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Longitudinal plasma amino acids during pregnancy and neonatal anthropometry: findings from the NICHD Fetal Growth Studies-Singleton Cohort

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Abstract

Background Amino acids (AAs) during pregnancy are crucial for fetal growth. Prior studies measured AA concentrations at single time points in pregnancy, despite their fluctuations throughout pregnancy. We measured plasma AA profiles in blood samples longitudinally collected from early through late pregnancy and evaluated their associations with neonatal anthropometry.

Methods Concentrations of plasma aromatic AAs, branched-chain AAs, and AAs involved in one-carbon metabolism were assessed at 10–14, 15–26, 23–31, and 33–39 gestational weeks (GW) among 321 women from a case–control study from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies-Singleton Cohort. Associations between AA concentrations in tertiles at each visit and neonatal anthropometric measures were assessed using weighted generalized estimating equations models, after adjusting for major confounders.

Results Women with higher concentrations of glutamine (3rd vs. 1st tertile) at 10–14 GW had offspring with greater birthweight *z*-score (β [95% CI] = 0.31 [0.06, 0.56], *p*-trend = 0.04) and birth length (1.35 cm [0.32, 2.37], *p*-trend = 0.04). Women with higher concentrations of aspartic acid (3rd vs. 1st tertile) at 23–31 GW, however, had offspring with smaller sum of skinfolds (− 3.9 mm [− 6.0, − 1.7], *p*-trend = 0.007). Similarly, women with higher concentrations of glycine (3rd vs. 1st tertile) at 10–14 GW had offspring with lower birthweight *z*-score (− 0.37 [− 0.65, − 0.08], *p*-trend = 0.04).

Conclusions Plasma AA concentrations during pregnancy appear to play a crucial role in neonatal anthropometry. Associations were observed as early as 10 GW and varied by type of AAs and gestational age.

Trial registration Clinical Trial Registry number: NCT 00912132.

Keywords Amino acids, Pregnancy, Neonatal anthropometry, Birthweight, Birth length, Sum of skinfolds

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Background

Optimal fetal growth is crucial for the development of the offspring and subsequent health [1]. Both neonatal undergrowth and overgrowth are associated with perinatal morbidity or mortality [2, 3] as well as short- and long-term adverse health consequences, including the development of cardiovascular diseases, type 2 diabetes, obesity, and neurological impairments [4–8]. Identifying factors, especially those potentially modifiable, related to fetal growth is key to improve lifelong health [9].

During pregnancy, women's metabolism undergoes dynamic physiological adaptations that are essential for fetal growth [10–12]. Among different metabolites, amino acids (AAs), notably aromatic AAs, branched-chain AAs (BCAAs), and AAs involved in one-carbon metabolism, play crucial roles in fetal growth as they are involved in protein synthesis and cellular function activation, such as in the mTOR signaling pathway, highlighting their potentially important role for fetal growth [13, 14]. The dynamic changes in plasma AA concentrations throughout pregnancy have been described previously: the concentrations of several AAs decrease, such as glycine, isoleucine, leucine, and valine, whereas others, like methionine, increase during pregnancy [10]. Several studies have reported that fetal plasma AA concentrations are higher than maternal plasma concentrations, indicating an active transport across the placenta, from the maternal to the fetal circulation [15–18].

Most previous studies examining the associations between maternal AAs and neonatal anthropometry have assessed maternal AAs at single time points during pregnancy [19–23] and thus were unable to pin down the most relevant timing of associations between AA concentrations across pregnancy and neonatal anthropometry. Further, some findings from these studies were inconsistent [19, 20], which could be partly explained by differences in the nature of the samples and the timing of AAs assessment. To the best of our knowledge, only one study assessed plasma AAs at multiple time points during pregnancy and examined their associations with neonatal anthropometry, based on a small sample population (50 pregnant women) [24]. To address this literature gap, we prospectively and longitudinally examined associations of plasma AA concentrations, with a specific focus on aromatic AAs, BCAAs, and AAs involved in one-carbon metabolism, with neonatal anthropometric measures. We first examined associations at each study visit to identify the most relevant timing in pregnancy for associations of plasma AA concentrations with neonatal anthropometry. In exploratory analyses, we further assessed trajectories of AA concentrations through pregnancy and their associations with neonatal anthropometry.

Methods

Study population

This study was based on data from the Fetal Growth Studies-Singleton Cohort (2009–2013) at the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Two thousand eight hundred two women with singleton pregnancies at 10–14 gestational weeks (GW), aged between 18 and 40 years, and without major pre-existing chronic diseases were enrolled at 12 clinical centers in the USA. Details of recruitment and study protocol can be found elsewhere [25, 26]. This study is registered in the Clinical Trial Registry (NCT 00912132). All clinical sites and the NICHD obtained approval from their respective institutional review boards. All participants provided written consent before data collection.

A nested case–control study was derived from the total cohort population to assess the associations between clinical biomarkers in early to mid-pregnancy and the subsequent risk of gestational diabetes mellitus (GDM). The current study is a planned ancillary analysis of this GDM nested case–control study, aiming to examine the associations between biomarkers and fetal growth, assessed by neonatal anthropometric measures. In the GDM case–control study, each GDM case ($n = 107$) was individually matched at a 1:2 ratio to non-GDM controls ($n = 214$) by age (± 2 years), race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, and Asian/Pacific Islander), and gestational age at blood collection (± 2 weeks). The flow chart of the study is presented in Additional file 1: Fig. S1.

