, self pay, or other	39 (36.5)	71 (33.1)	
Marital status, *n* (%)			0.12
Never married	11 (10.3)	35 (16.4)	
Married/living with a partner	92 (86.0)	167 (78.0)	
Divorced/separated	4 (3.7)	12 (5.6)	
Nulliparity, *n* (%)	48 (44.9)	96 (44.9)	>0.99
Family history of diabetes, *n* (%)	40 (37.4)	48 (22.4)	0.003
Prepregnancy BMI, *n* (%)			<0.001
Normal, 19.0–24.9	37 (34.6)	123 (58.0)	
Overweight, 25–29.9	35 (32.7)	56 (26.4)	
Obesity class 1, 30–34.9	20 (18.7)	17 (8.0)	
Obesity class 2, ≥35	15 (14.0)	16 (7.6)	
Smoking 6 months preconception, *n* (%)	4 (3.7)	1 (0.5)	0.06
Alcoholic beverage consumptions 3 months preconception, *n* (%)	61 (57.0)	137 (64.0)	0.22

NA, not applicable. **P* values were obtained by linear mixed models with associated likelihood ratio tests for continuous variables and binomial/multinomial logistic regression with generalized estimating equations for binary/multilevel categorical variables (Wald tests), accounting for matched case-control pairs. *P* values are not shown for matching variables (i.e., age and race and ethnicity).

ratio and some BCAAs or aromatic AAs (Table 2 and Fig. 1). Specifically, at 15–26 GW, the per-SD increment (adjusted OR, 95% CI) in alanine, aspartic acid, isoleucine, phenylalanine, and tyrosine was related to 50% (1.50, 1.11–2.02), 59% (1.59, 1.11–2.02), 64% (1.64, 1.19–2.27), 15% (1.15, 0.87–1.53), and 56% (1.56, 1.16–2.09), respectively, higher risk of GDM (all *P*-trend < 0.05). Consistent with this, summed aromatic AAs (i.e., phenylalanine and tyrosine) were related to 43% (1.43, 1.06–1.92) higher risk of GDM (*P*-trend = 0.03). Significantly higher risks of GDM were seen in higher-quartile groups of glutamic acid compared with the lowest-quartile group, despite a nonsignificant linear trend (*P*-trend = 0.17; per-SD increment [adjusted OR, 95% CI] 1.77, 1.07–2.91). In contrast, glycine and the glutamine–to–glutamic acid ratio were inversely associated with the risk of GDM: the per-SD increment (adjusted OR, 95% CI) in glycine and glutamine–to–glutamic acid ratio was associated with 44% (0.56, 0.38–0.82; *P*-trend < 0.001) and 50% (0.50, 0.26–0.94; *P*-trend = 0.03), respectively, lower risk of GDM.

Plasma AAs and Phospholipid FAs in Early Pregnancy and Midpregnancy in Association With Subsequent GDM Risk

Plasma AAs and phospholipid FAs were jointly and significantly associated with the risk of GDM (Table 3). In particular, AA was differentially associated with GDM by the status of phospholipid FA. The inverse associations of glycine at 10–14 GW and 15–26 GW were further enhanced in the copresence of a phospholipid FA profile. For example, at 10–14 GW, the lowest risk (adjusted OR, 95% CI) was observed in women with higher glycine (≥14.2 μmol/dL) and lower even-chain SFAs (<39.9%) than their counterparts (0.15, 0.06–0.37). On the other hand, the positive association of isoleucine at 15–26 GW was amplified in the copresence of a phospholipid FA profile. For instance, at 15–26 GW, the highest risk (adjusted OR, 95% CI) was seen in women with higher isoleucine (≥4.8 μmol/dL) and the lower n-6 PUFA DTA (<0.3%) (6.66, 2.21–20.05) (Table 3).

Correlation Between Plasma AAs With Markers of Glucose Homeostasis and Cardiometabolism

Consistent with findings described above, alanine, glutamic acid, BCAAs, aromatic AAs, and α-aminobutyric acid were overall positively correlated with markers of glucose homeostasis, including fasting glucose, HbA_{1c}, fasting insulin, C-peptide, hs-CRP, and HOMA of insulin resistance, whereas glycine, glutamine, asparagine, and taurine were inversely correlated with these markers (Supplementary Fig. 1). For markers of cardiometabolism, glycine, glutamine, glutamine–to–glutamic acid ratio, BCAAs, and aromatic AAs were generally correlated with a more favorable cardiometabolic profile (i.e., higher high-molecular-weight adiponectin and HDL cholesterol, lower LDL cholesterol, and lower triglycerides). In contrast, aspartic acid and glutamic acid were correlated with a less favorable cardiometabolic profile.

