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Associations of cord blood metabolites with early childhood obesity risk

Elvira Isganaitis¹, Sheryl L. Rifas-Shiman², Emily Oken², Jonathan Dreyfuss¹, Walt Gall³, Matthew W. Gillman², and Mary-Elizabeth Patti¹

¹Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA

²Obesity Prevention Program, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA

³Metabolon, Inc., Durham, NC, USA

Abstract

Background/Objective—Rapid postnatal weight gain is a potentially modifiable risk factor for obesity and metabolic syndrome. To identify markers of rapid infancy weight gain and childhood obesity, we analyzed the metabolome in cord blood from infants differing in their postnatal weight trajectories.

Methods—We performed a nested case-control study within Project Viva, a longitudinal cohort of mothers and children. We selected cases (n=26) based on top quartile of change in weight-for-age 0-6 mo and BMI >85th percentile in mid-childhood (median 7.7 years). Controls (n=26) were age- and sex-matched, had normal postnatal weight gain (2nd or 3rd quartile of change in weight-for-age 0-6 mo) and normal mid-childhood weight (BMI 25th-75th percentile). Cord blood metabolites were measured using untargeted LC/MS; individual metabolites and pathways differing between cases vs. controls were compared in categorical analyses. We adjusted metabolites for maternal age, maternal BMI, and breastfeeding duration (linear regression), and assessed whether metabolites improved the ability to predict case-control status (logistic regression).

Results—Of 415 detected metabolites, 16 were altered in cases vs. controls (T-test, nominal P<0.05). 3 metabolites were related to tryptophan: serotonin, tryptophan betaine, and tryptophyl leucine (46%, 48% and 26% lower in cases, respectively, P<0.05). Mean levels of 2 methyl donors, dimethylglycine and N-acetylmethionine, were also lower in cases (18% and 16% respectively, P=0.01). Moreover, the glutamine:glutamate ratio was reduced by 33% (P<0.05) in cases. Levels of serotonin, tryptophyl leucine, and N-acetylmethionine remained significantly different after adjustment for maternal BMI, age, and breastfeeding. Adding metabolite levels to logistic regression models including only clinical covariates improved the ability to predict case vs. control status.

Conflict of interest

W.G. formerly had a professional relationship with Metabolon, Inc., where he was employed as Program Manager for Diagnostic Development, and where the metabolomic analyses reported in this article were performed. There are no other potential conflicts of interest to disclose.

Supplementary information is available at International Journal of Obesity's website.

Conclusions—Several cord blood metabolites are associated with rapid postnatal weight gain. Whether these patterns are causally linked to childhood obesity is not clear from this cross-sectional analysis, but will require further study.

Introduction

Early infancy represents a window of developmental plasticity during which environmental exposures can modulate chronic disease risk. Accelerated postnatal weight gain trajectories are associated with increased risk of diabetes, obesity, and cardiovascular disease both in humans (1-3) and mammalian models (4;5). These data have implications for public health, as the postnatal period offers a window during which optimizing nutrition and/or growth rates could reap lifelong benefits. Thus, identifying early markers of rapid infancy weight gain, a potentially modifiable risk factor for obesity and type 2 diabetes (T2D), may eventually permit interventions targeting children at high risk for metabolic disease.

Metabolomic analysis allows comprehensive quantification of hundreds of nutrients, metabolic intermediates, and small molecules from biological samples and has proven a powerful tool for biomarker discovery. Recent metabolomic analyses have linked higher plasma levels of branched-chain and aromatic amino acids to subsequent development of T2D in adolescents and adults (6;7). We are not aware of any studies linking cord blood metabolomics to obesity or its early life risk factors. However, the cord blood metabolome does appear to be related to important clinical outcomes. For example, distinct cord blood signatures characterize infants with intrauterine growth restriction, notably higher phenylalanine and citrulline, and lower glutamine, choline, and glucose (8). Reductions in cord blood phospholipids have also been reported to predict early childhood type 1 diabetes risk (9).

We hypothesized that the cord blood metabolome would differ in newborns with subsequently development of early childhood obesity. To identify early biomarkers of rapid weight gain during infancy and childhood, we analyzed plasma metabolomic patterns in venous umbilical cord blood collected at delivery from infants who subsequently experienced or did not experience accelerated postnatal weight gain and childhood obesity.

Methods

Study population and measurements

We analyzed a subset of participants in Project Viva, a longitudinal cohort study of mothers and children. Detailed experimental protocols for Project Viva have been previously published (10). We recruited pregnant women during the first trimester from 8 obstetric offices of Harvard Vanguard Medical Associates in Boston, MA, between 1999 and 2002. Eligibility criteria included singleton pregnancy, ability to answer questions in English, and initial prenatal visit before 22 weeks gestation. In-person study visits occurred at recruitment (median 9.9 weeks gestation), mid-pregnancy (median 28.1 weeks gestation), just after delivery, during infancy (median 6.3 months), early childhood (median 3.3 years), and mid-childhood (median 7.7 years); detailed survey data were collected annually at intervening time points. At each visit, research assistants measured weight with a digital scale, and

length (newborn, infancy) or height (early childhood, mid-childhood) using a calibrated stadiometer. We used 2000 CDC reference data (<http://www.cdc.gov/growthcharts/>) to calculate weight-for-age Z-scores and BMI percentiles. We assessed body composition in mid-childhood by dual-energy X-ray absorptiometry (DXA, Discovery A, Hologic, Bedford, MA). The DXA scanner was monitored for quality control daily by scanning a standard synthetic spine. We used Hologic software QDR version 12.6 for analysis. A single trained investigator checked all scans for positioning, movement, and artifacts, and defined body regions for analysis. Intrarater reliability on duplicate measurements was high ($r=0.99$).

We obtained written informed consent from the mother at each encounter and verbal assent from children at the mid-childhood visit. The Harvard Pilgrim Health Care Institutional Review Board approved all procedures.

Identification of cases and controls

We identified 26 cases based on accelerated early postnatal weight gain, defined as the top-quartile of change in weight for age from 0-6 months in Project Viva and overweight status ($BMI > 85^{\text{th}}$ percentile according to CDC 2000) in mid-childhood. Controls were sex-matched and selected based on normal early postnatal weight gain, defined as the 2nd or 3rd quartile of change in weight for age from 0-6 months in Project Viva and normal weight (BMI between 25th and 75th percentile, CDC 2000) in mid-childhood.

Cord blood collection

Using a standard protocol, obstetricians and midwives collected blood from the umbilical cord vein at delivery. Whole blood was collected in EDTA tubes, refrigerated for <24 hours, and centrifuged at 2000 rpm at 4°C for 10 min. Plasma aliquots were stored in liquid nitrogen until shipment on dry ice to Metabolon (Durham, NC) for metabolomic analysis. Aliquots of cord blood were also used for analysis of adiponectin and leptin concentrations using commercial assay kits (RIA, Linco Research Inc., St. Charles, MO).

Metabolomic analysis

We performed untargeted metabolomic profiling using multi-platform mass spectroscopy (Metabolon, Durham, NC). Detailed descriptions of sample preparation, mass spectroscopy, and automated metabolite identification procedures have been previously published (11;12). We obtained semi-quantitative concentrations (expressed in arbitrary units) for 415 known biochemicals. We replaced undetectable values with 0.5*minimum value for each metabolite. We then $\log_2$ -transformed the metabolite concentrations to minimize the influence of outliers. We excluded from the analysis 27 metabolites with undetectable values in >50% of samples.

Statistical analysis

To determine which cord blood metabolites differed in cases vs. controls, we calculated fold-changes for each biochemical (calculated by exponentiating the mean $\log_2$ -metabolite level in cases, divided by the exponentiated mean $\log_2$ -metabolite level in controls) and performed 2-sided unpaired Student's T-tests using JMP Pro v10 software (SAS, Cary, NC). We calculated false discovery rate Q-values to account for multiple comparisons (<http://>

genomics.princeton.edu/storeylab/qvalue/) (13); Q-value < 0.25 was considered significant. We next performed metabolite set enrichment analysis to identify pathways enriched among the most differentially regulated metabolites between cases and controls (MetaboAnalyst 2.0, www.metaboanalyst.ca) (14).

To assess the overall variability within our dataset, we performed principal components analysis (PCA) using R (www.r-project.org), including only imputed, log-transformed metabolite concentrations and case/control status. Then, to determine to what extent the metabolome distinguished cases from controls, we performed unsupervised hierarchical clustering (MetaboAnalyst 2.0) (14). To assess which metabolites contributed most strongly to distinguishing cases from controls, we next used partial-least-squares discriminant analysis (PLS-DA) using R.

We adjusted for potential confounders through multivariate linear and logistic regression analysis (JMP Pro v 10, SAS, Cary, NC). After determining which clinical covariates were associated with case vs. control status using bivariate logistic regression, we next built multivariable models including those covariates with the strongest association with case-control status, namely, maternal pre-pregnancy BMI, maternal age, breastfeeding duration, and paternal BMI. Because paternal BMI did not contribute to the predictive power (whole-model R^2) of the multivariable models, we omitted it from subsequent models. We used linear regression to calculate metabolite concentrations adjusted for maternal BMI, maternal age, and breastfeeding duration. To test whether cord blood metabolite levels were associated with odds of case vs. control status after adjusting for clinical covariates, we then built models incorporating individual metabolites in addition to maternal BMI, maternal age and breastfeeding duration.

Results

Demographic, pregnancy, and hormonal characteristics in infants with accelerated postnatal weight gain

Mothers of cases were younger than controls (29.9 ± 5.4 vs. 34.9 ± 4.4 years), and had higher pre-pregnancy BMI (27.9 ± 6.7 vs. 23.6 ± 3.8 kg/m²). Paternal BMI was also higher among cases (27.6 ± 3.7 vs. 25.3 ± 3.5 kg/m²). 13 (50%) of the cases were non-white, vs. 7 (27%) of the controls.

Case infants tended to have lower birth weight (3275 ± 458 vs. 3487 ± 349 g), and a trend for lower birth weight Z-scores adjusted for gestational age and sex (-0.19 ± 0.79 vs. 0.11 ± 0.67). As expected based on our selection strategy, cases had significantly higher weight for age Z-score in infancy (1.25 ± 0.86 vs. 0.26 ± 0.59 , $P < 0.0001$) and higher BMI percentile in mid-childhood (95.5 ± 4.7 vs. 51.5 ± 15.5 , $P < 0.0001$). In mid-childhood, both total fat mass and lean mass were higher in cases vs. controls. Cases had a shorter duration of breastfeeding (4.0 ± 3.1 vs. 8.2 ± 4.0 months, $P < 0.001$). Demographic, pregnancy, and infancy characteristics are summarized in Table 1.

Unsupervised analysis: cord metabolites do not clearly discriminate cases and controls

We detected a total of 415 biochemicals, including 99 amino acids, 33 carbohydrates, 18 cofactors and vitamins, 42 xenobiotics, and 153 lipids in cord blood (full list, Supplemental Table 1). To better understand the structure of the cord blood metabolome in cases vs. controls, we used unsupervised principal components analysis (PCA) to identify metabolites contributing the most to observed differences in the dataset. PCA did not clearly separate the two groups (Supplemental Figure 1A). Similarly, unsupervised hierarchical clustering did not fully separate cases from controls, although visual inspection suggests that subsets of cases and controls did cluster together (Supplemental Figure 1B). We next used partial least squares discriminant analysis (PLS-DA) to identify metabolites predictive of case vs. control status. Two distinct clusters were observed (Supplemental Figure 1C), but the model was not statistically significant, with permuted p-value = 0.11.

Individual cord blood metabolites associated with case vs. control status

Mean levels of 16 biochemicals were significantly different between cases and controls (nominal $P < 0.05$); none reached a false-discovery-rate (FDR) threshold of < 0.25 . 2 metabolites, threonine and homostachydrine, were significantly higher in cases, while 14 metabolites were lower (Figure 1A-D and Table 2). The metabolite with the greatest fold-reduction (50% lower, $P = 0.01$) in cases was 4-vinylphenol sulfate. Two of the most significantly reduced metabolites in cases, dimethylglycine (18% lower, $P = 0.01$) and N-acetyl methionine (16% lower, $P = 0.01$), are methyl donors involved in one-carbon metabolism (Figure 1B). Three of the metabolites with lower levels in cases vs. controls are related to tryptophan or its metabolism: tryptophan betaine (48% lower, $P = 0.03$), tryptophyl leucine (26% lower, $P = 0.03$), and serotonin (46% lower, $P = 0.02$, Figure 1C). Three of the top-ranking metabolites were xenobiotics found in plants, including homostachydrine (abundant in alfalfa and citrus; 54% higher in cases, $P = 0.007$), N-methyl proline (citrus; 33% higher in cases, $P = 0.04$), and tryptophan betaine (legumes).

Given that several clinical characteristics linked to childhood obesity risk differed between cases and controls (i.e. maternal BMI, maternal age, and breastfeeding duration), we next used linear regression to calculate metabolite concentrations adjusted for these potential confounders. A total of 17 metabolites were significantly altered in cases vs. controls at adjusted $P < 0.05$ (Table 2B). Several of the metabolites identified in our raw comparison of cases vs. controls remained significant after adjustment, including homostachydrine, 4-vinylphenol sulfate, N-acetylmethionine, serotonin, tryptophyl leucine, and 1-stearoyl-GPE (adjusted $P < 0.05$). Of note, 5 of the 17 top-ranking metabolites in the adjusted analysis were related to tryptophan, including serotonin, tryptophan, tryptophyl leucine, N-acetyltryptophan, and indolelactate. We also observed lower levels of metabolites related to branched chain amino acids, including leucine, alpha-hydroxyisovalerate, and alpha-hydroxyisocaproate, in cases.

Pathway analysis of cord metabolites associated with case vs. control status

To test whether cord blood metabolites that differed in cases with accelerated postnatal weight gain vs. controls belonged to known biological pathways, we performed metabolite set enrichment analysis. Several pathways were enriched (false discovery rate < 0.25). The

most significantly enriched pathways were “Tryptophan Metabolism” ($P=0.02$) and “Excitatory neural signaling through 5HTR4/6/7 and serotonin” ($P=0.02$) (Supplemental Table 2). Also highly enriched were pathways associated with metabolism of other amino acids, including aspartate and beta-alanine ($P=0.02$ and $P=0.03$, respectively). Metabolites involved in metabolism of betaine, an organic osmolyte and methyl donor, were also significantly enriched ($P=0.02$).

Given that several pathways related to amino acid metabolism were altered, we examined the ratio of glutamine to glutamate, which reflects glutamine synthase activity, and has been linked to risk of obesity, insulin resistance and diabetes in humans (15). Plasma from cases tended to have a lower glutamine : glutamate ratio ($P=0.08$), a difference that became significant (33% lower, $P=0.046$) after excluding an outlier in the control group with glutamine levels >6 standard deviations below the mean (Figure 1E).

Cord blood metabolites predicted case vs. control status above and beyond clinical risk factors

Because infants with accelerated postnatal weight gain and overweight status in mid-childhood (‘cases’) differed from controls with regard to maternal pre-pregnancy BMI, maternal age, breastfeeding duration, and other covariates that could influence postnatal obesity risk, we next investigated whether cord blood metabolites were associated with the odds of being a case independent of these potential confounders. We therefore used logistic regression to identify clinical covariates predictive of case vs. control status and then tested whether cord blood metabolite levels independently contributed to the risk of being a case above and beyond the clinical risk factors. Using bivariate logistic regression, we found that breastfeeding duration (OR: 0.74, 95%CI: 0.62-0.89, $P=0.001$) and maternal age (OR: 0.81, 95%CI: 0.71-0.93, $P=0.003$) were associated with *reduced* odds of being a case, whereas pre-pregnancy maternal BMI (OR: 1.19, 95%CI: 1.04-1.36, $P=0.01$) and paternal BMI (OR: 1.21, 95%CI: 1.01-1.45, $P=0.04$) were the most significant *positive* predictors of case vs. control status (Table 3A). We next built stepwise multivariate logistic regression models, and found that breastfeeding duration, maternal age, and pre-pregnancy BMI were independently associated with case vs. control status, whereas the addition of covariates such as newborn weight, race, sex, and paternal BMI contributed little to the strength of the models. We sequentially added each of the top-ranking metabolites identified in our adjusted and unadjusted analyses (Table 2A/2B) to these logistic regression models to determine whether cord blood metabolites improved the ability to predict case vs. control status.

Many of the top-ranking metabolites substantially improved the strength of the logistic regression models. For example, adding cord blood levels of N-acetyl methionine to a model including breastfeeding duration, maternal age, and pre-pregnancy BMI improved the ability to discriminate cases from controls (R-squared for model *without* metabolite: 0.39, $P<0.0001$; R-squared for model with N-acetyl methionine: 0.54, $P<0.0001$, Table 3B). Similarly, adding metabolites related to tryptophan (e.g. tryptophyl leucine, tryptophan, indolelactate) also improved prediction ability, as did metabolites related to branched chain amino acid metabolism (e.g. alpha-hydroxyisocaproate, alpha-hydroxyisovalerate, leucine). Moreover, the associations between such metabolites and the odds of case vs. control status

were independent of the clinical covariates (Table 3B). Only the metabolites that improved the strength of the logistic regression models and contributed independently to the odds of case/control status are included in Table 3B.

Discussion

Among cases with rapid infancy weight gain and overweight in mid-childhood vs. controls without rapid weight gain and no childhood overweight, we found that mean levels of 16 cord blood metabolites were significantly different. Pathway analysis revealed that biochemicals related to tryptophan and one-carbon metabolism were enriched among the differentially-regulated metabolites. Moreover, the cord blood ratio of glutamine:glutamate was lower in cases vs. controls. Our analysis is limited by the fact that several clinical characteristics associated with childhood BMI, including maternal age, maternal BMI, and breastfeeding duration, differed between cases and controls. While many of the differentially regulated metabolites survived adjustment for identified covariates, we cannot exclude the possibility that additional factors related to maternal metabolism or breastfeeding that we did not measure or adjust for might have contributed to the observed differences. Moreover, no individual metabolites in our categorical analysis had significant false discovery rates. Logistic regression demonstrated that maternal age, pre-pregnancy BMI, and breastfeeding duration were associated with increased odds of being a case vs. a control; adding cord blood metabolites related to one-carbon and tryptophan metabolism to these prediction models improved the ability to differentiate cases with early childhood obesity risk from controls. Our results therefore suggest that cord blood metabolic signatures may be associated with early childhood obesity trajectories. However, the cross-sectional nature of our analysis and the potential influence of maternal or dietary factors on the cord metabolome preclude assessment of whether these metabolic pathways are causally related to infancy weight gain.

While many groups have used metabolomic profiling to identify disease biomarkers in adults at risk for insulin resistance (15), diabetes (7), and cardiovascular disease (16), few studies have examined plasma metabolomic patterns in infancy or childhood (6;17;18). A recent study found that cord blood phospholipids were predictive of early-onset type 1 diabetes (9). Cord blood metabolomic markers are also linked to neonatal hypoxia, prematurity, and intrauterine growth retardation (8;19;20). To our knowledge, our study is the first to characterize the cord blood metabolome of infants at risk for childhood obesity, prior to obesity onset.

Our data suggest that differences in maternal diet are associated with postnatal weight gain trajectories in infancy. Indeed, several of the top-ranking metabolites altered between cases and controls were food constituents – mostly plant products. For example, homostachydrine, higher in cases, is linked to dietary intake of citrus (21) and dairy products (22); N-methyl proline, reduced in cases, is linked to citrus consumption (22); 4-vinylphenol sulfate, lower in cases, is a styrene metabolite found in wine, cider, berries and peanuts (22); and tryptophan betaine, reduced in cases, is abundant in legumes. Moreover, pyridoxate, a vitamin B6 metabolite reduced in cases, is linked to multivitamin consumption, and maltose, reduced in cases, is a disaccharide abundant in many foods. Another metabolite with lower

levels in cases, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), is a uremic toxin, but also correlates with dietary intake of green vegetables, garlic, onions, and fish (22). CMPF has been described to compete with penicillin for binding to the renal tubule transporter rOat3; it is unclear whether a trend for higher penicillin G levels in cases vs. controls (fold-change: 2.83, $P=0.22$, not shown) might have contributed to observed reductions in CMPF in cases (23). Interestingly, elevations in CMPF have recently been described in the setting of impaired glucose tolerance, GDM, and T2DM; in mice, CMPF directly inhibits beta cell insulin secretion (24). Such data contrast with our observation of decreased CMPF levels in cord blood from cases despite a similar prevalence of GDM in both groups. Future studies will be required to assess transplacental passage and metabolism of this potentially important metabolite. Taken together, the numerous food and plant components among the most differentially regulated metabolites between cases and controls are largely consistent with the literature linking maternal dietary patterns (both during and after pregnancy) with offspring obesity (25).

A unique feature of our data is that it implicates specific nutrients and metabolites as potential contributors to early childhood obesity risk. To what degree such signatures might stem from differences in maternal BMI, which differed in cases and controls, or from differences in feeding/fasting in the hours leading up to delivery, vs. long-term dietary patterns, is not clear.

Our results point to an association between cord blood levels of metabolites related to tryptophan and one-carbon metabolism, and accelerated postnatal weight gain. Three of the 16 significantly altered metabolites in cases vs. controls are structurally related to or metabolized from tryptophan. Moreover, 2 of the 16 metabolites that differed in cases and controls were methyl donors, and metabolite set enrichment analysis revealed “Betaine Metabolism” as one of the top-ranking pathways altered between cases vs. controls (betaine is diet-derived methyl donor). Associations of biochemicals related to tryptophan and obesity trajectories in our analysis are intriguing in light of the multiple lines of evidence linking serotonin to appetite regulation, beta cell development, and brown adipose tissue thermogenesis, as well as links between diet-derived tryptophan betaine and sleep regulation (26-30). Metabolites involved in one-carbon metabolism (e.g. dimethylglycine, methionine, betaine) have also recently been implicated in the pathogenesis of obesity, insulin resistance, and fatty liver (15)(31), in addition to an established role in the regulation of epigenetic marks (e.g. DNA and histone methylation). However, it would be premature to conclude, from our analysis of cord blood, that these metabolite patterns are causally linked to obesity pathogenesis. Rather, these relationships may result from differences in dietary patterns among the mothers of cases, or from other differences between cases and controls that were not accounted for in our adjusted analyses. It will be important for future studies to further explore whether tryptophan or one-carbon metabolism are linked to childhood obesity risk.

Because our categorical and pathway analyses highlighted differences in the levels of several amino acids and their derivatives, we examined whether the glutamine:glutamate ratio – a marker for the activity of glutamine synthetase – differed in cases vs. controls. Cases had a reduced glutamine:glutamate ratio. Other groups have described reductions in cord blood glutamine levels and in the glutamine:glutamate ratio in LBW infants (8;32), but

this has not, to our knowledge, previously been assessed in the setting of early childhood obesity risk. Plasma glutamine levels fluctuate in response to stress, and are reduced during acute infection or trauma (33); the reduction in cord glutamine:glutamate ratio we observed in cases vs. controls might therefore reflect greater stress around the time of delivery. These associations are intriguing in light of an emerging literature linking reductions in plasma glutamine, and in the glutamine:glutamate ratio, to increased risk of diabetes, insulin resistance, and hypertension (15), as well as a recent study linking genetic polymorphisms in glutamate metabolism to cardiovascular risk in individuals with T2D (34). Because we did not measure cord blood insulin or c-peptide levels, we could not directly examine associations between infant hyperinsulinemia or cord HOMA-IR and glutamine:glutamate ratio, or other metabolites. This will be an important area for future analyses.

Our study has several limitations. First, observational nature of the analysis precludes causal inferences. Second, relatively few metabolites were significantly altered by case vs. control status – only 16 out of 415 detected metabolites were altered at $P < 0.05$ in our unadjusted analysis, and only 17 were significantly altered ($P < 0.05$) after adjustment for covariates. The small sample size likely limited our power to detect differences. Clinical heterogeneity between groups, such as differences in maternal BMI, may have contributed to differences in the cord blood metabolome. Future studies using propensity-matched cases and controls could address this possibility. We recognize the possibility that the cord blood metabolome may have limited “predictive” significance, instead providing a snapshot of the immediate developmental and metabolic status of the infant. This possibility is supported by the observation that relatively few metabolites ($n=13$) were significantly correlated with the change in weight from 0-6 months, yet large numbers of cord blood metabolites were significantly associated with infant adiposity ($n=42$) and newborn T4 ($n=34$) (not shown). It is also unclear to what extent the cord blood metabolome is influenced by maternal diet immediately prior to delivery as opposed to maternal, fetal or placental metabolism. Despite these limitations, pathway analysis allowed us to identify concordant regulation of both methyl donor and tryptophan-related metabolites. Some of the strengths include the detailed assessments of mothers during pregnancy, and the extensive information captured about clinical and environmental factors that may influence childhood obesity risk.

In summary, we examined the cord blood metabolome in infants with and without accelerated early childhood weight gain to better understand the nutritional and metabolic determinants of early-onset obesity risk. While we found few overall differences in the cord metabolome of cases vs. controls, several metabolites linked to tryptophan and one-carbon metabolism were significantly reduced in cord blood from cases with accelerated postnatal weight gain. Moreover, the ratio of glutamine to glutamate, which has recently been linked to insulin resistance and cardiometabolic risk, was lower in cases vs. controls. Our data raise the possibility that perturbed metabolite patterns may already be present in cord blood of infants at risk for rapid postnatal weight gain, prior to obesity onset. Elucidating the mechanistic basis for these metabolite alterations and their potential role in obesity pathogenesis will require future study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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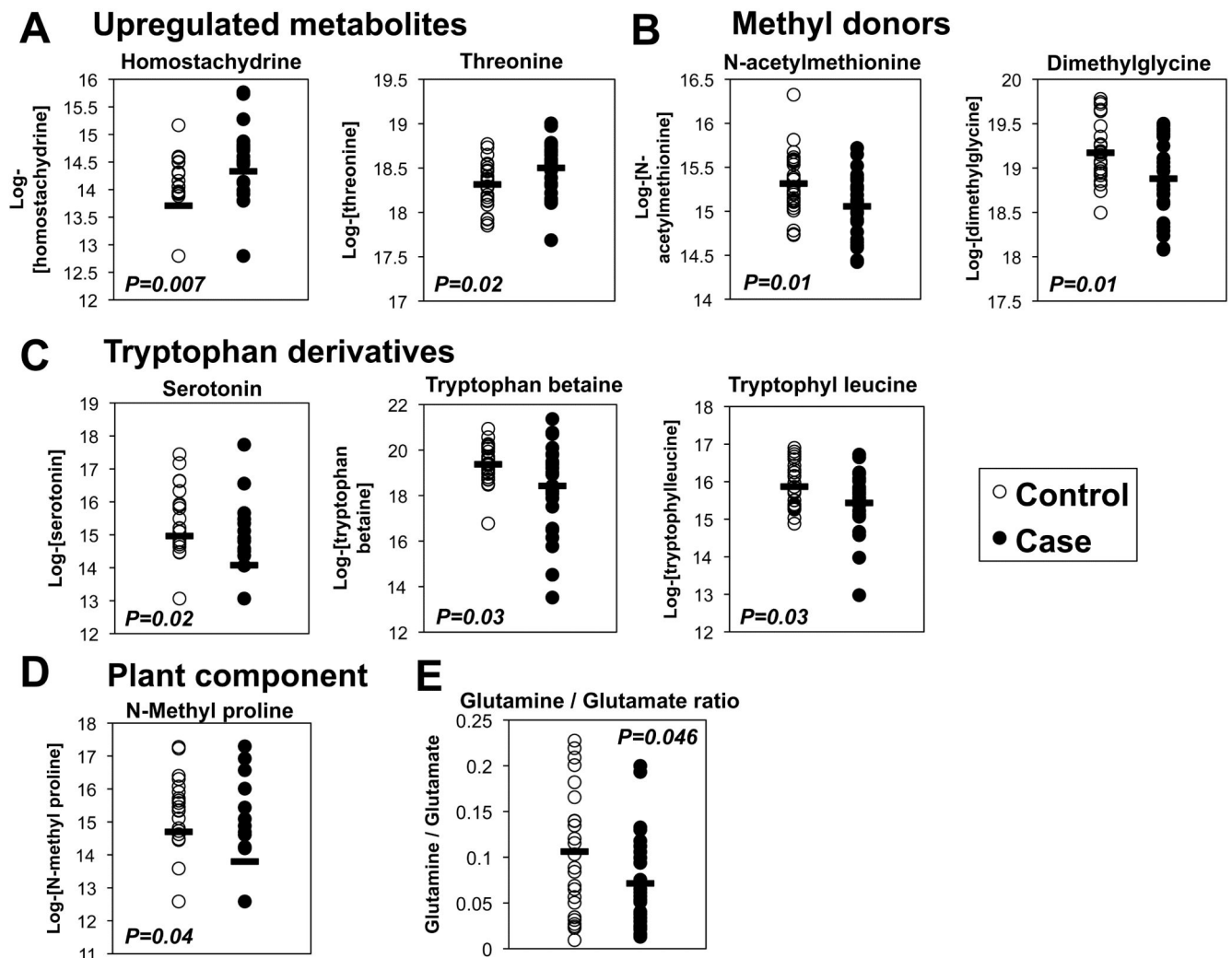


Figure 1. Selected top-ranking metabolites in categorical analysis of cases vs. controls

- A. Upregulated metabolites. N=26/group, $P < 0.05$.
- B. Methyl donors. N=26/group, $P < 0.05$.
- C. Tryptophan derivatives. N=26/group, $P < 0.05$.
- D. Plant components. N=26/group, $P < 0.05$.
- E. Glutamine to glutamate ratio. N=25 controls and 26 cases, $P < 0.05$.

Open circles denote controls; filled circles denote cases.

Table 1
Maternal, pregnancy, and early childhood characteristics

Maternal, pregnancy, and early childhood characteristics of 26 cases of accelerated postnatal weight gain and 26 controls with normal postnatal growth from Project Viva. If not otherwise noted, P-value refers to unpaired Student's T-test. For categorical data, P-value calculated using Fisher's exact test.

<i>Maternal & pregnancy characteristics</i>	Cases (n=26)	Controls (n=26)	P-value
	Mean (SD) or N (%)		
Maternal age, years	29.9 (5.4)	34.9 (4.4)	0.001
Race/ethnicity, N (%)			
White	13 (50)	19 (73)	0.13
Black	8 (31)	6 (23)	
Hispanic	4 (15)	0 (0)	
Other or more than 1 race	1 (4)	1 (4)	
Smoking status, N (%)			
Never	17 (65)	19 (73)	0.58
Former smoker	4 (15)	5 (19)	
Smoked during pregnancy	5 (19)	2 (8)	
Pre-pregnancy BMI, kg/m ²	27.9 (6.7)	23.6 (3.8)	0.01
Pre-pregnancy BMI category, N (%)			
Lean	11 (42)	20 (77)	0.04
Overweight	7 (27)	3 (11.5)	
Obese	8 (31)	3 (11.5)	
Gestational weight gain, kg	15.5 (5.5)	15.8 (5.2)	0.82
Glucose challenge test, mg/dl	110 (22)	113 (25)	0.67
Glucose tolerance, N (%)			
Normal	22 (85)	23 (88)	0.7
GDM	3 (12)	1 (4)	
Transient hyperglycemia	1 (4)	2 (8)	
Blood pressure status, N (%)			
Normal blood pressure	19 (79)	24 (96)	0.11
Gestational hypertension	4 (15)	1 (4)	
Pre-eclampsia	1 (4)	0 (0)	
Caesarian delivery			
No, N (%)	22 (85)	22 (85)	1
Yes, N (%)	4 (15)	4 (15)	
Father's BMI, kg/m ² (SD)	27.6 (3.7)	25.3 (3.5)	0.03
<i>Early childhood characteristics</i>			
Gestational age, weeks	39.2 (2.1)	39.8 (1.5)	0.2

<i>Maternal & pregnancy characteristics</i>	Cases (n=26)	Controls (n=26)	P-value
Neonatal anthropometry			
Birth weight, grams	3275 (458)	3487 (349)	0.07
Birth weight for gestational age and sex, Z-score	-0.19 (0.79)	0.11 (0.67)	0.15
Length for age and sex, Z-score	-0.38 (0.81)	0.00 (0.58)	0.16
Weight for length, Z-score	0.37 (0.94)	0.43 (0.78)	0.85
Breastfeeding duration, months	4.0 (3.1)	8.2 (4.0)	0.0002
Thyroid hormone (T4), microg/dl, newborn	18.7 (6.1)	17.8 (3.9)	0.68
Leptin, ng/ml, cord blood	6.4 (3.3)	8.0 (4.9)	0.19
Adiponectin, microg/ml, cord blood	27.4 (6.3)	26.9 (7.9)	0.83
Anthropometry, age 6 months			
Weight for age, Z-score	1.25 (0.86)	0.26 (0.59)	<0.0001
Length for age, