

BASIC SCIENCE ARTICLE


Associations of maternal plasma and umbilical cord plasma metabolomics profiles with birth anthropometric measures

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BACKGROUND: The metabolomics profiles of maternal plasma during pregnancy and cord plasma at birth might influence fetal growth and birth anthropometry. The objective was to examine how maternal plasma and umbilical cord plasma metabolites are associated with newborn anthropometric measures, a known predictor of future health outcomes.

METHODS: Pregnant women between 24 and 28 weeks of gestation were recruited as part of a prospective cohort study. Blood samples from 413 women at enrollment and 787 infant cord blood samples were analyzed using the Biocrates AbsoluteIDQ® p180 kit. Multivariable linear regression models were used to examine associations of cord and maternal metabolites with infant anthropometry at birth.

RESULTS: In cord blood samples from this rural cohort from New Hampshire of largely white residents, 13 metabolites showed negative associations, and 10 metabolites showed positive associations with birth weight Z-score. Acylcarnitine C5 showed negative association, and 4 lysophosphatidylcholines showed positive associations with birth length Z-score. Maternal blood metabolites did not significantly correlate with birth weight and length Z-scores.

CONCLUSIONS: Consistent findings were observed for several acylcarnitines that play a role in utilization of energy sources, and a lysophosphatidylcholine that is part of oxidative stress and inflammatory response pathways in cord plasma samples.

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IMPACT:

- The metabolomics profiles of maternal plasma during pregnancy and cord plasma at birth may influence fetal growth and birth anthropometry.
- This study examines the independent effects of maternal gestational and infant cord blood metabolomes across different classes of metabolites on birth anthropometry.
- Acylcarnitine species were negatively associated and glycerophospholipids species were positively associated with weight and length Z-scores at birth in the cord plasma samples, but not in the maternal plasma samples.
- This study identifies lipid metabolites in infants that possibly may affect early growth.

INTRODUCTION
Background

Anthropometric measures at birth such as birth weight and length are associated with an increased risk of childhood obesity^{1–4} as well as cardiovascular disease and diabetes later in life.^{5–8} Studies have also reported birth anthropometry to be associated with risks of childhood cancer and leukemia,⁹ breast cancer among women,^{10–14} and colorectal cancer.¹⁵

During pregnancy, adaptive metabolic processes occur over the course of gestation to foster fetal growth and development.^{16,17} The early life environment and metabolism may have long-term effects on disease risks later in life.^{18–20} Metabolomic profiling is a powerful tool to capture and identify low molecular weight

metabolites present in the metabolome of a cell, tissue, organ, or organism.^{21,22} This approach is used to assess maternal metabolomics profiles across gestation and newborn outcomes^{23–29} as well as neonatal adiposity.^{30,31} These studies have identified different classes of metabolites associated with gestational age, birth weight, macrosomia, low birth weight, sum of skinfolds, and body fat percentage. Fewer studies have investigated the association between the cord blood metabolome and newborn anthropometrics. There have been controversial findings regarding certain classes of metabolites including but not limited to acyl/acetylcarnitines, phospholipids, nonesterified fatty acids, and amino acids (AA) associated with low birth weight, small-for-gestational age (SGA), macrosomia, birth weight, and body fat

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percentage.^{32–38} It is also known that maternal factors including pre-pregnancy body mass index (BMI), pregnancy weight gain, age, multiparity, diabetes, and infant sex, and diet can affect birth size outcomes.^{31,39–41} However, the independent effects of both maternal gestational and infant cord blood metabolomes on anthropometry measures at birth including birth weight and length after consideration of these factors have been less studied. Identifying metabolic markers from mothers during pregnancy and from the cord blood of infants at birth that are associated with birth weight, length and weight-for-length Z-score of infants at birth could allow prediction of pregnancy outcomes and lead to the development of interventions leading to improved infant and child health.

Therefore we sought to clarify (1) how metabolites measured in both maternal plasma samples collected during pregnancy and umbilical cord plasma samples collected at birth relate to infant anthropometry at birth and (2) potential effect modification of any observed associations by child sex and maternal pre-pregnancy weight status.

METHODS

Study participants

The New Hampshire Birth Cohort Study (NHBCS) is a prospective cohort study that recruited pregnant women between approximately 24 and 28 weeks of gestation through prenatal clinics in New Hampshire beginning in January 2009.⁴² As one of the initial goals of the NHBCS was to examine how child health outcomes were related to a wide range of exposures, in particular through drinking water, recruitment was initially restricted to women who reported using a private and unregulated water system at their home. Maternal blood samples were collected from women once at enrollment and umbilical cord blood samples were collected from infants at the time of birth. Anthropometric data were collected via medical record review. In total, 413 mothers and 787 infants had analyzed with blood metabolomics and anthropometry data. Participants provided written informed consent and all study procedures were approved by the Institutional Review Board at Dartmouth College.