In the sensitivity analyses, additional adjustment of the fasting status did not influence the associations of AAs at 10–14 GW with GDM (results not shown). After post hoc FDR correction for multiple comparisons, the positive associations for alanine and tyrosine and the inverse association for glycine at 15–26 GW remained statistically significant (Table 2). At 10–14 GW, further adjustment of glucose or HbA_{1c} did not materially alter the strengths of the associations for significant AAs (Supplementary Table 3). At 15–26 GW, however, the associations were attenuated and became nonsignificant after the adjustment of fasting glucose, except for glycine; additional adjustment of HbA_{1c} largely did not influence the results (Supplementary Table 4). In the analyses stratified by major risk factors, similar patterns of the associations for those significant AAs were observed across categories of race and ethnicity, nulliparity, and prepregnancy BMI overall. The associations appeared to be stronger

Table 2—Crude and adjusted ORs of GDM associated with quartile groups of plasma AAs at 10–14 and 15–26 GW in the NICHD Fetal Growth Studies–Singleton Cohort

	10–14 GW		15–26 GW	
AAs and quartile groups	Crude OR	Adjusted OR*	Crude OR	Adjusted OR
Glycine				
Q2 vs. Q1 (reference)	0.42 (0.21, 0.84)	0.44 (0.21, 0.95)	0.58 (0.29, 1.15)	0.66 (0.31, 1.40)
Q3 vs. Q1	0.29 (0.14, 0.61)	0.39 (0.17, 0.90)	0.31 (0.15, 0.65)	0.35 (0.15, 0.81)
Q4 vs. Q1	0.19 (0.09, 0.43)	0.25 (0.10, 0.62)	0.17 (0.07, 0.41)	0.18 (0.07, 0.51)
P-trend†	<0.001	0.004	<0.001	<0.001
FDR critical *P* value†		0.002		0.0025**
Alanine				
Q2 vs. Q1 (reference)	1.50 (0.68, 3.31)	1.92 (0.79, 4.65)	1.22 (0.57, 2.60)	1.10 (0.45, 2.67)
Q3 vs. Q1	2.24 (1.07, 4.70)	2.78 (1.19, 6.46)	1.36 (0.62, 3.01)	1.46 (0.56, 3.77)
Q4 vs. Q1	2.67 (1.28, 5.57)	3.32 (1.43, 7.74)	2.50 (0.18, 5.29)	3.59 (1.48, 8.72)
P-trend	0.005	0.005	0.01	0.002
FDR critical *P* value		0.004		0.0075**
Aspartic acid				
Q2 vs. Q1 (reference)	1.05 (0.49, 2.26)	1.17 (0.49, 2.81)	2.15 (1.04, 4.44)	2.83 (1.20, 6.66)
Q3 vs. Q1	1.69 (0.80, 3.56)	1.63 (0.69, 3.84)	1.28 (0.54, 3.01)	1.76 (0.66, 4.70)
Q4 vs. Q1	2.19 (1.10, 4.36)	2.40 (1.07, 5.39)	3.59 (1.41, 9.16)	3.62 (1.26, 10.30)
P-trend	0.008	0.01	0.02	0.05
FDR critical *P* value		0.006		
Asparagine				
Q2 vs. Q1 (reference)	0.97 (0.52, 1.81)	0.88 (0.44, 1.75)	0.54 (0.23, 1.24)	0.51 (0.18, 1.43)
Q3 vs. Q1	0.63 (0.32, 1.24)	0.69 (0.32, 1.45)	0.45 (0.17, 1.19)	0.51 (0.16, 1.67)
Q4 vs. Q1	0.51 (0.25, 1.06)	0.49 (0.21, 1.15)	0.39 (0.15, 1.03)	0.52 (0.16, 1.75)
P-trend	0.05	0.09	0.08	0.40
Arginine				
Q2 vs. Q1 (reference)	1.10 (0.55, 2.20)	1.14 (0.52, 2.48)	0.45 (0.18, 1.13)	0.28 (0.10, 0.83)
Q3 vs. Q1	1.22 (0.62, 2.38)	1.24 (0.58, 2.64)	1.43 (0.68, 2.97)	1.01 (0.43, 2.33)
Q4 vs. Q1	1.66 (0.84, 3.29)	1.75 (0.81, 3.75)	2.06 (1.01, 4.21)	1.32 (0.59, 2.98)
P-trend	0.12	0.14	0.07	0.15
Glutamine				
Q2 vs. Q1 (reference)	1.09 (0.56, 2.11)	0.91 (0.43, 1.93)	0.46 (0.19, 1.10)	0.62 (0.23, 1.68)
Q3 vs. Q1	1.06 (0.49, 2.28)	1.27 (0.52, 3.11)	0.83 (0.20, 3.40)	0.98 (0.22, 4.26)
Q4 vs. Q1	0.90 (0.39, 2.07)	1.00 (0.38, 2.60)	0.34 (0.07, 1.56)	0.31 (0.06, 1.52)
P-trend	0.89	0.91	0.05	0.10
Glutamic acid				
Q2 vs. Q1 (reference)	1.06 (0.52,2.15)	0.86 (0.39, 1.89)	3.09 (1.26, 7.62)	3.89 (1.34, 11.30)
Q3 vs. Q1	1.78 (0.81, 3.93)	1.62 (0.67, 3.90)	4.70 (1.15, 19.2)	4.04 (0.87, 19.0)
Q4 vs. Q1	2.66 (1.07, 6.59)	2.45 (0.89, 6.67)	7.20 (1.60, 32.3)	5.15 (1.00, 26.5)
P-trend	0.02	0.04	0.04	0.17
FDR critical *P* value		0.008		
Threonine				
Q2 vs. Q1 (reference)	1.14 (0.57, 2.32)	0.93 (0.42, 2.07)	1.19 (0.59, 2.42)	0.95 (0.41, 2.20)
Q3 vs. Q1	1.15 (0.58, 2.29)	0.83 (0.38, 1.82)	1.30 (0.65, 2.62)	1.13 (0.51, 2.50)
Q4 vs. Q1	1.85 (0.92, 3.71)	1.61 (0.73, 3.56)	1.36 (0.67, 2.77)	1.09 (0.47, 2.54)
P-trend	0.08	0.25	0.37	0.73
Serine				
Q2 vs. Q1 (reference)	0.72 (0.38, 1.34)	0.74 (0.48, 0.60)	0.79 (0.39, 1.61)	0.97 (0.43, 2.18)
Q3 vs. Q1	0.54 (0.28, 1.04)	0.48 (0.22, 1.03)	0.76 (0.37, 1.55)	0.83 (0.37, 1.87)
Q4 vs. Q1	0.51 (0.24, 1.05)	0.60 (0.27, 1.38)	1.01 (0.48, 2.13)	1.20 (0.50, 2.89)
P-trend	0.04	0.13	0.94	0.72
Methionine				
Q2 vs. Q1 (reference)	1.14 (0.58, 2.24)	0.98 (0.45, 2.13)	1.27 (0.57, 2.81)	1.66 (0.66, 4.16)
Q3 vs. Q1	1.27 (0.67, 2.40)	1.24 (0.62, 2.50)	1.73 (0.76, 3.94)	2.36 (0.92, 6.01)
Q4 vs. Q1	0.96 (0.49, 1.86)	0.87 (0.40, 1.87)	2.36 (1.01, 5.50)	2.16 (0.84, 5.59)
P-trend	0.85	0.79	0.03	0.11
Histidine				
Q2 vs. Q1 (reference)	1.25 (0.67, 2.34)	1.82 (0.89, 3.73)	1.26 (0.62, 2.55)	1.08 (0.48, 2.42)
Q3 vs. Q1	0.77 (0.40, 1.50)	0.71 (0.33, 1.54)	1.10 (0.53, 2.25)	0.95 (0.43, 2.09)
Q4 vs. Q1	0.63 (0.30, 1.31)	0.68 (0.29, 1.58)	0.58 (0.27, 1.27)	0.44 (0.17, 1.11)
P-trend	0.13	0.18	0.17	0.10

Continued on p. 727

Table 2—Continued

AAs and quartile groups	10–14 GW		15–26 GW	
	Crude OR	Adjusted OR*	Crude OR	Adjusted OR
Lysine				
Q2 vs. Q1 (reference)	0.61 (0.30, 1.24)	0.53 (0.25, 1.16)	1.02 (0.49, 2.11)	1.10 (0.49, 2.44)
Q3 vs. Q1	0.97 (0.50, 1.86)	0.90 (0.43, 1.88)	0.98 (0.48, 2.01)	0.83 (0.38, 1.85)
Q4 vs. Q1	1.14 (0.58, 2.24)	1.10 (0.51, 2.40)	1.49 (0.76, 2.94)	1.18 (0.54, 2.59)
P-trend	0.44	0.49	0.2	0.76
Proline				
Q2 vs. Q1 (reference)	1.14 (0.59, 2.20)	1.23 (0.60, 2.55)	0.91 (0.42, 1.99)	0.80 (0.33, 1.95)
Q3 vs. Q1	0.62 (0.30, 1.31)	0.73 (0.33, 1.65)	1.13 (0.55, 2.34)	1.33 (0.58, 3.04)
Q4 vs. Q1	0.96 (0.50, 1.82)	1.05 (0.50, 2.19)	0.65 (0.30, 1.41)	0.53 (0.22, 1.28)
P-trend	0.66	0.87	0.37	0.30
Valine				
Q2 vs. Q1 (reference)	1.09 (0.54, 2.19)	1.02 (0.47, 2.23)	1.51 (0.70, 3.25)	1.65 (0.66, 4.16)
Q3 vs. Q1	1.22 (0.58, 2.56)	1.03 (0.44, 2.41)	1.49 (0.71, 3.11)	0.97 (0.39, 2.40)
Q4 vs. Q1	1.32 (0.67, 2.59)	1.19 (0.56, 2.53)	1.97 (0.97, 4.02)	1.71 (0.72, 4.06)
P-trend	0.40	0.63	0.07	0.39
Leucine				
Q2 vs. Q1 (reference)	1.42 (0.73, 2.78)	1.45 (0.70, 3.01)	0.72 (0.33, 1.58)	0.72 (0.29, 1.78)
Q3 vs. Q1	1.07 (0.52, 2.19)	0.91 (0.41, 2.02)	1.01 (0.50, 2.07)	0.79 (0.35, 1.79)
Q4 vs. Q1	1.16 (0.58, 2.30)	1.13 (0.52, 2.43)	1.66 (0.81, 3.41)	1.30 (0.57, 2.99)
P-trend	0.88	0.99	0.09	0.39
Isoleucine				
Q2 vs. Q1 (reference)	1.36 (0.67, 2.77)	1.18 (0.54, 2.58)	1.28 (0.58, 2.82)	1.46 (0.59, 3.63)
Q3 vs. Q1	1.58 (0.77, 3.24)	1.29 (0.58, 2.86)	1.46 (0.67, 3.16)	1.27 (0.51, 3.13)
Q4 vs. Q1	1.67 (0.83, 3.34)	1.52 (0.69, 3.33)	3.11 (1.14, 1.94)	3.15 (1.29, 2.27)
P-trend	0.17	0.30	0.002	0.01
FDR critical *P* value				0.01
Phenylalanine				
Q2 vs. Q1 (reference)	1.34 (0.66, 2.71)	1.29 (0.59, 2.84)	1.22 (0.56, 2.63)	1.20 (0.50, 2.86)
Q3 vs. Q1	2.09 (1.03, 4.26)	2.11 (0.97, 4.58)	1.87 (0.87, 4.01)	1.82 (0.76, 4.32)
Q4 vs. Q1	1.26 (0.62, 2.59)	1.25 (0.56, 2.82)	2.55 (1.20, 5.43)	2.22 (0.95, 5.17)
P-trend	0.48	0.52	0.008	0.04
FDR critical *P* value				0.015
Tyrosine				
Q2 vs. Q1 (reference)	1.82 (0.86, 3.84)	1.27 (0.56, 2.90)	0.86 (0.32, 2.29)	0.66 (0.23, 1.94)
Q3 vs. Q1	2.23 (1.08, 4.60)	2.00 (0.91, 4.37)	1.60 (0.73, 3.50)	1.16 (0.47, 2.83)
Q4 vs. Q1	2.34 (1.13, 4.85)	1.72 (0.76, 3.86)	3.75 (1.78, 7.89)	2.78 (1.24, 7.21)
P-trend	0.04	0.18	<0.001	0.001
FDR critical *P* value				0.0025**
Taurine				
Q2 vs. Q1 (reference)	0.50 (0.22, 1.12)	0.42 (0.17, 1.06)	1.52 (0.72, 3.23)	1.50 (0.65, 3.44)
Q3 vs. Q1	0.93 (0.46, 1.89)	0.89 (0.40, 1.98)	1.43 (0.64, 3.15)	1.57 (0.64, 3.86)
Q4 vs. Q1	1.41 (0.77, 2.58)	1.36 (0.68, 2.71)	1.65 (0.80, 3.40)	1.51 (0.66, 3.46)
P-trend	0.06	0.08	0.26	0.45
Citrulline				
Q2 vs. Q1 (reference)	0.71 (0.35, 1.42)	0.75 (0.35, 1.61)	0.82 (0.42, 1.63)	0.70 (0.32, 1.53)
Q3 vs. Q1	0.86 (0.43, 1.74)	0.86 (0.38, 1.95)	0.70 (0.33, 1.48)	0.52 (0.21, 1.25)
Q4 vs. Q1	1.18 (0.60, 2.30)	1.33 (0.64, 2.78)	1.17 (0.57, 2.40)	1.13 (0.47, 2.73)
P-trend	0.51	0.39	0.74	0.93
Ornithine				
Q2 vs. Q1 (reference)	0.89 (0.46, 1.73)	0.66 (0.31, 1.40)	0.58 (0.26, 1.29)	0.60 (0.23, 1.54)
Q3 vs. Q1	1.04 (0.53, 2.06)	0.89 (0.41, 1.95)	1.76 (0.84, 3.68)	1.70 (0.70, 4.13)
Q4 vs. Q1	1.30 (0.66, 2.56)	1.04 (0.47, 2.28)	1.21 (0.57, 2.56)	1.02 (0.43, 2.44)
P-trend	0.36	0.67	0.33	0.73
Hydroxyproline				
Q2 vs. Q1 (reference)	0.79 (0.39, 1.61)	0.66 (0.30, 1.45)	0.96 (0.46, 2.01)	1.21 (0.49, 3.00)
Q3 vs. Q1	1.44 (0.71, 2.93)	1.44 (0.64, 3.29)	1.12 (0.56, 2.24)	1.18 (0.53, 2.64)
Q4 vs. Q1	1.34 (0.66, 2.74)	1.28 (0.56, 2.92)	1.00 (0.48, 2.08)	1.09 (0.46, 2.59)
P-trend	0.32	0.36	0.90	0.91