Plasma biomarkers assessment

Blood samples were collected longitudinally before the delivery date, following a standardized protocol at 10–14 GW (enrollment), 15–26 GW, 23–31 GW, and 33–39 GW [26]. At 15–26 GW, women fasted for 8–14 h overnight before the blood collection. Random blood samples were collected at the other visits. At both 10–14 and 15–26 GW, blood samples were collected for all the participants of the nested case–control study. At 23–31 and 33–39 GW, blood samples were collected for all the women with GDM but only for one of the two non-GDM controls, who were randomly selected (i.e., $n = 107$ GDM cases and $n = 107$ non-GDM controls). All the blood samples were processed immediately after collection and blood components (plasma, RBCs, and buffy coat) were stored at − 80 °C until analysis. All assays were performed without knowledge of the case–control status.

Plasma concentrations (μmol/dL) of BCAAs (i.e., isoleucine, leucine, and valine), aromatic AAs (i.e., phenylalanine and tyrosine), AAs involved in one-carbon metabolism (i.e., methionine, serine, and glycine),

and other AAs (i.e., arginine, aspartic acid, glutamine, hydroxyproline, and taurine) were quantified using an Amino Acid analyzer (Hitachi L-8900). As described previously, samples were deproteinized, acidified, and injected into a high-performance liquid chromatography system [10]. Each AA was quantified relative to standards of known concentrations. The inter-assay coefficients of variation for assessed AAs were all less than 5.0%. The grouped BCAAs (i.e., the sum of isoleucine, leucine, and valine), the total aromatic AAs (i.e., the sum of phenylalanine and tyrosine), and the total of AAs involved in one-carbon metabolism (i.e., the sum of methionine, serine, and glycine) were also calculated.

Several other maternal biomarkers that are crucial for fetal growth [27, 28] have been quantified in the Fetal Growth Studies. For instance, plasma concentrations of glucose and individual fatty acids, including polyunsaturated fatty acids (PUFAs), were also assessed at the same four study visits. As described previously [29], glucose concentration was measured by enzymatic assays with the COBAS 6000 Chemistry Analyzer and PUFAs were measured using a Hewlett Packard 5890 gas chromatography system. Similar to a previous analysis [27], we calculated the sum of *n*-3 PUFAs, which included the following: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), *n*-3 docosapentaenoic acid (*n*-3 DPA), and docosahexaenoic acid (DHA). In addition, insulin resistance at 15–26 GW was assessed by calculating the homeostatic model assessment for insulin resistance (HOMA-IR): fasting plasma insulin [mU/L] ×fasting plasma glucose [mg/dL])/405 [30, 31].

Neonatal anthropometric measures

Neonatal anthropometric measurements were measured by trained and certified study personnel. Birthweight and gestational age at birth were extracted from medical records, as described previously [32]. Neonatal birth length (i.e., the distance from the infant's feet to the top of the head) was measured in centimeters using an infantometer (SECA 416 Infantometer). Skinfold measurements in millimeters were taken using a Lange skinfold caliper (Beta Technology, Inc., Santa Cruz, CA) on the right side of the body at the abdominal flank, anterior thigh, subscapular, and triceps. The skinfold measurements were summed up as an indicator of total neonatal adiposity [33]. All measurements were obtained before discharge, 12–24 h after delivery. When it was impossible to obtain measurements at birth because of neonatal intensive care unit admission or neonatal complications, infants born very preterm (≤ 32 weeks) were measured at 32 completed weeks of gestation-corrected age, and infants born moderately preterm (33–36 weeks) were measured once stabilized. Measurements were obtained in duplicates; a third measurement was taken if the difference between the first two measurements exceeded prespecified tolerances based on expected technical errors of measurement [34–36], and the two closest measurements were averaged.

Gestational age- and sex-specific birthweight *z*-scores were derived using a United States national reference [37]. Birthweight, birth length, and the sum of skinfolds were analyzed in crude measurements. Birthweight was further used to derive the following dichotomous neonatal anthropometry measures: low birthweight (LBW, defined as a birthweight of less than 2500 g), macrosomia (birthweight above 4000 g), small for gestational age (SGA, birthweight below the 10th percentile for gestational age), and large for gestational age (LGA, birthweight above the 90th percentile for gestational age). As described previously [38], SGA and LGA were calculated using the data of the main cohort, based on US sex-specific birthweight references [39].

Covariates

Covariates were selected a priori, based on previous literature on their associations with both exposures and outcomes. Information on maternal age, education level, parity, and infant sex was collected using self-reported questionnaires and medical records. The gestational age of participants was confirmed with a screening ultrasonography examination performed between 10 and 13 GW. Women were included in the study if their last menstrual period data and ultrasound date matched within 5 days for gestation estimates between 8^{+0} and 10^{+6} weeks, 6 days for those between 11^{+0} and 12^{+6} weeks, and 7 days for estimates between 13^{+0} and 13^{+6} weeks [26]. Pre-pregnancy body weight was self-reported by participants, and height was directly measured by trained staff at the enrolment visit, corresponding to visit 0. Pre-pregnancy BMI was then calculated by dividing the self-reported weight (kg) by the square of height (m^2). Self-reported weight was highly correlated with staff-measured weight in the study (correlation coefficient $r = 0.97$) [40]. As only 5 women reported smoking 6 months before the index pregnancy, smoking status was not included as a covariate in the current analysis [27].

Gestational weight gain was not included as a covariate, as it may lie in the causal pathway between plasma AA concentrations and neonatal anthropometry. Additionally, dietary intake was not considered a potential confounder, as our objective was to investigate the associations between overall plasma AA concentrations and neonatal anthropometry. Adjusting for dietary intake would have restricted the interpretation of beta coefficients of AAs to AAs from non-dietary sources.