Metabolomics measurements

Targeted metabolomics data were acquired using the commercially available AbsoluteIDQ[®] p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). This mass spectrometry (MS) method is comprised of two separate parts that are analyzed by multiple reaction monitoring (MRM) tandem MS analysis. The first part is a high-performance liquid chromatography (LCMS/MS)-based method that can separate and quantify 43 metabolites (21 AA, 21 biogenic amines, and 1 monosaccharide), and the second part is a flow injection analysis (FIA-MS/MS) that can simultaneously quantify up to 145 metabolites, which are all lipids. These include 40 acylcarnitine species (AC) including free carnitine, glycerophospholipids species (GL) including 38 acyl/acyl side chain phosphatidylcholines (PCaa), 38 acyl/alkyl side chain phosphatidylcholines (PCae), and 14 lysophosphatidylcholines (lysoPC), and 15 sphingolipids (SL) in the positive (+) polarity mode, and the total concentration hexoses in the negative (–) polarity mode. A full list of metabolites that are measured by the p180 kit can be found here: <https://biocrates.com/wp-content/uploads/2022/02/biocrates-p180-list-of-metabolites-v2-2021.pdf>. The detailed preparation of the kit components, samples and the kit plate are stated in the AbsoluteIDQ[®] p180 user's manual (*UM_p180_Sciex_13*). Ten μ L of the stable labeled internal standard mixture and 10 μ L of blood plasma were pipetted onto the filter paper in each of the sample wells. After drying samples under high-purity N₂ gas, the sample metabolites were derivatized with phenol isothiocyanate, and then extracted, split into two separate analysis plates, and then diluted in preparation for analyses. MS-based analyses were performed on a 4000 Q-Trap[®] ESI-LCMS/MS System (Sciex, Framingham, MA) equipped with an Agilent 1200 Series HPLC (Agilent Technologies, Palo Alto, CA) using an Agilent Zorbax[®] Eclipse XDB-C₁₈ (3.5 μ m) 3.0 \times 100 mm column. The flow rate was set to 500 μ L/min. Mobile phase A was 100% LCMS-grade water with 0.2% formic acid. Mobile phase B was 100% acetonitrile with 0.2% formic acid. The elution gradient was as follows: 100% A held constant from 0.0 to 0.50 min; linear gradient from 100% to 5% A from 0.5 to 5.5 min; 5% A held constant from 5.5 to 6.5 min; linear gradient from 5% A to 100% A from 6.5 to 7.0 min; 100% A 7.0–9.5 min. The FIA mobile phase

was 100% LCMS-grade methanol combined with the proprietary additive that was provided in the p180 kit. The gradient was isocratic, and the flow rate was as follows: 30 μ L/min from 0.0 to 1.60 min; 30–200 μ L/min from 1.60 to 2.40 min; 200 μ L/min to 2.80 min; 200–30 μ L/min to 3.00 min. The LCMS system was operated by a workstation PC installed with Analyst[®] 1.6.2 software (Sciex LP, Ontario, Canada). The MS parameter settings for both LCMS and FIA analysis were CAD medium 6; CUR 20; Gas1 40; Gas2 50; TEM (500 LCMS and 200 FIA); ISTD (5500 LCMS and 5500 positive polarity and –4500 negative polarity); x-axis 5; y-axis (LCMS 2 and FIA 10). A unique MRM ion pair (precursor MS1 and product MS2 “transition” ions), which are specifically unique for each analyte, was used to measure the analytes and their stable labeled internal standards. The internal standard was used to determine absolute and/or relative quantification. All raw data were processed using a combination of Analyst[®] 1.6.2 (Sciex LP, Ontario, Canada) instrument control and data processing software, and MetIDQ Carbon 6.4.8-DB105-2809 Laboratory Information Management System software (Biocrates Life Sciences AG, Innsbruck, Austria).

Assay performance was monitored using quality control (QC) samples. We analyzed three kit-provided QC samples that were at three known levels of concentration for each metabolite, as well as four replicates of the mid-level QC (QC2) to normalize via known target values of the QC2 level analytes across the samples analysis plates, and six Children's Health Exposure Analysis Resource Consortium⁴³ standard reference material replicates. QC assessments within an analytical run as well as pre- and post-analysis system suitability checks were also performed. The QC procedures involved reviewing the quality of each plate run, including both LCMS/MS and FIA-MS/MS. The quality metrics included the signal stability of the internal standards, retention time drifts of the standards and their effects, if any, on peak integration, and accuracy of points on the standard curves of each analyte in the LC-based analysis as well as the regression model used and the graph weighting to generate the curves. This research included masked QC samples and was completed without knowledge of study participants' birth outcomes.