Continued on p. 728

Table 2—Continued

AAs and quartile groups	10–14 GW		15–26 GW	
	Crude OR	Adjusted OR*	Crude OR	Adjusted OR
α-Aminobutyric acid				
Q2 vs. Q1 (reference)	0.73 (0.37, 1.41)	0.63 (0.30, 1.33)	1.01 (0.47, 2.18)	0.69 (0.28, 1.72)
Q3 vs. Q1	0.49 (0.23, 1.04)	0.36 (0.15, 0.89)	1.26 (0.62, 2.58)	1.10 (0.48, 2.53)
Q4 vs. Q1	1.09 (0.56, 2.15)	0.88 (0.41, 1.85)	1.02 (0.48, 2.17)	0.67 (0.28, 1.61)
P-trend	0.75	0.78	0.87	0.58
Cystine‡				
T2 vs. T1 (reference)	1.95 (0.88, 4.32)	2.37 (0.97, 5.81)	1.20 (0.52, 2.75)	0.89 (0.34, 2.36)
T3 vs. T1	2.12 (0.81, 5.52)	1.97 (0.66, 5.85)	3.41 (1.21, 9.58)	2.44 (0.79, 7.50)
P-trend	0.12	0.20	0.02	0.12
Grouped AAs				
Glutamine–to–glutamic acid ratio				
Q2 vs. Q1 (reference)	0.61 (0.31, 1.20)	0.57 (0.27,1.22)	0.53 (0.24, 1.18)	0.71 (0.28, 1.78)
Q3 vs. Q1	0.55 (0.25, 1.21)	0.47 (0.19, 1.16)	0.58 (0.16, 2.08)	0.84 (0.20, 3.44)
Q4 vs. Q1	0.49 (0.20, 1.17)	0.57 (0.21, 1.56)	0.23 (0.05, 1.05)	0.27 (0.05, 1.39)
P-trend	0.17	0.43	0.02	0.03
Summed BCAAs§				
Q2 vs. Q1 (reference)	1.14 (0.57, 2.30)	1.10 (0.51, 2.38)	1.39 (0.67, 2.88)	1.41 (0.61, 3.26)
Q3 vs. Q1	1.33 (0.66, 2.69)	1.09 (0.49, 2.40)	0.63 (0.27, 1.48)	0.46 (0.17, 1.23)
Q4 vs. Q1	1.33 (0.68, 2.59)	1.15 (0.54, 2.47)	2.23 (1.12, 4.42)	1.89 (0.83, 4.28)
P-trend	0.41	0.73	0.04	0.16
Summed aromatic AAs‖				
Q2 vs. Q1 (reference)	2.97 (1.33, 6.66)	2.32 (0.97, 5.57)	2.66 (1.19, 3.52)	3.46 (1.31, 9.13)
Q3 vs. Q1	3.35 (1.53, 7.30)	3.33 (1.42, 7.81)	1.33 (0.55, 3.23)	1.12 (0.41, 3.11)
Q4 vs. Q1	2.31 (1.00, 5.34)	1.83 (0.73, 4.62)	3.04 (1.23, 7.52)	3.30 (1.12, 9.71)
P-trend	0.20	0.36	0.002	0.03