Statistical methods

As women with GDM were overrepresented in this nested GDM case–control study, the sample was reweighted to represent the full cohort (e.g., in the reweighted sample, 4% of women had GDM as opposed to 33% in the non-weighted sample). Sampling weights were calculated for each included woman by the inverse of her sampling probability, following the idea of pseudolikelihood in (Samuelsen 1997) [41]. The sampling probability of each non-GDM control was calculated from a logistic regression in the full cohort, excluding GDM cases. Predictors included matching factors for selecting controls: age, race/ethnicity, and GW at blood collection. GDM cases had a sampling probability of 1, as described in our previous publications [27]. Using sampling weights allowed us to assess the associations of AAs with neonatal anthropometric measures, irrespective of a woman's GDM status, as GDM is potentially on the pathway between maternal AAs and neonatal anthropometry.

Descriptive statistics were used to summarize the weighted characteristics of the women at enrollment (i.e., 10–14 GW) and their infants at birth. The weighted medians of individual AA concentrations were calculated at each study visit. Pearson correlation was used to calculate correlation coefficients among AAs at each study visit and across the four visits.

Primary analyses

Adjusted weighted generalized estimating equation models, with weights calculated using inverse sampling probability and the clustering variable corresponding to the matching variable between GDM cases and non-GDM controls, were used to estimate the associations between individual AAs at each study visit and each continuous neonatal anthropometric measure (i.e., birthweight, birthweight z-score, birth length, and sum of skinfolds). Each AA was categorized into tertiles. The lowest tertile was used as the reference group. Tertile-specific medians were calculated and entered into the models as continuous variables to estimate the *p*-value for trend. All models were adjusted for maternal age (continuous), education level (high school degree or less, some college/associate degree, or 4-year college degree or higher), nulliparity (yes or no), pre-pregnancy BMI (continuous), gestational age at blood collection (continuous), and infant sex. Models of birth length and sum of skinfolds were further adjusted for the number of days post-delivery when the measurements were made. Moreover, we examined the associations between plasma AAs (considered as continuous variables, due to the relatively small sample size) and dichotomous neonatal anthropometric measures: LBW, macrosomia, SGA, and LGA. These associations were assessed using weighted generalized equation models with a log link function, accounting for the nested case–control study design, adjusted for the same covariates presented above. One model was performed by individual maternal AA at each study visit and for each neonatal anthropometric measure. We adjusted the level of statistical significance using the Benjamini–Hochberg false discovery rate (FDR) controlling method to account for the multiplicity of the tests [42]. FDR correction was applied to the *p*-values obtained for the tertiles of the 13 individual amino acids. In the context of the lack of previous studies, we selected a cut-off value of 0.25 to balance the control of the false discoveries with the identification of potentially significant associations, as described in previous epidemiological studies [43, 44].

Exploratory analysis: amino acid trajectories across pregnancy and neonatal anthropometry (n = 214)

When a significant association was found between individual AAs and continuous neonatal anthropometric measures for at least one study visit, we studied the AA trajectory across pregnancy and its potential association with neonatal anthropometry. To study how individual AAs changed across pregnancy, we performed a weighted latent class analysis (i.e., a flexible data-driven semiparametric approach, PROC TRAJ procedure, SAS software) to identify the trajectories of individual maternal AAs in pregnancy [45]. Gestational age in days, rather than the study visit number, was used as the time variable to account for less-biased time-specific variability. To choose the most suitable trajectory groups for each AA, we used the following decision criteria. A complex model (i.e., a higher number of trajectory groups—model B) was selected over a simpler model (A) only in case of a higher Bayesian Information Criteria (BIC), defined as follows: 2*(BIC model B − BIC model A) > 10. Then to identify the shape of the AA trajectory groups across pregnancy, we used the Average Posterior Probability (should be ≥ 0.7), the difference between the observed and the estimated prevalence (should be the closest to 0), and the Odds of Correct Classification (should be > 5) (Additional file 1: Table S1). Results of bivariate analyses comparing maternal characteristics among AA trajectory groups are reported in Additional file 1: Tables S2 and S3. This analysis was performed only among individuals with AA measured at all four study visits ($n = 214$; i.e., 107 non-GDM controls and 107 GDM cases). We chose the final trajectories that included at least 10% of participants in each group. Associations between trajectory groups of each maternal AA and each continuous neonatal anthropometric measure were then assessed using weighted generalized estimating equation models accounting for the nested case–control study design and adjusted for maternal age, education level, nulliparity, pre-pregnancy

BMI, and infant sex. As above, models of birth length and sum of skinfolds were further adjusted for the number of days post-delivery when measurements were made.

Sensitivity analyses

Multiple sensitivity analyses were conducted to assess the robustness of our results. First, as previous literature highlighted significant associations of both plasma glucose and *n*-3 PUFA concentrations with fetal growth or neonatal anthropometric measures [27, 28], we tested whether the associations between AAs and neonatal anthropometric measures persisted after further adjustment for glucose and the sum of *n*-3 PUFAs (continuous variables, assessed at the same corresponding study visits as AAs). One model was performed for each further adjustment and another model was assessed by further adjusting for both glucose and the sum of *n*-3 PUFA concentrations. Second, we excluded women with preterm delivery (< 37 gestational weeks, $n = 22$), to ensure that the results were not driven by preterm birth. Third, as previous studies showed that several AAs are involved in insulin resistance [46], we assessed whether the associations between AAs at 15–26 GW and neonatal anthropometry were still significant after further adjustment for homeostatic model assessment for insulin resistance (HOMA-IR: continuous variable, assessed at 15–26 GW). One model was performed by individual maternal AA at each study visit and for each neonatal anthropometric measure. All analyses were performed using SAS software (version 9.4; SAS Institute Inc.), with a 2-sided *p*-value of < 0.05 as the significance level.