Of the 232 metabolites and custom metabolite indicators (CMI) that were quantified or semi-quantified, phenylethylamine was removed for having <1% non-zero measurements and also spermine, spermidine, and related derivatives (four features) were removed at the suggestion of the manufacturer. A total of 227 metabolites (183 metabolites and 44 CMI) were included in the further analyses. Then, the ComBat function from the *sva* R package was used to correct batch-related differences.⁴⁴ The residuals of the metabolites and CMI were mean-centered and unit-scaled.

Anthropometric measures

Infant weight (g), and length (cm) were abstracted from medical records at birth by trained study staff. World Health Organization reference curves were used to calculate sex- and age-standardized weight, length, and weight-for-length Z-scores.⁴⁵ Age- and sex-adjusted birth weight percentiles were used to classify infants' size-for-gestational age as SGA (\leq 10th percentile), appropriate- (11–89th percentile) (AGA), or large-for-gestational age (\geq 90th percentile) (LGA).

Other measures

Maternal education, age at enrollment, parity, race/ethnicity, alcohol use and tobacco use during pregnancy were obtained from self-reported questionnaires. Gestational age at birth, delivery type, and infant sex were collected from medical records. Maternal pre-pregnancy BMI was self-reported, and was used to ascertain pre-pregnancy weight status, which was categorized as underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), and overweight/obese (\geq 25 kg/m²).

Statistical and multivariate analyses

Descriptive statistics were assessed for characteristics of study participants for cord and maternal plasma samples. Mean values and standard deviations (SD) were calculated for continuous variables, and number and percentages were used to describe categorical and ordinal variables. Participant characteristics were then stratified by infant sex and maternal pre-pregnancy weight status to observe the differences across these variables. Seven maternal samples and 16 cord blood samples were associated with mothers with a pre-pregnancy BMI classified as underweight (<18.5 kg/m²) and were excluded from further analysis due to the small sample size.

To assess the correlation between metabolites and CMI in cord and maternal plasma samples, Pearson's correlation coefficients were

Table 1. Characteristics of study participants.

N (%) or mean (SD)	Cord^a (N = 787)	Maternal^a (N = 413)
Infant sex		
Male	391 (49.7%)	206 (49.9%)
Female	396 (50.3%)	207 (50.1%)
Maternal pre-pregnancy weight status		
Normal weight	387 (49.2%)	213 (51.6%)
Overweight/obese	363 (46.1%)	184 (44.6%)
Missing	37 (4.7%)	16 (3.9%)
Maternal age at enrollment (years)	31.1 (4.85)	31.2 (5.00)
Maternal BMI (kg/m ²)	26.1 (5.83)	26.0 (5.68)
Missing	19 (2.4%)	9 (2.2%)
Gestational age at birth	39.1 (1.46)	39.51 (1.51)
Missing	0 (0%)	9 (2.2%)
Parity		
0	314 (39.9%)	187 (45.3%)
1	296 (37.6%)	143 (34.6%)
>2	173 (22.0%)	78 (19.0%)
Missing	4 (0.5%)	5 (1.2%)
Hispanic/non-Hispanic (infant)		
Hispanic/Latino	9 (1.1%)	5 (1.2%)
Non-Hispanic	778 (98.9%)	407 (98.5%)
Missing	0 (0%)	1 (0.2%)
White/non-white (infant)		
White	785 (99.7%)	370 (89.6%)
Non-white	2 (0.3%)	3 (0.7%)
Missing	0 (0%)	40 (9.7%)
Maternal weight gain during pregnancy	35.3 (14.7)	34.2 (15.2)
Missing	30 (3.8%)	17 (4.1%)
Highest grade of school completed		
<High school degree	7 (0.9%)	3 (0.7%)
High school graduate	80 (10.2%)	46 (11.1%)
Some college	151 (19.2%)	72 (17.4%)
College graduate	294 (37.4%)	126 (30.5%)
Postgraduate schooling	194 (24.7%)	109 (26.4%)
Missing	61 (7.8%)	57 (13.8%)
Type of delivery		
Vaginal	513 (65.2%)	274 (66.3%)
C-section	264 (33.5%)	131 (31.7%)
Missing	10 (1.3%)	8 (1.9%)
Alcohol during pregnancy		
Yes	117 (14.9%)	63 (15.3%)
No	608 (77.3%)	325 (78.7%)
Missing	62 (7.9%)	25 (6.1%)
Ever-smoker		
Yes	90 (11.4%)	43 (10.4%)
No	630 (80.1%)	309 (74.8%)
Missing	67 (8.5%)	61 (14.8%)
Size for gestational age		
SGA	39 (5.0%)	13 (3.1%)
AGA	614 (78.0%)	281 (68.0%)