AA concentrations were measured as μmol/dL. *Models were adjusted for family history of diabetes (yes, no), nulliparity (yes, no), prepregnancy BMI (<25.0, 25.0–29.9, 30.0–34.9, and 35.0–44.9 kg/m^2), maternal age (years), and GW 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. FDR was performed on the 24 individual plasma AAs. Individual AAs were ranked based on their *P* values for trend. Critical *P* value was calculated for individual AAs using the equation critical *P* value = (rank/total number of test) × FDR rate, with an FDR rate of 0.05 and compared with the *P* value. Significant AAs with *P* value < critical *P* value were considered statistically significant after the FDR adjustment and are marked with double asterisks. ‡Tertiles instead of quartiles were modeled for cystine due to the large number of zero values (n = 96 at 10–14 GW and n = 129 at 15–26 GW). §BCAA: summed valine, leucine, and isoleucine. ‖Aromatic AA: summed tyrosine and phenylalanine.

among women without a family history of diabetes than for those with a family history of diabetes (Supplementary Tables 5 and 6).

CONCLUSIONS

In this longitudinal prospective study of a multiracial pregnancy cohort, several glucogenic AAs at both 10–14 GW and 15–26 GW and isoleucine and aromatic AAs at 15–26 GW were prospectively associated with the development of GDM. Among the glucogenic AAs, alanine, aspartic acid, and glutamic acid were positively associated with GDM risk, whereas glycine and the glutamine–to–glutamic acid ratio were inversely associated with GDM risk. The BCAA isoleucine and aromatic AAs were positively associated with GDM risk. The inverse associations for glycine and the positive association for isoleucine appeared to be enhanced in the copresence of phospholipid FA profile.

Prospective studies on circulating AAs with GDM risk are emerging, although findings are largely inconsistent. We identified seven studies that prospectively evaluated AAs at one time point during gestation (9–15) and one study that assessed AAs at two gestational windows (9–13 GW and 16–19 GW) (16) with GDM risk. Among glucogenic AAs, positive associations of alanine in the first trimester (10,13,15,16) and second trimester (12) have been reported. In contrast, an inverse association of glycine with the risk of GDM was seen in the first trimester (9) and the second trimester (12). Findings on aspartic acid, glutamine, and glutamic acid were less clear: no evidence of associations was reported for glutamine or aspartic acid; mixed findings were reported for glutamic acid (11–13). Because glutamine is a derivative of glutamic acid, the conflicting findings on glutamic acid may be due to a lack of proper consideration of glutamine. Indeed, our observations on the associations of glutamic acid vs. those of glutamine in opposite directions and the inverse association of the glutamine-to-glutamic-acid ratio suggest the importance of examining their relative rather than individual contributions to the risk of GDM. Although evidence of association between glucogenic AAs with GDM is still limited, literature findings on their associations with type 2 diabetes have generally been more robust and were consistent with directions of the associations observed in our study (27). Taken together, our study suggests that several glucogenic AAs are implicated in the etiology of GDM starting from early pregnancy. Alternatively, the altered glucogenic AAs may be a manifestation of the underlying altered insulin sensitivity starting in early pregnancy.

So far, most of the studies evaluating BCAAs and aromatic AAs in early pregnancy and/or midpregnancy reported positive associations between these AAs with risk of GDM (10–14,16). In our study, although we found that concentrations of these AAs at 10–14 GW tended