Results

Description of the population

Characteristics of the study population are presented in Table 1. Additional file 1: Table S4 presents the weighted medians (IQR) of the plasma AA concentrations at the four study visits. For the majority of individual AAs, concentrations at each study visit were positively correlated with concentrations of the same AAs at the prior visit: the highest correlations were observed for glycine ($r > 0.70$).

Table 1 Characteristics of the study sample in the nested case–control study ($n = 321$)

Characteristics	*N* (%) or median (IQR)[a]
Maternal characteristics	
Age, years	27.6 (23.2, 31.9)
Race/ethnicity	
Non-Hispanic White	75 (30.9)
Non-Hispanic Black	45 (23.3)
Hispanic	123 (27.2)
Asian and Pacific Islander	78 (18.5)
Education	
High school or less	81 (25.1)
Some college/associate degree	117 (35.2)
4-year college degree or higher	123 (39.8)
Nulliparous	144 (51.1)
Cigarette smoking[b]	5 (0.7)
Pre-pregnancy BMI, kg/m^2 [c]	24.6 (22.0, 27.4)
Normal weight: 19.0–24.9	156 (51.7)
Overweight: 25.0–29.9	99 (33.1)
Obese: ≥ 30	66 (15.2)
Gestational diabetes	107 (3.9)
Infant characteristics	
Male	166 (52.0)
Birthweight, grams	3400 (3100, 3700)
Birthweight, *z*-score	− 0.1 (− 0.7, 0.5)
Low birthweight	17 (7.3)
Macrosomia	30 (6.0)
Small for gestational age	13 (4.7)
Large for gestational age	34 (7.1)
Birth length, cm	50.6 (48.4, 52.0)
Sum of skinfolds, mm	18.5 (16.0, 23.0)

[a] Continuous and categorical variables were summarized using weighted median (IQR) or numbers (weighted percentage)

[b] Smoking women without obesity before pregnancy were ineligible for the study

[c] No participant was underweight

Associations between amino acids at each visit and neonatal anthropometry

After adjustment for major confounders, most individual maternal AAs (11/13 AAs, 84.6%) and AA groups (2/3 AA groups, 66.7%) were significantly associated with birthweight *z*-score, birthweight, birth length, or sum of skinfolds for at least one of the four study visits (Figs. 1, 2, and 3, Additional file 1: Fig. S2, and Table S5). Similarly, most individual AAs (8/13 AAs, 61.5%) and AA groups (2/3 AAs groups, 66.7%) were significantly associated with LBW, macrosomia, SGA, or LGA for at least one of the four study visits. The associations between each maternal AA at each study visit and neonatal anthropometry varied by AA type, neonatal anthropometric measure, and study visit.

Aromatic amino acids

Plasma aromatic AAs at different study visits were inversely associated with birthweight and birth length. Higher maternal phenylalanine and tyrosine concentrations at 15–26 GW were associated with a smaller birth length (Fig. 2 and Additional file 1: Table S5). Coherently, higher maternal phenylalanine concentrations at 23–31 GW and 33–39 GW were respectively associated with a