Table 1. continued

N (%) or mean (SD)	Cord^a (N = 787)	Maternal^a (N = 413)
LGA	126 (16.0%)	58 (14.0%)
Missing	8 (1.0%)	61 (14.8%)
Weight-for-age Z-score	0.350 (1.01)	0.342 (0.973)
Missing	8 (1.0%)	61 (14.8%)
Length-for-age Z-score	0.835 (1.41)	0.816 (1.38)
Missing	10 (1.3%)	62 (15.0%)
Weight-for-length Z-score	-0.439 (1.44)	-0.443 (1.47)
Missing	18 (2.3%)	64 (15.5%)

N number, SD standard deviation.

^a356 dyads provided both cord and maternal samples.

computed to test all pairwise correlations between the same metabolites in cord and maternal samples.

Associations between metabolites as independent variables and each anthropometric measure at birth as dependent variables were assessed with linear regression analysis. Normality of the distributions of metabolite concentrations was explored through visual inspection of histograms, and all metabolite concentrations were normalized prior to regression. The main exposure of the regression is 227 metabolites and CMLs, and the main outcomes of the regression include birth weight Z-score, birth length Z-score, and birth weight-for-length Z-score. In addition to crude, unadjusted models, we selected a set of a priori covariates including infant sex, gestational age, and delivery mode, maternal age, parity, pre-pregnancy BMI, ever-smoker, and alcohol use during pregnancy and fit-adjusted models. As an exploratory analysis, linear regression analyses were also performed after stratifying by infant sex and by pre-pregnancy maternal weight status to examine the interaction. All stratified models were also adjusted for the same set of covariates: infant sex, gestational age, delivery mode, maternal age, parity, pre-pregnancy BMI, ever-smoker, and alcohol use during pregnancy with the exception of the stratification variable (i.e., infant sex or maternal pre-pregnancy weight status). To address the issue of multiple comparisons, a Benjamini-Hochberg method was applied to control the false discovery rate for the testing of 227 metabolites and CMLs accounting for all three birth size outcomes. Results were also visualized using Manhattan plots.

Of the metabolites that showed statistical significance in the linear regression model after correcting for multiple comparisons, logistic regression models were used to further confirm the metabolic differences in terms of healthy-weight infants versus SGA and LGA age infants.

Statistical and pathway enrichment analyses were performed using MetaboAnalyst software.⁴⁶ A list of metabolites that showed significant associations across three outcome measures in either cord or maternal plasma samples after multiple testing correction was compared with a list of 335 reference metabolites obtained from Biocrates Life Sciences AG based on human metabolic pathways contained in the curated library.

Unsupervised principal components analysis (PCA) of all metabolites was conducted using both the cord and maternal plasma to visualize any clustering of the data and identify possible outliers. PCA was also stratified by maternal pre-pregnancy weight status and infant sex in separate analyses to observe any modification of clustering of metabolites by these variables. Supervised orthogonal partial least-squares discriminant analysis (OPLS-DA) was used to construct predictive models to discriminate between infant size for gestational age (AGA and SGA) and identify metabolites that were important to differentiating the phenotype. The quality of these models was assessed with the following parameters: R²X, R²Y, and Q². R²X and R²Y represent the variation of the X and Y matrix, respectively, and Q² describes the predictive accuracy of the model.

All analyses were completed using R: A Language and Environment for Statistical Computing, version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). The R packages that were utilized for the analyses include: dplyr,⁴⁷ ggpubr,⁴⁸ ggrepel,⁴⁹ sva,⁴⁴ tableone,⁵⁰ and ropls.⁵¹

RESULTS

In total, 413 maternal first-trimester blood samples and 787 cord blood samples with corresponding infant anthropometry

measurements at birth were included in the analyses, of which 356 had both maternal and cord blood samples. Table 1 shows the participant characteristics and birth outcomes for both the maternal and cord study samples. The NHBCS cohort is mostly non-Hispanic (98.9% for cord samples; 98.5% for maternal samples), white (99.7% for cord samples; 89.6% for maternal samples). About 30% of mothers had less than a college degree (30% in cord samples and 29% in maternal samples). Of mothers included in the cord and maternal study samples, 46.1% and 44.6% had a pre-pregnancy BMI that was in the overweight or obese category, respectively. The mean and standard deviation of overall maternal age at enrollment was 31.1 ± 4.9 years and gestational age at birth was 39.1 ± 1.5 weeks. The means and standard deviations of age- and sex-adjusted birth weight, birth length, and weight-for-length Z-scores were 0.35 ± 1.0 , 0.84 ± 1.4 , 0.50 ± 1.4 , -0.44 ± 1.4 for cord blood samples. Participant characteristics stratified by maternal pre-pregnancy weight status and infant sex are presented in more detail (Supplementary Table 1).