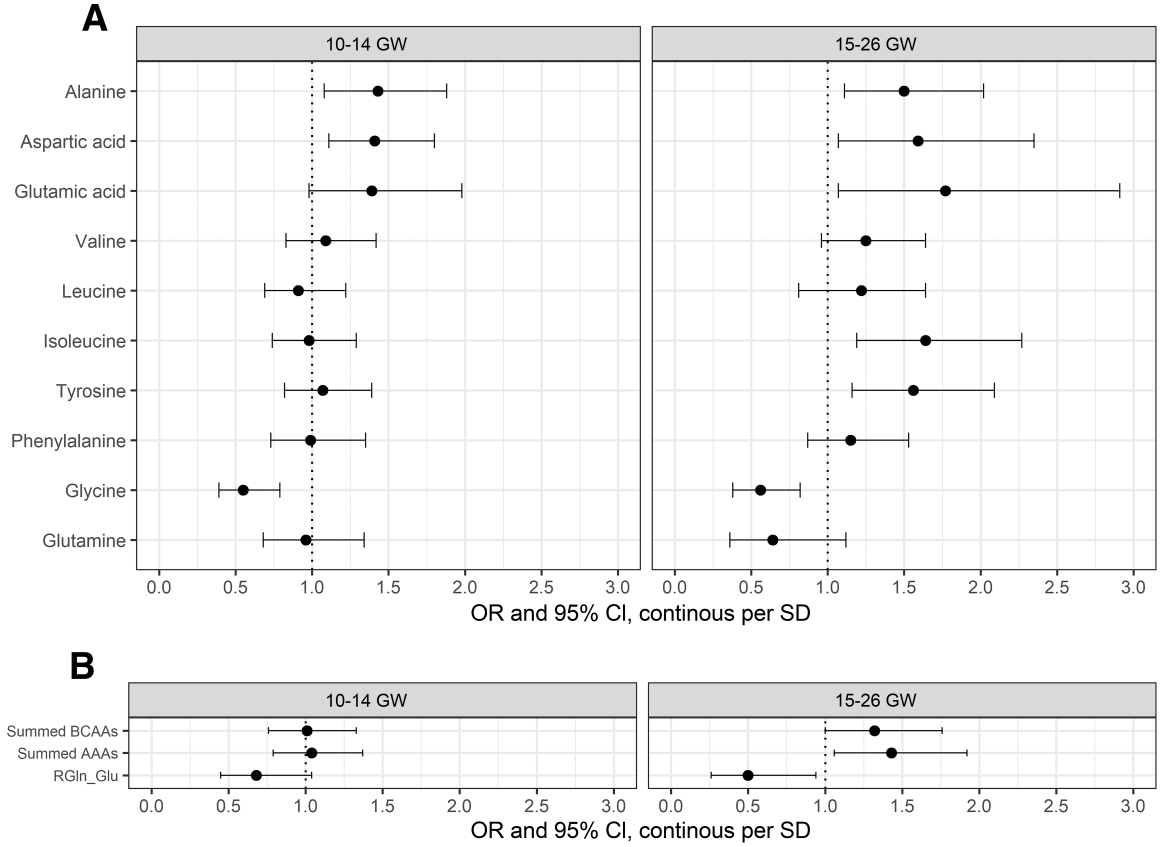

Figure 1—Adjusted ORs of GDM risk associated with individual plasma AAs (*A*) and grouped plasma AAs (*B*) with *P*-trend < 0.05 (continuous increase per SD) at 10–14 and 15–26 GW from the NICHD Fetal Growth Studies–Singleton Cohort. Multivariable logistic regression models were adjusted for family history of diabetes (yes, no), nulliparity (yes, no), prepregnancy BMI (<25.0, 25.0–29.9, 30.0–34.9, and 35.0–44.9 kg/m^2), maternal age (years), and GW at the time of blood draw. AAAs, aromatic AAs; RGln_Glu, glutamine–to–glutamic acid ratio.

to be positively related to GDM risk, the associations were not statistically significant. However, we did observe significant and positive associations of these AAs in midpregnancy with subsequent GDM risk. BCAAs and aromatic AAs have been extensively examined with diabetes-related markers and diabetes progression (6,28); prospective associations of BCAAs and aromatic AAs with type 2 diabetes risk were reported among diverse populations (8,27). The observed null associations of BCAAs and aromatic AAs in early pregnancy with GDM risk should not exclude the possibility of their involvement in the early stage of GDM development, given their prospective correlations at 10–14 GW with glucose- and/or cardiometabolism-related markers.

Circulating AAs are reflective of and are critical for glucose homeostasis, insulin activity, inflammation, and oxidative stress, which are key hypothesized pathways involved in GDM etiology (29). For glucogenic AAs, glycine may act as an insulin secretagogue; inadequate glycine may result in decreased pancreatic insulin secretion (29). Alanine inhibits hepatic autophagy, which is important for maintaining blood glucose concentrations (29,30). Glutamate and aspartic acid are involved in *N*-methyl-D-aspartate (NMDA) activation; inhibition of NMDA increases glucose-stimulated insulin secretion, improving glucose tolerance (29,31). Glutamine is a derivative of glutamate and itself may additionally act on pancreatic β-cell function as an anaplerotic substrate to enhance glucose oxidation (31). BCAAs may activate mammalian target of rapamycin complex (mTOR); prolonged elevation of BCAA concentrations results in hyperactivation of mTOR signaling and subsequently β-cell dysfunction (28). Dyslipidemia is induced during the development of GDM; BCAAs are associated with altered lipid metabolism (32,33). A surplus of aromatic AAs, particularly tyrosine, could weaken blood glucose clearance and increase gluconeogenesis (34). Our observations on the correlations between AAs and markers of glucose and cardiometabolism are in line with these proposed mechanisms of AAs on glucose control, insulin resistance, and lipid metabolism. Furthermore, the persistent associations of some significant AAs with GDM risk after further adjustment of glucose or HbA_{1c} support their involvement in pathway(s) in addition to that of glucose homeostasis, such as inflammation and oxidative stress. Future studies with the appropriate study design may conduct formal mediation analysis to investigate the underlying mechanisms of AAs on the development of GDM.