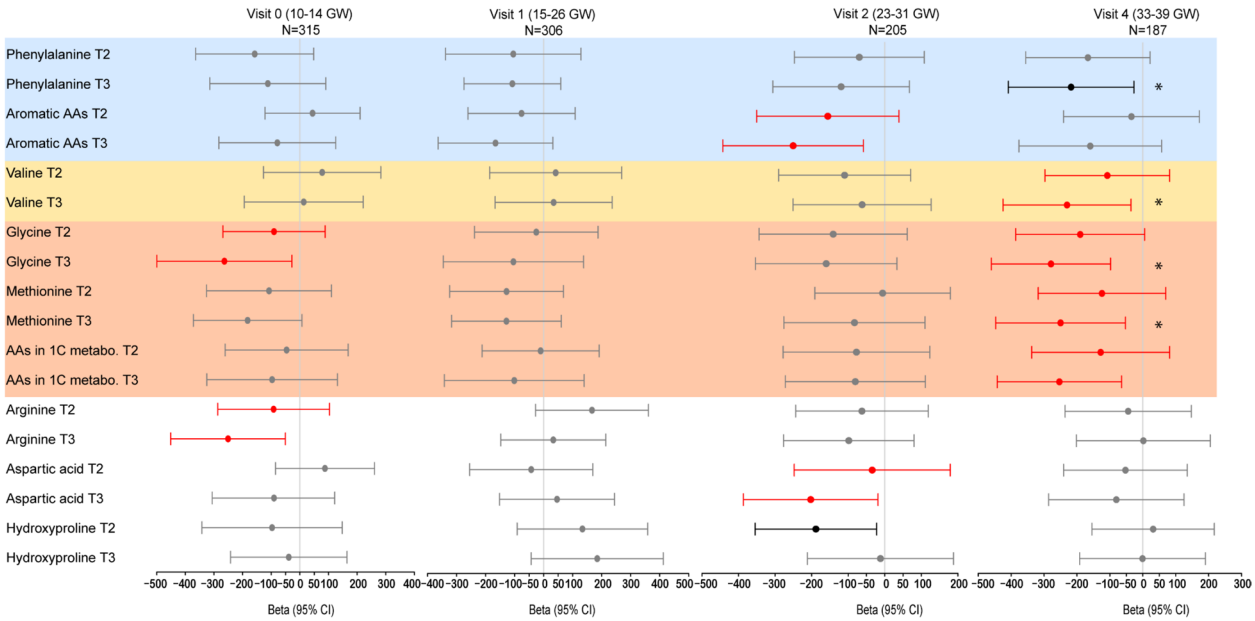

Fig. 1 Adjusted beta coefficients (95% CI) for the associations between individual amino acids by tertiles at each study visit and birthweight (in grams). Abbreviations: AAs: amino acids, AAs in 1C metabo.: AAs involved in one-carbon metabolism, T: tertile. Footnote: Associations between each amino acid at each visit and birthweight (in grams) were assessed using weighted generalized estimating equations models, adjusted for maternal age (continuous), education level (high school degree or less, some college/associate degree, or 4-year college degree or higher), nulliparity (yes, no), pre-pregnancy BMI (continuous), gestational age at blood collection (continuous), and infant sex. The lowest tertile (i.e., T1) was considered as the reference group. Tertile-specific medians were calculated and entered into the models as continuous variables to estimate the *p*-value for trend: *p*-trends<0.05 are presented in red. *P*-values <0.05 are presented in black and non-significant *p*-values are in grey. Aromatic amino acids are highlighted in blue. Branched-chain amino acids are highlighted in yellow and amino acids involved in one-carbon metabolism are in orange. FDR correction was applied to the *p*-values obtained for the tertiles of the 13 individual plasma AAs. The *p*-values of AA tertiles were ranked in ascending order. Critical *p*-value was calculated for individual AA tertiles using the equation: Critical *p*-value= (rank/total number of tests) * FDR rate, with a FDR rate of 0.25. The observed *p*-values were compared to their respective critical *p*-values. Tertiles of AAs with *p*-values below the corresponding critical *p*-value were considered statistically significant after FDR adjustment, and are marked with *

higher risk of SGA (RR [95% CI] = 1.92 [1.22, 3.04]) and a lower risk of LGA (RR [95% CI] = 0.51 [0.28, 0.93]).

Branched-chain amino acids

Globally, maternal BCAAs were not significantly associated with neonatal anthropometric measures. However, some inverse associations were observed. Women with a higher isoleucine concentration at 15–26 GW or valine concentration at 33–39 GW had offspring with a smaller birth length and lower birthweight, respectively (Figs. 1 and 2, and Additional file 1: Table S5).

Amino acids involved in one-carbon metabolism

Maternal AAs involved in one-carbon metabolism were inversely associated with neonatal anthropometry measures. Women with higher glycine concentrations in early and late pregnancy had offspring with a lower birthweight (Fig. 1 and Additional file 1: Table S5). Moreover, higher glycine concentrations at 23–31 GW were associated with a smaller sum of skinfolds (Fig. 3 and Additional file 1: Table S5). Women with higher glycine concentration at 33–39 GW also had offspring with smaller birth length (Fig. 2 and Additional file 1: Table S5). Higher methionine concentration at 33–39 GW was associated with lower birthweight (Fig. 1, Additional file 1: Table S5).

Other amino acids

Women with a higher aspartic acid concentration at 23–31 GW had offspring with smaller sum of skinfolds (Fig. 3 and Additional file 1: Table S5), and higher risks of LBW (RR [95% CI] = 7.84 [2.40, 25.60]) and SGA (7.77 [1.35, 44.9]). Higher arginine concentration at 10–14 GW was associated with a lower birthweight (Fig. 1, Additional file 1: Table S5). Higher maternal glutamine concentration at 10–14 GW was associated with a lower risk of SGA (RR [95% CI] = 0.93 [0.88, 0.98]) (Fig. 2, Additional file 1: Table S5 and Additional file 1: Fig. S2). Women with higher hydroxyproline concentration at 15–26 GW also had offspring with higher risk of

