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Cord Blood Metabolites Associated with Newborn Adiposity and Hyperinsulinemia

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Abstract

Objective—To evaluate the association between cord blood amino acid and acylcarnitine profiles and measures of adiposity and hyperinsulinemia in healthy newborns.

Study design—A cross-sectional study of 118 full-term infants born to mothers without gestational diabetes was performed. Cord blood leptin, C-peptide, acylcarnitine, and amino acid levels were measured. Body composition was measured by air displacement plethysmography. Multivariate linear regression and principal component analysis (PCA) were used to analyze associations of cord blood metabolites with newborn anthropometrics, leptin, and C-peptide.

Results—Acylcarnitines C2, C4-DC/Ci4-DC, and C8:1-OH/C6:1-DC were positively associated with leptin, and C14, C14:1, C16, C18, and C18:2 were negatively associated with C-peptide (p 0.0016). PCA revealed a positive association between factor 1 (C2, C3, C5, C4/Ci4, C4-OH, C4-DC/Ci4-DC, glutamate/glutamine, and glycine) and adiposity measures.

Conclusion—The positive association of C2 and C4-DC/Ci4-DC levels with leptin may reflect excess fat stores, higher fatty acid oxidation rate, and mitochondrial dysfunction leading to accumulation of acylcarnitine intermediates. Principal component analysis revealed a positive

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association between BCAA and ketone body metabolites and adiposity, confirming prior findings in adults. Cord blood acylcarnitine profiles may identify at-risk children before obesity or insulin resistance develops.

Newborn adiposity may be an important predictor of later childhood obesity.(2, 3) Understanding the early life contributors to and predictors of newborn body composition is essential for delineating risk factors and mechanisms leading to excess newborn adiposity, and may inform earlier implementation of childhood obesity prevention efforts.

Metabolite levels in cord blood may represent transfer of maternal metabolites, fetal synthesis of metabolites in response to cues from the intrauterine environment, or a combination. A unique cord blood metabolite signature may emerge as a representation of various *in utero* factors impacting fetal fat deposition and insulin sensitivity.

There are few existing studies of metabolomics as related to obesity or insulin resistance in children and even fewer in newborns. In adults, studies have demonstrated a well-known association between branched chain amino acids (BCAA), aromatic amino acids (AAA), and insulin resistance.(4) A similar associations of BCAAs with insulin resistance and obesity has been demonstrated in adolescents.(5) However, it is unclear whether elevations in these metabolites precede or follow the development of adiposity and/or insulin resistance. Higher levels of BCAA and AAA are associated with future insulin resistance and diabetes in adolescents and adults (5–8), with more recent studies suggesting that BCAA and AAA levels increase subsequent to the development of adiposity and insulin resistance.(9, 10) It is currently unknown whether these metabolite-phenotype associations are present at birth or develop in later childhood.

In this study, we examined associations between amino acids, acylcarnitines (AC), and measures of adiposity and hyperinsulinemia at birth in a cohort of healthy, full term neonates born to mothers with normal glucose tolerance in order to provide insight into the early life pathogenesis of metabolic disease. We hypothesized that newborns with higher adiposity have a unique metabolomic signature that parallels metabolite associations in adolescents and adults with obesity and insulin resistance.

Methods

We selected a subset of 118 newborns from a larger cohort of 168 healthy maternalnewborn pairs recruited from 2011–2014 at a large academic medical center.(11) In brief, women carrying a singleton pregnancy with normal glucose tolerance (as evidenced by normal results on a 2-hour 75g oral glucose tolerance test performed between 24 and 28 weeks gestation) were eligible for enrollment into the study (12). The subset of 118 maternal-newborn pairs selected for this particular analysis was chosen based on the availability of cord blood on which to perform metabolomics assays. Early gestational weight gain was calculated by evaluating the difference between measured maternal weight at a mid-pregnancy visit and self-reported pre-pregnancy weight. Women were noted to have excessive early gestational weight gain if their weight gain exceeded the maximum recommended weight gain based on their pre-pregnancy BMI, using the Institute of Medicine's 2009 guidelines on weight gain during pregnancy.(13) Women were excluded if

they had a history of greater than 3 term pregnancies or smoking during pregnancy, as these factors are associated with excess and restricted growth, respectively.(14)

All infants were full-term and were excluded if they required intensive care, were too ill to undergo body composition measurements within the first 24–72 hours of life, or if they had congenital anomalies, as some of these are independently associated with abnormal fetal growth. Cord blood was collected after birth by labor and delivery staff, processed within 30 minutes, and stored at –70°C until laboratory assays were performed. Of the 118 neonates included in the final analysis, 114 had cord blood leptin levels, 111 had cord blood C-peptide levels, and 109 had body composition data available. This study was approved by the Northwestern University Institutional Review Board and each mother provided written informed consent for herself and her neonate at the time of study enrollment.

Samples assayed for C-peptide and leptin were batched and measured in duplicate with a radioimmunoassay kit (Millipore Corp, Billerica, MA). The inter- and intra-assay coefficients of variation were 2.9–4.4% and 2.1–3.4%, respectively for C-peptide. The inter- and intra-assay coefficients were 3.7–5.9% and 3.0–4.0% respectively for leptin.

Targeted metabolomics assays for acylcarnitines and amino acids were performed at the Duke Molecular Physiology Institute Metabolomics Core Laboratory. Acylcarnitines and amino acids were analyzed using stable isotope dilution techniques. The measurements were made by flow injection tandem mass spectrometry using sample preparation methods described previously (15, 16). The data were acquired using a Waters triple quadrupole detector equipped with Acquity UPLC system and controlled by MassLynx 4.1 software platform (Waters, Milford, MA).

Infant length, weight, and body composition were measured by one of two trained clinicians. Length was obtained using a hard surface measuring board, recorded to the nearest 0.1cm, and the mean of duplicate measurements used for the final research measure. Weight and body composition were measured utilizing an air displacement plethysmography system (PeaPod, Cosmed, Rome, Italy) as follows: first, the machine was calibrated prior to use according to manufacturer guidelines. Second, the infant was undressed and placed on the calibrated PeaPod scale and weight recorded to the nearest 0.0001 kg. Next, the infant was placed inside the PeaPod volume chamber for 2 minutes to determine body volume. Density was calculated after which age- and sex-specific fat-free mass density values were used to determine absolute fat-free mass and fat mass. Finally, percent body fat was calculated from these values (17). In accordance with prior studies, body composition measurements were obtained between 24–72 hours of life to mitigate the effects of any possible fluid shifts or weight loss common in the immediate post-birth period.(18)

Statistical Analyses

Variables were summarized using means and standard deviations for continuous variables and frequencies for categorical variables. Acylcarnitine levels were log transformed for statistical analysis to improve normality. Separate linear regression models were used to examine associations between individual metabolites and newborn outcomes, adjusting for newborn sex, gestational age, race, maternal pre-pregnancy BMI, early excessive weight

gain, and maternal fasting glucose at OGTT, applying Bonferroni correction within each outcome. Nominal $P = 0.0016$ was used to indicate statistical significance, maintaining overall Type I error of 0.10 for each phenotype and ~60 metabolites used in this discovery analysis. Principal components analyses were then conducted using all metabolites with <10% missing values. Metabolites with highest loadings were identified to assist with description and interpretation of each principal component. Linear regression was then used to examine associations between the first three principal components and the newborn outcomes. Three models were examined for analysis of principal components and outcomes. Model A adjusted for newborn sex, gestational age, and race; Model B included Model A covariates, maternal pre-pregnancy BMI, and early excessive weight gain; Model C included Model B covariates and maternal fasting glucose at OGTT.

Results

Maternal and newborn characteristics of the cohort are displayed in Table I. Of note, 61.9% of mothers had a normal pre-pregnancy BMI and 34.8% were overweight or obese. All mothers had normal glucose tolerance on a 75g OGTT performed between 24–28 weeks of gestation. Neonates were full term, and the majority had a birth weight that was appropriate for gestational age. Mean newborn percent body fat was $11.0 \pm 3.8\%$ and was normally distributed.

When examining associations of individual acylcarnitines with newborn outcomes (Table 2), acetylcarnitine (AC C2), AC C4-DC/Ci4-DC, and AC C8:1-OH/C6:1-DC were positively associated with cord blood leptin, a measure of newborn fat tissue. We found that select medium-chain and long-chain acylcarnitines (AC C14, AC C14:1, AC C16, AC C18, AC C18:2) were negatively associated with cord C-peptide. There were no individual associations of acylcarnitines with birth weight or percent body fat and of cord blood amino acids with birth weight, percent body fat, or cord blood leptin.

Principal component analyses were undertaken in order to determine which groups of highly correlated metabolites best explained the variation in measures of newborn adiposity and hyperinsulinemia (Table 4; available at www.jpeds.com). Metabolites represented in each of the first four principal component factors were biologically related to each other and together, explained 56% of the total variance in the data. As depicted in Table 3 and the Figure, Factor 1 was positively associated with newborn percent body fat, fat mass, and leptin in models adjusting for early excessive gestational weight gain and maternal fasting glucose (Models B, C). The association with leptin was also present in model A. There was no association with cord C-peptide. Factor 1 included eight metabolites, including acetylcarnitine (AC C2), branched chain amino acid metabolites (AC C3, AC C5, AC C4-DC/Ci4-DC), a ketone body metabolite (AC C4-OH) and glutamine/glutamine. Together, these eight metabolites explained 28% of the variance in newborn adiposity and may work synergistically to contribute to newborn fat deposition, even when adjusting for important confounders of newborn size such as maternal BMI and excessive gestational weight gain.

Factor 2 largely contained a variety of medium-chain acylcarnitines and did not demonstrate an association with newborn outcomes. Factor 3 included medium-chain and longchain

acylcarnitines, and was positively associated with newborn percent body fat and fat mass in Model C and negatively associated with cord C-peptide in model A.

Discussion

The current study demonstrates associations of cord blood amino acids and acylcarnitines with newborn measures of adiposity and hyperinsulinemia in a cohort of infants born to mothers with normal glucose tolerance. These findings suggest that some of the known associations of amino acids and acylcarnitines with obesity and insulin resistance that have been previously reported in adolescents and adults (4–8) may already be present at birth.

Acetylcarnitine (AC C2) is a product of fatty acid beta oxidation and was positively associated with cord blood leptin, a surrogate marker of adiposity. Acetylcarnitine is a shortchain acylcarnitine previously shown to be elevated in insulin resistant states (4) and thought to play an important role in the regulation of mitochondrial fuel switching in response to nutritional circumstances.(19, 20) In the absence of adequate fuel switching, mitochondria become overburdened with substrate, leading to tissue accumulation of acylcarnitines of various chain lengths that can impair insulin action and sensitivity.(19) AC C4-DC/Ci4-DC and its association with cord blood leptin in our study is another notable finding. This acylcarnitine is derived from BCAA metabolism and has been positively correlated with basal glucose and hemoglobin A1c levels in adults.(21) The associations of acetylcarnitine and AC C4-DC/Ci4-DC with cord blood leptin but not C-peptide in our study may represent preferential impact on fat tissue quantity before insulin resistance develops, particularly as none of the infants in our cohort were exposed to gestational diabetes *in-utero*. Longitudinal assessment of metabolite levels and metabolic outcomes is necessary to evaluate for changes in associations over time.

Higher levels of medium-chain and long-chain acylcarnitine elevations have been described in adults with obesity and/or Type 2 diabetes.(21, 22) The accumulation of long-chain acylcarnitines may also directly interfere with cellular insulin signaling.(22) In our cohort, we found that the medium-chain and long-chain acylcarnitines AC C14, AC C14:1, AC C16, AC C18, and AC C18:2 were negatively associated with C-peptide, a marker of hyperinsulinemia. This finding is contrary to what has been reported in the literature. The range of C-peptide measures in our cohort was relatively narrow, as infants were all born to mothers with normal glucose tolerance and without fetal hyperinsulinemia. Our results suggest that medium-chain and long-chain acylcarnitines may be associated with body composition independent of the presence of hyperinsulinemia, a well-known contributor to fetal growth.

The principal component analysis (PCA) in this study revealed a group of eight metabolites in Factor 1, that together, were associated with newborn percent body fat, fat mass, and leptin. The association with leptin was present in all models, and the association with percent body fat and fat mass occurred when adjusting for early excessive gestational weight gain and maternal fasting glucose. The eight metabolites in Factor 1 included acetylcarnitine, a by-product of the first step of BCAA metabolism (glutamate/glutamine),

derivatives of BCAA metabolism (AC C3, AC C5, AC C4-DC/Ci4-DC), and the carnitine ester of 3-hydroxybutyrate (AC C4OH).

Acetylcarnitine has previously been linked to insulin resistance.(4) BCAA are also associated with obesity and insulin resistance in adolescents and adults (5, 23), thus the association of BCAA metabolites and newborn size may be a downstream reflection of higher maternal levels of BCAAs. Interestingly, the positive association between Factor 3, largely composed of medium-chain and long-chain acylcarnitines, and percent body fat and fat mass is similar to reports in adults (21); although the negative association between Factor 3 and C-peptide is a contrary finding though consistent with the individual associations previously reported in this study. We hypothesize that the paradoxical association of Factor 3 with C-peptide in this cohort is due to the narrow range of cord blood C-peptide in these infants who were not exposed to maternal hyperglycemia during pregnancy.

We did not find an association between BCAA and AAA levels and measures of newborn adiposity and hyperinsulinemia in our per-metabolite or principal component analyses. These previously reported relationships in adolescents and adults may not be evident in this cohort without significant hyperinsulinemia.

There are few studies in the literature relating cord blood metabolic profiles to newborn adiposity. Similar to our findings, a study of 1600 mother-newborn pairs from the Hyperglycemia and Adverse Pregnancy Outcome Study reported an association of medium-chain acylcarnitines with measures of adiposity, specifically both birth weight and sum of skinfolds. (24). The current study also revealed an association of medium-chain acylcarnitines with adiposity, though our reported association is with leptin, a biomarker of adiposity as opposed to an anthropometric measure. A study of 126 mother-newborn pairs from Project Viva described an association of a group of branched-chain amino acids and related metabolites with newborn size measured as birth weight.(25) We report comparable associations of branched-chain amino acid metabolites with adiposity and the anthropometric measures used in our study (percent body fat and fat mass), are more direct measures of adiposity than birth weight. Furthermore, our analysis accounted for maternal glycemia and excessive gestational weight gain, two independent risk factors for newborn size. Patel et al investigated cord blood metabolites in offspring born specifically to obese women. The authors demonstrated associations between lipid metabolites, birth weight, and sum of skinfolds but no associations were found between acylcarnitines, amino acids, and anthropometrics at birth or at 6 months of age.(26) We recognize that some of our study findings differ from previous reports; this may be partially attributable to phenotypic differences between cohorts and our adjustment for maternal glucose. Specifically, the mothers in our study all had normal glucose tolerance in contrast to those in the HAPO cohort who had a range of pre-pregnancy BMIs across weight categories unlike the study by Patel et al. Further study in comparable populations are necessary to confirm or refute these associations.

Limitations of this study include its cross-sectional design, only allowing us to report associations rather than causality. Measurement of metabolites at just one time point provides only a glimpse into metabolite-phenotype associations and does not allow for a

comprehensive understanding of how fetal metabolite levels change throughout gestation and in the postnatal period; all time periods important for growth. The use of cord blood samples for metabolite analysis also limits our ability to definitively know whether the metabolite profile is fully fetal or maternal in origin. Lastly, our sample size is relatively small and further studies in larger cohorts are needed to validate our findings.

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Figure 1: Associations of Factor 1 and Measures of Newborn Adiposity.

Each plot depicts the joint association of the interrelated metabolites that comprise Factor 1 with a measure of newborn adiposity, % fat (A), fat mass (B), and cord blood leptin (C). The blue dots represent lower levels of each measure while the red dots represent higher levels.

The regression line is depicted in green and the lowess curve in black.

Table 1:**Maternal and Newborn Characteristics**

Demographics (n=118)	
Maternal Race	62% White 38% Non-White
Maternal Characteristics (n=118)	
	Mean ± SD
Age at Delivery	32 ± 3.8 years
Pre-Pregnancy BMI	25.1 ± 6.1 kg/m ²
Underweight (BMI <18.5 kg/m ²)	40%
Normal Weight (BMI 18.5–24.9 kg/m ²)	61.90%
Overweight (BMI 25–29.9 kg/m ²)	13.60%
Obese (BMI ≥30 kg/m ²)	21.20%
OGTT Fasting Glucose	76 ± 5 mg/dl (4.2 ± 0.28 mmol/L)
OGTT 1-hour Glucose	117 ± 26 mg/dl (6.5 ± 1.4 mmol/L)
OGTT 2-hour Glucose	100 ± 19 mg/dl (5.6 ± 1.05 mmol/L)
Newborn Characteristics (n=118, unless noted)	
	Mean ± SD
Sex	52% Male 48% Female
Gestational Age	39.5 ± 1 week
Birth Weight	3499 ± 508 g
Fat Mass (n=109)	375 ± 168 g
% Body Fat (n=109)	11 ± 3.8 %
Cord Blood Leptin (n=111)	10.8 ± 8.8 ng/ml (0.675 ± 0.55 nmol/L)
Cord Blood C-peptide (n=111)	0.65 ± 0.36 ng/ml (0.22 ± 0.12 nmol/L)

Table 2:

Individual Significant Associations of Acylcarnitines and Newborn Outcomes

Acylcarnitine ($\mu\text{mol/L}$)	Beta	p
Associations with Cord Blood Leptin		
AC C2	0.24	<0.001
AC C4-DC/Ci4-DC	0.24	0.001
AC C8:1-OH/C6:1-DC	0.25	0.002
Associations with Cord Blood C-peptide		
AC C14	−0.40	<0.001
AC C14:1	−0.45	<0.001
AC C16	−0.32	<0.001
AC C18	−0.44	0.002
AC C18:2	−0.30	<0.001

Acylcarnitines were log-transformed for statistical analysis. Linear regression models included adjustment for newborn sex, gestational age, race, maternal pre-pregnancy BMI, early excessive gestational weight gain, and maternal fasting glucose at OGTT.

Table 3:

Associations of Principal Component Analysis Factor 1 with Newborn Outcomes

Factor 1 Metabolites: C2, C3, C5, C4/Ci4, C4-OH, C4-DC/Ci4-DC, Glutamate/Glutamine, Glycine												
% Body Fat				Fat Mass			Leptin			C-peptide		
Model (beta, p)				Model (beta, p)			Model (beta, p)			Model (beta, p)		
A	B	C		A	B	C	A	B	C	A	B	C
0.99,	1.3,	1.25,		0.04,	0.05,	0.05,	0.11,	0.13,	0.12,	0.01,	0.05,	0.03,
0.08	0.02*	0.02*		0.14	0.03*	0.03*	0.01*	<0.01*	<0.01*	0.85	0.45	0.59

Beta represents the association of the principal component with the newborn adiposity measure Model A adjusted for newborn sex, gestational age, and race. Model B adjusted for Model A covariates, maternal pre-pregnancy BMI, and early excessive gestational weight gain. Model C adjusted for Model B covariates and maternal fasting glucose at OGTT

Table 4:

Principal Component Analysis

Factor	Metabolites	Description	Eigenvalue	Variance	Cumulative Variance
1	C2, C3, C5, C4/Ci4, C4-OH, C4-DC/Ci4-DC, Glutamate/Glutamine, Glycine	BCAA and ketone body metabolites and acetylcarnitine Polar amino acids (Glu/Gln) and nonpolar amino acid (Gly)	13.8	0.28	0.28
2	C6-DC/C8-OH, C8, C8:1, C10:1, C10-OH/C8-DC, C10:3, C12, C12:1	Medium-chain acylcarnitines	6.3	0.13	0.40
3	C8:1-DC, C14-OH/C12-DC, C14:1-OH, C14:2, C18-OH/C16-DC, C18:1OH/C16:1-DC, C18:1-DC, C20:4, C20-OH/C18-DC	Medium-chain and long-chain acylcarnitines	4.2	0.08	0.49
4	Valine, Tyrosine, Leucine/Isoleucine, Phenylalanine, Methionine, Proline, Arginine	Branched chain amino acids, aromatic amino acids, neutral amino acids (Met, Pro) and basic amino acids (Arg)	3.62	0.07	0.56
5	Ornithine, Serine, C16, C16:1-OH/C14:1-DC, C18, C18:1, C18:2	Ornithine, Neutral amino acid (Ser) Long-chain acylcarnitines	2.24	0.04	0.6
6	Alanine, C14, C16:1	Non-polar amino acid (Ala), longchain acylcarnitines	1.83	0.04	0.64
7	Histidine, Citrulline	Basic amino acid (His), Citrulline	1.51	0.03	0.67
8	Aspartate/Asparagine, C5:1	Polar amino acids (Asp/Asn), BCAA metabolite	1.37	0.03	0.70
9	C18:2-OH	Long-chain acylcarnitine	1.20	0.02	0.72
10	C22	Long-chain acylcarnitine	1.16	0.02	0.75
11	C8:1-OH/C6:1-DC, C5-DC	Medium and shortchain acylcarnitines, BCAA metabolite	1.04	0.02	0.76