Recent studies have linked altered profiles of circulating phospholipid FAs with insulin resistance and risk of GDM (18–20). We extended our prior work and examined the joint associations of AAs and phospholipid FAs with GDM (18,19). As one of the first lines of evidence, we show potential synergistic interplay between key AAs and phospholipid FAs on the development of GDM. In particular, magnitudes of the associations for some significant AAs

Table 3—Joint associations of plasma AAs and phospholipid FAs at GW 10–14 and 15–26 with risk of GDM from the NICHD Fetal Growth Studies–Singleton Cohort

		Adjusted OR (95% CI)* at:	
AA	FA	10–14 GW	15–26 GW
Glycine (− at 10–14 and 15–26 GW)†	Even-chain SFA (+ at 10–14 and 15–26 GW)†		
Low	Low	0.38 (0.16, 0.89)	0.48 (0.21, 1.08)
Low	High	REF (OR = 1.00)‡	REF (OR = 1.00)
High	Low	0.15 (0.06, 0.37)	0.19 (0.08, 0.50)
High	High	0.50 (0.23, 1.12)	0.42 (0.17, 1.01)
	P-interaction§	0.51	0.09
Glycine (− at 10–14 and 15–26 GW)	Odd-chain SFA (− at 10–14 and 15–26 GW)		
Low	Low	REF (OR = 1.00)	REF (OR = 1.00)
Low	High	0.28 (0.11, 0.66)	0.50 (0.23, 1.09)
High	Low	0.24 (0.10, 0.58)	0.50 (0.22, 1.13)
High	High	0.23 (0.09, 0.52)	0.13 (0.05, 0.36)
	P-interaction	0.06	0.86
Glycine (− at 10–14 and 15–26 GW)	PUFA GLA (+ at 10–14 GW)		
Low	Low	0.50 (0.24, 1.05)	0.83 (0.38, 1.79)
Low	High	REF (OR = 1.00)	REF (OR = 1.00)
High	Low	0.22 (0.09, 0.52)	0.37 (0.16, 0.91)
High	High	0.38 (0.17, 0.85)	0.31 (0.13, 0.76)
	P-interaction	0.06	0.23
Glycine (− at 10–14 and 15–26)	PUFA DGLA (+ at 10–14 and 15–26 GW)		
Low	Low	0.51 (0.23, 1.15)	0.62 (0.26, 1.50)
Low	High	REF (OR = 1.00)	REF (OR = 1.00)
High	Low	0.27 (0.11, 0.64)	0.21 (0.08, 0.52)
High	High	0.31 (0.13, 0.71)	0.48 (0.19, 1.21)
	P-interaction	0.06	0.92
Glycine (− at 10–14 and 15–26 GW)	PUFA DTA (− at 15–26 GW)		
Low	Low	REF (OR = 1.00)	REF (OR = 1.00)
Low	High	0.78 (0.32, 1.89)	0.44 (0.19, 1.05)
High	Low	0.39 (0.17, 0.89)	0.45 (0.19, 1.07)
High	High	0.30 (0.11, 0.83)	0.15 (0.05, 0.42)
	P-interaction	0.75	0.43
Isoleucine (+ at 15–26 GW)	Even-chain SFA (+ at 10–14 and 15–26 GW)		
Low	Low	REF (OR = 1.00)	REF (OR = 1.00)
Low	High	3.37 (1.35, 8.43)	2.29 (0.85, 6.17)
High	Low	1.40 (0.58, 3.36)	1.59 (0.64, 3.99)
High	High	4.29 (1.86, 9.91)	3.59 (1.50, 8.57)
	P-interaction	0.93	0.43
Isoleucine (+ at 15–26 GW)	Odd-chain SFA (− at 10–14 and 15–26)		
Low	Low	1.25 (0.51, 3.06)	2.52 (1.00, 6.31)
Low	High	REF (OR = 1.00)	REF (OR = 1.00)
High	Low	2.90 (1.19, 7.07)	3.75 (1.63, 8.64)
High	High	0.93 (0.39, 2.22)	1.51 (0.58, 3.95)
	P-interaction	0.07	0.15
Isoleucine (+ at 15–26 GW)	PUFA GLA (+ at 10–14 GW)		
Low	Low	REF (OR = 1.00)	REF (OR = 1.00)
Low	High	1.75 (0.81, 3.79)	0.64 (0.28, 1.48)
High	Low	1.26 (0.57, 2.78)	1.23 (0.57, 2.64)
High	High	2.55 (1.22, 5.32)	1.77 (0.79, 3.96)
	P-interaction	0.97	0.45
Isoleucine (+ at 15–26 GW)	PUFA DGLA (+ at 10–14 and 15–26 GW)		
Low	Low	REF (OR = 1.00)	REF (OR = 1.00)
Low	High	1.49 (0.65, 3.41)	1.35 (0.53, 3.43)
High	Low	1.47 (0.66, 3.26)	1.13 (0.46, 2.77)
High	High	2.14 (0.98, 4.70)	3.81 (1.52, 9.55)
	P-interaction	0.91	0.18

Continued on p. 731

Table 3—Continued

AA	FA	Adjusted OR (95% CI)* at: 10–14 GW	15–26 GW
Isoleucine (+ at 15–26 GW)	PUFA DTA (− at 15–26 GW)		
Low	Low	1.71 (0.70, 4.18)	5.14 (1.68, 15.71)
Low	High	REF (OR = 1.00)	REF (OR = 1.00)
High	Low	1.67 (0.67, 4.17)	6.66 (2.21, 20.05)
High	High	2.14 (1.03, 4.44)	3.41 (1.23, 9.47)
	P-interaction	0.15	0.65

REF, reference. *Models were adjusted for family history of diabetes (yes, no), nulliparity (yes, no), prepregnancy BMI (<25.0, 25.0–29.9, 30.0–34.9, and 35.0–44.9 kg/m^2), maternal age (years), and GW at the time of blood draw. †Observed direction of the associations in previous studies was noted, + for positive association and − for inverse association. Median value among the non-GDM control subjects was assigned as the binary cutoff for AAs and FAs at each visit. At 10–14 GW, the binary cutoff value was 14.2 μmol/dL for glycine, 5.0 μmol/dL for isoleucine, 39.9% for even-chain SFA, 0.7% for odd-chain SFA, 0.07% for GLA, 3.4% for DGLA, and 0.5% for DTA. At 15–26 GW, the binary cutoff value was 14.0 μmol/dL for glycine, 4.8 μmol/dL for isoleucine, 40.7% for even-chain SFA, 0.7% for odd-chain SFA, 0.07% for GLA, 3.4% for DGLA, and 0.3% for DTA. ‡For each combination of AA and phospholipid FA, binary groups were created for AA and FA based on median value. The category with lower values was always set as the reference group for AA to be consistent with the main analysis. Given the observed direction of the associations (i.e., lower glycine was associated with higher GDM risk and lower isoleucine was associated with lower GDM risk), the high-risk group was set as the reference group for glycine–FA combination, and the low-risk group was set as the reference group for isoleucine–FA combination. §*P* value of the interaction term between each pair of AA and phospholipid FA.

may be modulated by the profile of phospholipid FAs. An earlier study examined individual BCAAs and SFA 16:0 (palmitic acid). The highest GDM risk was observed among women with higher leucine or higher isoleucine and higher SFA 16:0; evidence of additive interactions between high BCAAs and high SFA 16:0 was suggested (14). We examined the joint association of isoleucine and SFA 16:0 with GDM and observed joint associations consistent with those noted in the earlier study. While the underlying mechanisms remain to be determined, our findings on the joint roles of AAs and FAs are biologically plausible. Mechanisms of AAs and phospholipid FAs are implicated in inflammatory lipotoxicity and the destruction of pancreatic cells (21,35). Glycine and several phospholipid FAs are involved in NMDA signaling (24,36). Both BCAAs and FAs may act on mTOR signaling (14). In this regard, it is possible that several AAs and phospholipid FAs have synergistic effects on β-cell functioning and insulin signaling.

Circulating AAs are under tight homeostatic control, yet they are affected by diet and other exogenous factors (29). The majority of circulating AAs share both endogenous and exogeneous origins, except for nine essential AAs that must come from diet (29). High-fat diets or diets rich in animal proteins, particularly red or processed meats, have been linked to altered AA metabolism (37), particularly elevated circulating BCAAs and aromatic AAs (38), suggesting potential modifiable features of AAs by diet. Meanwhile, levels of circulating phospholipid FAs also reflect and are subject to dietary intake. A joint consideration of AAs and phospholipid FAs may bring additional insights into the underlying mechanisms of GDM compared with the consideration of either of them alone. Modifiability of circulating AAs by diets and ideally in the context of other key metabolites, and whether or not such modifications would lead to reduction in GDM risk, warrants investigation in future studies.

Our study has several notable strengths. The longitudinal design with multiple biospecimen collections and detailed covariate assessment enabled us to perform a comprehensive prospective investigation of plasma AAs in early pregnancy and midpregnancy with the development of GDM. We investigated associations of AAs with GDM in the context of phospholipids FAs, which have been implicated in the development of GDM. Several potential limitations merit discussion. First, due to the logistical challenges, fasting blood samples were collected only during the follow-up visit at 15–26 GW. Random nonfasting blood samples were collected at 10–14 weeks, which may introduce more variations in plasma AA concentrations. However, our findings at 10–14 GW based on the random plasma samples may better reflect clinical settings given the practical challenge of asking pregnant women to fast. Because fasting durations prior to biospecimen collection at both visits were nondifferential to the case-control status, variations would be nondifferential. Any differences in the distributions of the fasting status at 10–14 and 15–26 GW may partially contribute to differences in the observed associations between the two visits. Second, although we matched by design or adjusted for major risk factors, the study was subject to residual or unmeasured confounding. Despite being one of the largest prospective studies that evaluates AAs with GDM risk and the matched 1:2 case-control design to improve statistical precision, this study may have been insufficiently powered to detect some significant associations due to the relatively small number of GDM case subjects. However, even with the relatively moderate sample size, strong and significant associations were identified for several AAs, which implicates a potentially important role of AAs in the development of GDM. Given the limited sample size, results of the stratified analyses by major covariates need to be examined in future studies. Studies of larger sample size are warranted to confirm our findings.

In conclusion, plasma glucogenic AAs, BCAAs, and aromatic AAs were significantly and prospectively associated with the risk of GDM. Several AAs and phospholipid FAs were significantly and jointly associated with the risk of GDM. These findings suggested potential roles of

these AAs in the development of GDM starting in early pregnancy.

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Authors Contributions. J.Y. and J.W. conducted statistical analysis and designed the figures. J.Y. drafted the manuscript. C.Z. conceptualized the study design. C.Z. obtained funding and supervised data collection for the *Eunice Kennedy Shriver* NICHD Fetal Growth Study. All authors contributed to the interpretation of the results and revision of the manuscript for important intellectual content and approved the final version of the manuscript. C.Z. and J.Y. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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