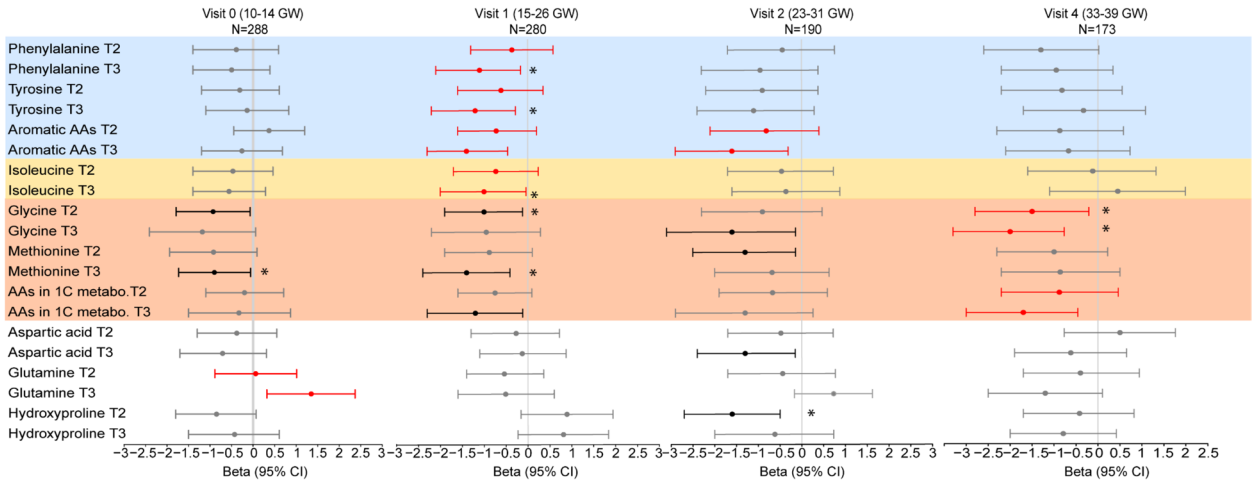

Fig. 2 Adjusted beta coefficients (95% CI) for the associations between individual amino acids by tertiles at each study visit and birth length (in cm). Abbreviations: AAs: amino acids, 1C metabo.: one-carbon metabolism, T: tertile. Footnote: Associations between each amino acid at each visit and birth length (in cm) were assessed using weighted generalized estimating equations models, adjusted for maternal age (continuous), education level (high school degree or less, some college/associate degree, or 4-year college degree or higher), nulliparity (yes, no), pre-pregnancy BMI (continuous), gestational age at blood collection (continuous), number of days post-delivery when the measurements were made (continuous), and infant sex. The lowest tertile (i.e., T1) was considered as the reference group. Tertile-specific medians were calculated and entered into the models as continuous variables to estimate the *p*-value for trend: *p*-trends <0.05 are presented in red. *P*-values <0.05 are presented in black and non-significant *p*-values are in grey. Aromatic amino acids are highlighted in blue. Branched-chain amino acids are highlighted in yellow and amino acids involved in one-carbon metabolism are in orange. FDR correction was applied to the *p*-values obtained for the tertiles of the 13 individual plasma AAs. The *p*-values of AA tertiles were ranked in ascending order. Critical *p*-value was calculated for individual AA tertiles using the equation: Critical *p*-value= (rank/total number of tests) * FDR rate, with a FDR rate of 0.25. The observed *p*-values were compared to their respective critical *p*-values. Tertiles of AAs with *p*-values below the corresponding critical *p*-value were considered statistically significant after FDR adjustment, and are marked with *

macrosomia (RR [95% CI] =1.77 [1.02, 3.08]), and lower risk of SGA (RR [95% CI] =0.23 (0.07, 0.77).

Exploratory analysis: amino acid trajectories across pregnancy and associations with neonatal anthropometry

We identified one trajectory for glutamine, hydroxyproline, phenylalanine, and isoleucine; 2 distinct trajectories for total aromatic AAs, tyrosine, glycine, serine, arginine, and aspartic acid; and 3 distinct trajectories for methionine, taurine, valine, and for total AAs involved in one-carbon metabolism (Additional file 1: Fig. S3). For each AA, we defined the reference trajectory (referred to as "Group 1") as the most stable trajectory or the lowest concentration trajectory across pregnancy. When relevant, "Group 2" and "Group 3" referred to trajectories of higher or less stable concentrations of individual or grouped AAs across pregnancy.

Women in the trajectories of less stable or higher concentrations of tyrosine or total aromatic AAs (including tyrosine) throughout pregnancy had offspring with lower birthweight, birthweight *z*-score, and smaller sum of skinfolds (for tyrosine only) (Additional file 1: Table S6). Women with a trajectory of higher glycine concentration across pregnancy had offspring with lower birthweight, birthweight *z*-score, and smaller birth length. Similarly, women presenting higher or less stable concentrations of the total AAs involved in one-carbon metabolism throughout pregnancy had offspring with lower birthweight and birthweight *z*-score, a smaller length, and a smaller sum of skinfolds.

Sensitivity analyses

Similar results were found after further adjustment for glucose, sum of *n*-3 PUFAs, or for both glucose and the sum of *n*-3 PUFAs at the different visits. The same results were also observed after excluding women with preterm delivery and after further adjustment for HOMA-IR (data not shown).

Discussion

In this prospective study, we assessed plasma aromatic AAs, BCAAs, and AAs involved in one-carbon metabolism longitudinally during pregnancy and found that several AA profiles were associated with neonatal anthropometry. The magnitude of associations varied by different trimesters of pregnancy.

Previous studies on the associations of maternal AAs with neonatal anthropometry have yielded inconsistent results [19, 20], and most of the studies assessed AAs

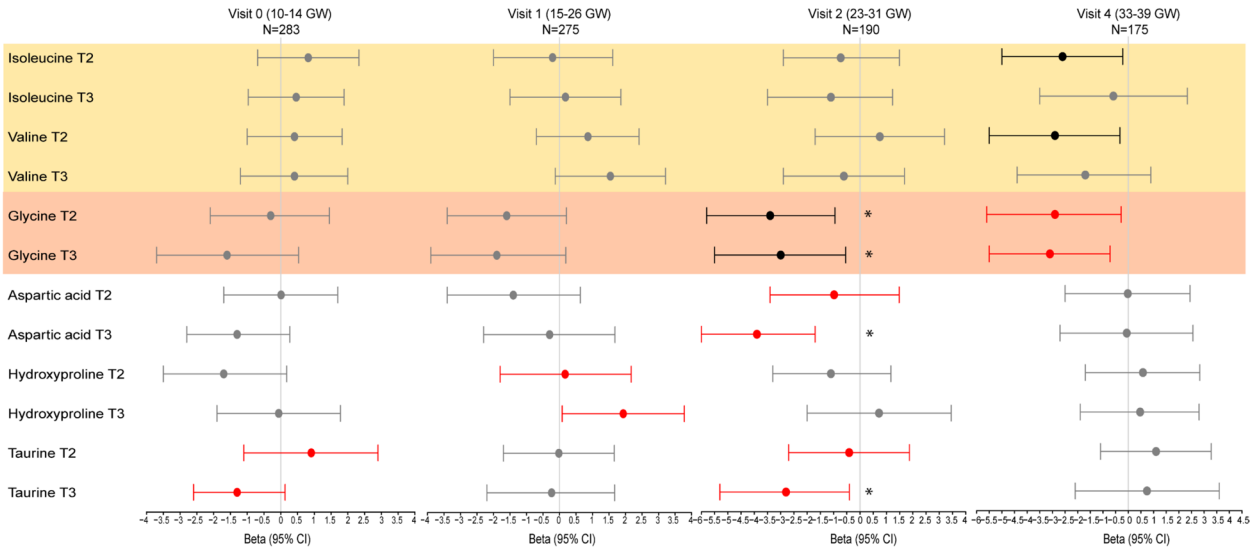

Fig. 3 Adjusted beta coefficients (95% CI) for the associations between individual amino acids by tertiles at each study visit and sum of skinfolds (in mm). Abbreviations: T: tertile. Footnote: Associations between each amino acid at each visit and sum of skinfolds (in mm) were assessed using weighted generalized estimating equations models, adjusted for maternal age (continuous), education level (high school degree or less, some college/associate degree, or 4-year college degree or higher), nulliparity (yes, no), pre-pregnancy BMI (continuous), gestational age at blood collection (continuous), number of days post-delivery when the measurements were made (continuous), and infant sex. The lowest tertile (i.e., T1) was considered as the reference group. Tertile-specific medians were calculated and entered into the models as continuous variables to estimate the *p*-value for trend: *p*-trend <0.05 are presented in red. *P*-values <0.05 are presented in black and non-significant *p*-values are in grey. Branched-chain amino acids are highlighted in yellow and amino acids involved in one-carbon metabolism are in orange. FDR correction was applied to the *p*-values obtained for the tertiles of the 13 individual plasma AAs. The *p*-values of AA tertiles were ranked in ascending order. Critical *p*-value was calculated for individual AA tertiles using the equation: Critical *p*-value = (rank/total number of tests) × FDR rate, with a FDR rate of 0.25. The observed *p*-values were compared to their respective critical *p*-values. Tertiles of AAs with *p*-values below the corresponding critical *p*-value were considered statistically significant after FDR adjustment, and are marked with *

using one point-in-time measurement [19–23]. To the best of our knowledge, this is the first study to assess the associations between longitudinal plasma AAs across pregnancy (i.e., at four different time points) and neonatal anthropometry. The current study is also one of the first to further account for other primary metabolites previously shown to be involved in fetal growth, such as maternal glucose and total *n*-3 PUFAs.

Our findings confirmed previous studies that observed mothers with a higher glutamine concentration in early pregnancy had offspring with larger neonatal anthropometry [47, 48]. These findings align with a previous study that concluded that placental intake of glutamine decreased in case of fetal growth restriction and identified that the mammalian target of rapamycin (mTOR) signaling pathway (i.e., a crucial nutrient sensor that supports placental growth and is notably involved in embryonic development [49]) was an involved mechanism [50].

Our results on plasma aromatic AAs align with a recent study showing that higher plasma phenylalanine at 24–28 GW was associated with lower birthweight [47] and with previous literature indicating that high maternal phenylalanine concentrations and maternal phenylketonuria are associated with lower birthweight and higher risk of SGA [51, 52].

Our results on maternal concentrations of AAs involved in one-carbon metabolism are consistent with a previous study highlighting inverse associations between plasma glycine and methionine concentrations at 26–28 GW and the sum of skinfolds [21]. Moreover, our results align with previous studies showing an inverse association between maternal homocysteine concentration (i.e., a product of methionine) and neonatal anthropometry [53]. Nevertheless, results from past studies are rather varied. A previous study reported no significant association between maternal glycine or methionine concentrations and birthweight [47], whereas others showed positive associations [19, 22, 23, 54].

Overall, we did not find substantial evidence supporting associations between maternal BCAAs across pregnancy and neonatal anthropometry. Results of previous studies on the associations between maternal BCAAs and neonatal anthropometry are sparse and inconsistent, suggesting both inverse [21, 47, 55] or positive [19] associations. Future longitudinal studies of larger sample sizes are therefore required.

In the current study, we used a latent-class (data-driven) approach to identify the specific groups of how maternal AAs change across pregnancy and their association with neonatal anthropometry. The AA trajectories reported in the current manuscript were globally consistent with those reported previously [10]. Blood samples collected at 15–26 GW are fasting samples, which could partly explain the decrease in AA concentrations during this time window, especially for essential AAs, whose major sources come from diet. In general, the significant results of the associations between AA trajectory groups and neonatal anthropometry were consistent with our main analyses.

The majority of previous studies lacked longitudinal AA measurements across pregnancy to assess the relevance of timing in the associations between maternal AA concentrations and neonatal anthropometry. The current study tried to address this literature gap by considering AAs at four time-points during pregnancy and by accounting for the dynamic changes of AA concentrations across pregnancy (using trajectories) to assess their associations with neonatal anthropometry. The inconsistent findings of the literature may be due to different factors that could be related to the assessment of AAs, such as differences in sample size, nature of the sample, timing of sample collection, AAs assessment approach analysis, or the study population, such as socio-demographic and medical characteristics. While our findings warranted further replication in other populations, they underscore the potential importance of plasma AAs at different trimesters of pregnancy as biomarkers for fetal growth. These AAs could potentially help identify pregnancies at-risk and guide timely interventions.

This study had several strengths. First, the prospective and longitudinal design of this study allowed us to examine the temporal associations between plasma AAs at four time-points during pregnancy and neonatal anthropometry and to highlight the relevance of timing in this association. Second, our analyses were able to account for blood glucose and omega-3 fatty acids, which play crucial roles in fetal growth and neonatal anthropometry. Even though these analyses may be over-adjusted, the current results at least highlight the importance of maternal AA concentrations for neonatal anthropometry. Third, the objective measurements of AAs in pregnancy using plasma samples limited the bias due to self-reported data assessment. Moreover, considering binary neonatal anthropometry measures in addition to continuous measures allowed us to explore several indicators of suboptimal fetal growth that may be more clinically relevant. Future studies of greater sample sizes are warranted to confirm our results. Women included in this nested case–control study had diverse ethnicities, which improved the generalization of our results.

Nevertheless, our study also presented some potential limitations. Even though this analysis is one of the largest prospective studies assessing the longitudinal associations between plasma AAs and neonatal anthropometry, the relatively small sample size may have led to false negative findings. The use of an FDR threshold of 0.25 may increase the risk of false positive results. However, given the existing literature gap on the longitudinal associations between plasma AA concentrations and neonatal anthropometry, this threshold was chosen to maximize the sensibility of detecting potential significant associations by balancing between Type I errors and statistical power, while avoiding an undue increase in false positives. While external validation would be ideal, the availability of appropriate longitudinal datasets remains a challenge. The current findings should, therefore, be considered as promising hypotheses for future studies, including those aiming at external validation. Weighted analyses were applied to address the overrepresentation of GDM cases in our study sample and it may be linked to certain limitations, such as larger variances. Nonetheless, similar trends and directions were observed in the analyses performed on non-GDM controls only. By design, women included in this study were at low risk. Therefore, the small number of women who reported smoking 6 months before pregnancy ($n = 5$) did not allow us to account for smoking status in the current analyses, where it could be a potential confounder. Moreover, we cannot exclude the possibility of other unmeasured confounders that may have an impact on the current results. Further longitudinal studies of greater sample sizes or other populations are warranted to confirm our results.

Conclusions

In conclusion, we observed that plasma concentrations of several amino acids during pregnancy, such as aromatic amino acids and glycine, are inversely associated with neonatal anthropometry, whereas women with higher glutamine concentration in early pregnancy had offspring with a higher birthweight *z*-score and birth length. Future studies with a larger sample size and on other populations would be warranted to confirm our findings. Further, the fact that our findings from multiple time points during the pregnancy were able to confirm previous findings in the literature (from a single point) shows that plasma amino acid concentrations in pregnancy may play a role as additional biomarkers for the monitoring of fetal growth.

Abbreviations

AA	Amino acid
BCAAs	Branched-chain amino acids
FGR	Fetal growth restriction
GDM	Gestational diabetes mellitus
GW	Gestational week
HOMA-IR	Homeostatic model assessment for insulin resistance
LBW	Low birthweight
LGA	Large for gestational age
NICHD	National Institute of Child Health and Human Development
PUFA	Polyunsaturated fatty acid
SGA	Small for gestational age

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04146-3.

Additional file 1: Fig. S1-S3 and Table S1-S6. Fig. S1. Study flow chart. Fig. S2. Adjusted beta coefficients (95% CI) for the associations between individual amino acids at each study visit and birthweight z-score. Fig. S3. Maternal amino acids trajectory groups throughout pregnancy. Table S1. Model criteria for longitudinal AA trajectory groups throughout pregnancy. Table S2. Non-adjusted associations between maternal characteristics and amino acid trajectory group (n=211, chi-square test). Table S3. Non-adjusted associations between maternal characteristics and amino acid trajectory group (n=211, t-test). Table S4. Weighted median (IQR) of individual and grouped plasma amino acid concentrations (μmol/L) by study visit. Table S5. Exact estimations [β (95% CI)] of the associations of individual amino acids at each study visit and neonatal anthropometric measures. Table S6. Exact estimations [β (95% CI)] for the associations between trajectory groups of amino acids and neonatal anthropometric measures.

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Authors' contributions
CG: Conceptualization, methodology, software, formal analysis, writing - original draft, writing - review & editing. JW: Methodology, formal analysis, software, writing - review & editing. RL: Methodology, software, writing - review & editing. JG: Data curation, writing - review & editing. GY: Methodology, writing - review & editing. LJL: Methodology, writing - review & editing. JY: Methodology, writing - review & editing. WWP: Methodology, writing - review & editing. DDW: Methodology, writing - review & editing. NLW: Investigation, writing - review & editing. ZC: Data curation, methodology, writing - review & editing. MYT: Investigation, methodology, writing - review & editing. CZ: Funding, conceptualization, methodology, investigation, data curation, writing - reviewing & editing, study supervision. CG and CZ have full access to the raw data. All authors read and approved the final manuscript.

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Data availability
The data are not publicly available because they contain information that could compromise research participants' privacy/consent. However, the data that support the findings of this study and the statistical code used for all the models will be made available from the corresponding author upon reasonable request with a signed data access agreement.

Declarations

Ethics approval and consent to participate
All clinical sites and the NICHD obtained approval from their respective institutional review boards. All participants provided written consent before data collection. The IRB approval number is NICHD (09-CH-N152).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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