Pairwise correlations across the entire sets of 227 metabolites and CMI between maternal and cord samples were conducted (Supplementary Table 2). There were 83 pairs of metabolites and CMI with significant correlations in the positive direction after the FDR correction, indicating that there are metabolites and CMI non-randomly varying between cord and maternal samples. However, all of the significantly correlated pairs of metabolites and CMI are either moderately or weakly correlated (2 CMI with $r > 0.4$).

PCA score plots for the overall, infant sex-stratified, and maternal pre-pregnancy weight status-stratified analyses are presented for all metabolites (Supplementary Fig. 1). PCA score plots showed complete overlap between male and female infants and between normal weight and overweight/obese populations in both cord and maternal samples ($R^2X = 0.54$ and 0.53 for cord and maternal samples respectively). Principal components 1 and 2 combined explained 41.5 and 36.5% of the variation of metabolites in the cord and maternal samples, respectively. Principal components 1 and 2 were modeled as predictors in a linear regression with three continuous measures: birth weight, length, and weight-for-length Z-scores; however, none were statistically significant for either maternal and cord samples (for PC1, $p > 0.8$, $p > 0.8$, $p > 0.5$ for weight, length, and weight-for-length Z-scores respectively for cord samples; $p > 0.8$, $p > 0.5$, $p > 0.3$ for weight, length, and weight-for-length Z-scores respectively for maternal samples). In addition, OPLS-DA modeling did not show a clear separation between infants who were born as

SGA and AGA. The metabolomic features did not meaningfully predict birth outcomes (i.e., model $Q^2 = -0.07$ for maternal samples and model $Q^2 = 0.02$ for cord samples); therefore, principal components 1 and 2 were not modeled as predictors in further analyses (Supplementary Fig. 2).

The results from multivariable linear regression models that show the individual associations of each metabolite as an independent variable associated with three anthropometric measures (birth weight Z-score, length Z-score, and weight-for-length Z-score) in the cord blood samples are presented (Supplementary Table 3). Three types of multivariable linear regression models (unstratified, infant sex-stratified, and maternal pre-pregnancy weight status-stratified) were evaluated. Figure 1 presents the Manhattan plots for the metabolites that showed statistical significance after an FDR correction (q value < 0.05) from the adjusted linear regression models.

Table 2 presents the summary of the metabolites that had significant associations with each outcome after the multiple testing correction. Twenty-three metabolites/CMI measured in the infant cord blood samples were associated (q value < 0.05) with birth weight Z-score. Thirteen of these metabolites had a negative association with birth weight Z-score including 11 AC, 1 LysoPC, and 1 CMI, the ratio of polyunsaturated fatty acid to monounsaturated fatty acid. Ten metabolites with a positive association with birth weight Z-score included 7 LysoPCs and 3 CMIs (the ratios of total lysophosphatidylcholine to total phosphatidylcholine, monounsaturated fatty acid to saturated fatty acid, and total lysophosphatidylcholine). Six metabolites measured in the infant cord blood samples were associated (q value < 0.05) with birth length Z-score. One AC was negatively associated, and 5 metabolites (4 lysoPCs and 1 CMI, the ratio of Total to over total phosphatidylcholine) were positively associated with birth length Z-score. No statistically significant associations were found between cord plasma metabolites and birth weight-for-length Z-score.

Compared to several ACs and GLs having significant associations with birth weight and length Z-scores in the cord samples, metabolites in the maternal samples did not show any significant associations with weight and length Z-scores. However, one CMI, the ratio of Kynurenine to Tryptophan, measured in the maternal blood samples was negatively associated with birth weight-for-length Z-score.

The results from the models stratified by infant sex and maternal pre-pregnancy weight status are consistent with the

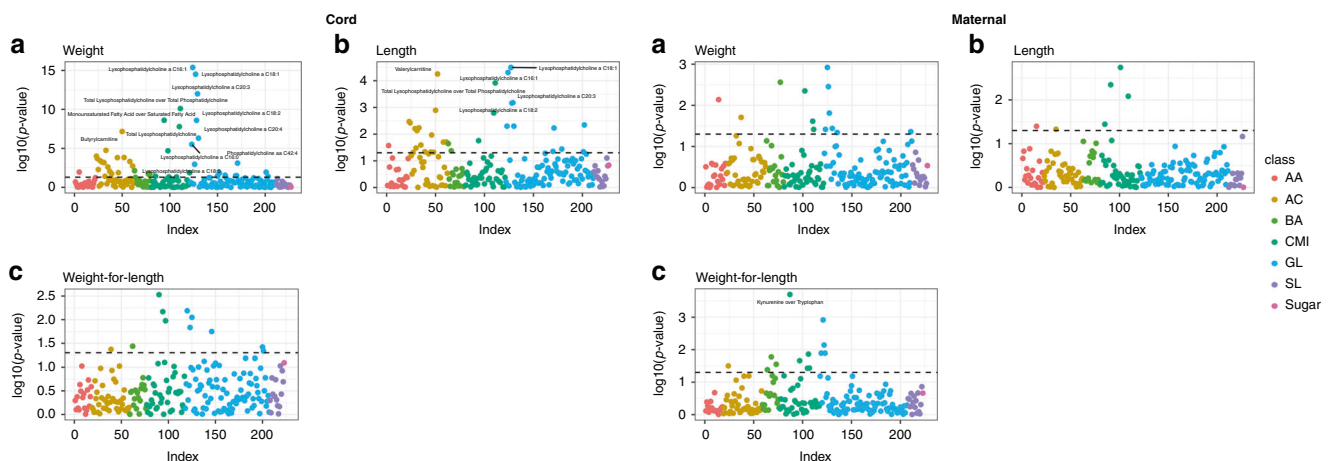


Fig. 1 Three plots present $\log_{10}(p$ value) for the association between each metabolite or custom indicator/ratio and three newborn outcome measures (weight, length, and weight-for-length Z-scores) from the linear regression models. The X-axis indicates the metabolites separated by classes and Y-axis indicates $-\log_{10}(p$ value) for each metabolite. Statistical significance is shown by dashed lines (p value = 0.05) in all plots, and the metabolites below the FDR-adjusted q value at 0.05 threshold are named. All models are adjusted for infant sex, gestational age, and delivery mode, maternal age, parity, pre-pregnancy BMI, ever-smoker, and alcohol use during pregnancy.

Table 2. Summary of metabolites with significant associations (adjusted *p* value < 0.05) from adjusted linear regression models.

	Outcome (Z-scores)	Negative association		Positive association		
		Metabolite	Class	Metabolite	Class	
Cord samples	Weight	L-Carnitine (C0)	AC	Lysophosphatidylcholine a C16:0	GL	
		L-Acetylcarnitine (C2)	AC	Lysophosphatidylcholine a C16:1	GL	
		Valerylcarnitine (C5)	AC	Lysophosphatidylcholine a C18:0	GL	
		Butyrylcarnitine (C4)	AC	Lysophosphatidylcholine a C18:1	GL	
		Dodecenoylcarnitine (C12:1)	AC	Lysophosphatidylcholine a C18:2	GL	
		Decanoylcarnitine (C10)	AC	Lysophosphatidylcholine a C20:3	GL	
		Hexanoylcarnitine (C6 (C4:1-DC))	AC	Lysophosphatidylcholine a C20:4	GL	
		Dodecanoylcarnitine (C12)	AC	Total lysophosphatidylcholine over total phosphatidylcholine	CMI	
		Tetradecenoylcarnitine (C14:1)	AC	Total lysophosphatidylcholine	CMI	
		Tetradecanoylcarnitine (C14)	AC	Monounsaturated fatty acid to saturated fatty acid	CMI	
		Tetradecadienylcarnitine (C14:2)	AC			
		Phosphatidylcholine aa C42:4	GL			
		Polyunsaturated fatty acid to monounsaturated fatty acid	CMI			
		Length	Valerylcarnitine (C5)	AC	Lysophosphatidylcholine a C16:1	GL
					Lysophosphatidylcholine a C18:1	GL
			Lysophosphatidylcholine a C18:2	GL		
			Lysophosphatidylcholine a C20:3	GL		
			Total lysophosphatidylcholine to total phosphatidylcholine	CMI		
Maternal samples	Weight-for-length	None	None			
	Weight	None	None			
	Length	None	None			
	Weight-for-length	Kynurenine to tryptophan	CMI	None		

overall models in that ACs were negatively associated and lysoPCs were positively associated with birth weight Z-scores (Supplementary Table 4). We further observed that the results for lysoPCs were largely unchanged and similar for both males and females when stratifying cord plasma samples by infant sex; however, the associations of ACs differed by infant sex and were only statistically significant in females. Only one AA, methionine, was positively associated with newborn weight Z-score, and one CMI, the ratio of kynurenine to tryptophan was negatively associated with newborn weight-for-length Z-score among mothers classified as overweight/obese before pregnancy in the maternal samples.

Metabolic sets enrichment analyses are presented graphically displaying the enrichment ratio on the x-axis and the *p* values (Supplementary Fig. 3). Across three outcomes in maternal and cord plasma samples, we identified the enrichment of seven different metabolic pathways. Two enrichment pathways including the beta-oxidation of very long-chain fatty acids (*p* value = 0.002) and the oxidation of branched-chain fatty acids (*p* value = 0.006) showed statistical significance; however, the statistical significance of the enrichment did not remain after multiple testing correction.

Finally, logistic regression was conducted with the metabolites that were found to have significant associations with weight Z-score in the cord samples. Twenty-three metabolites/CMI were used as exposures and SGA and LGA were included as outcomes in separate logistic regression models. An increase in metabolite concentration was protective (odds ratio <1) against LGA for the same metabolites/CMI that showed negative associations with

weight Z-score, and had positive associations (odds ratios >1) for those that showed positive associations with weight Z-score. Similarly, an increase in the metabolite concentration was protective against SGA for the metabolites/CMI that showed positive associations with weight Z-score, and was positively associated with SGA for those that had negative associations with weight Z-score (Supplementary Table 5).

DISCUSSION

In our pregnancy cohort study from rural New Hampshire, USA, higher acylcarnitines measured in the infant cord blood samples were negatively associated with both newborn measures of weight Z-score and length Z-score, and higher lysophosphatidylcholines were positively associated with both newborn measures of weight Z-score and length Z-score. Carnitine is known to play a crucial role in mitochondrial metabolic pathways especially the beta-oxidation of long-chain fatty acids into the mitochondria.⁵² Inside the cell, carnitine and acetylcarnitine, which is the shortest form of acylcarnitine, are readily converted by enzymes according to metabolic needs.⁵³ The main source of fetal carnitine is placental transfer during early pregnancy and via breastfeeding during the postnatal period.⁵⁴ The metabolism of fatty acids enhances a newborn's ability to utilize diverse energy sources, which may affect its long-term growth and metabolism. It is known that the carnitine concentration in plasma of pregnant women decreases over gestation and is the lowest near the end of gestation;^{37,55} therefore, carnitine concentrations in maternal

plasma at birth are not only correlated with each other but also the lowest at delivery.⁵⁶ Perhaps this could explain the negative associations observed in AC among the cord plasma samples since the majority of the newborns in our cohort were full-term healthy infants. In addition, short-chain acylcarnitine concentrations are higher in cord blood plasma samples than maternal plasma samples at delivery,⁵⁶ which may explain the greater number of significant acylcarnitine associations found in the cord plasma, but not in the maternal plasma.

Previous studies have shown that low birth weight, preterm, and SGA newborn infants have different levels of carnitine concentrations. Some studies found lower concentrations of acylcarnitines in low birth weight^{57,58} and preterm infants,⁵⁹ however, some studies found higher concentrations of acylcarnitines in low birth weight,³⁴ extreme macronomia,³⁴ SGA,⁵⁸ and preterm infants.⁶⁰ One study that investigated 481 cord blood samples at delivery in four cohorts across Europe also found that lower levels of multiple AC (C4, C6, C8, C10, C12, C14, and C16) were positively associated with birth weight.⁶¹ Another study that assessed the trajectories of 28 acylcarnitines throughout gestation and their associations with birth anthropometry found that women with decreasing C12 levels across gestation had offspring with significantly lower birth weight, birth weight Z-score, and birth length.³⁷ Most literature that examined the associations between cord or maternal metabolomics profiles and newborn anthropometric measures were based on specialized cohorts of infants with abnormal birth weight such as SGA, macrosomia, or low birth weight. Our findings of significant acylcarnitine perturbations are consistent with current literature for small for gestational age, however, more research is needed on healthy, full-term infants in order to examine further underlying mechanisms and replicate our findings.

Lysophosphatidylcholine is lysophospholipid in which one acyl chain is lacking and one hydroxyl group of the glycerol backbone is acylated from glycerophospholipids.⁶² The class of lysophosphatidylcholine was positively associated with newborn weight Z-score and length Z-score. Lysophosphatidylcholine is a class of lipid biomolecule that activates the signaling pathways involved in oxidative stress and inflammatory responses.⁶³ There is growing evidence that shows lysophosphatidylcholines playing a role in fatty acid transport from the maternal plasma to the placenta.⁶⁴ To our best knowledge, this is the third large-scale study that examined the associations between the lysophosphatidylcholine profiles in cord plasma and birth weight. Evidence from two previous studies also show that cord plasma lysophosphatidylcholines species are positively associated with birth weight. In the German birth cohort study, LISApplus, 753 cord blood samples that were collected showed significant positive associations between birth weight and cord plasma lysophosphatidylcholines such as LPC14:0, LPC 16:1, and LPC 18:1,⁶⁵ which is consistent with our findings. In addition, 226 newborns and their mothers enrolled from the Department of Obstetrics, Charité Universitätsmedizin Berlin found a strong association between birth weight and LPC 16:1.⁶⁶ Our study was not able to identify significant associations between the metabolomic profiles and newborn length and weight-for-length Z-scores. However, further research is required to understand and confirm the potential associations between the metabolomic profiles and newborn length and weight-for-length Z-scores at delivery.

Pairwise correlation tests between the metabolites and CMI in maternal and cord plasma samples from the same mother–infant dyad showed that there is statistical dependence between some metabolites and CMI; however, maternal and cord blood metabolomic profiles contain distinct information that is likely reflective of different metabolic states at different periods of gestation as well as physiological states at maternal metabolome during pregnancy and maternal/infant metabolome at birth.

Our study has limitations that should be noted. The cord and maternal plasma metabolites were measured at just one

time-point, so we were unable to assess changes in metabolite level throughout gestation in relation to anthropometry at birth. Second, the maternal plasma profiles reflect the metabolites at 24–28 weeks of gestation and cord at birth, and gestation is known to affect both metabolism and the transmission of metabolites across the placenta.⁵⁵ Third, while the Biocrates AbsoluteIDQ platform enabled standardized quantitative analysis of 227 different metabolites and custom metabolic indicators, these molecules represent only a small proportion of the blood metabolome. Future analyses can expand our study framework across additional classes of metabolites using untargeted approaches. Fourth, there is an imbalance in the numbers of infant and maternal plasma samples with 356 dyad samples contributing to both datasets. Finally, the exploratory interaction analyses were limited due to sample size, and the maternal weight interaction analysis may have been further limited by the maternal self-report of weight. Women have been shown to underestimate their self-reported pre-pregnancy weight, which could have resulted in some women being misclassified as having a normal pre-pregnancy weight,⁶⁷ thus biasing results towards the null. Nevertheless, to our knowledge, this is the first study that examined both maternal and cord metabolomics to study the independent associations (with adjustment for infant sex, gestational age, and delivery mode, maternal age, parity, pre-pregnancy BMI, ever-smoker, and alcohol use during pregnancy) with three different newborn measures including weight Z-score, length Z-score, and weight-for-length Z-score among full-term and healthy infants and mothers. These results elucidate potential metabolites that may explain the associations between exposure and birth outcomes. Further research is needed to discern how these metabolites mediate the exposure and birth outcomes.

CONCLUSIONS

Our study identified cord blood metabolites associated with newborn sex- and age-adjusted weight and length Z-scores when accounting for infant sex, maternal age, parity, maternal pre-pregnancy BMI, gestational age, delivery mode, ever-smoker, and alcohol during pregnancy. Our results were consistent by infant sex and maternal pre-pregnancy weight status. Exploring growth measures throughout early childhood and examining associations with both maternal and cord metabolomics profiles using untargeted approaches in further studies will allow us to better understand the associations between metabolic profiles and child growth more comprehensively. Future studies should include the measurement of serum metabolites on multiple occasions throughout pregnancy for maternal samples as well as throughout the infancy of newborns in order to investigate the longitudinal effect of the metabolomics profiles on fetal and early growth.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon request and completion of a satisfactory data transfer agreement.

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AUTHOR CONTRIBUTIONS

M.R.K. and S.S. designed and supervised the data collection. A.G.H., D.G.-D., S.S., S.M. and M.R.K. conceptualized the study. D.Y. analyzed the data and wrote the first draft of the manuscript. All co-authors (D.G.-D., B.D., M.C., D.S., D.K., S.M., S.S., M.R.K., and A.G.H.) read, edited several draft versions, and approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All study procedures were approved by the Center for the Protection of Human Subjects at Dartmouth College (protocol code STUDY00020844). All participants provided written informed consent after they were explained the aims of the study, the procedure of the data collection, and voluntary nature of participation that they were free to withdraw from the study at any time.

ADDITIONAL INFORMATION

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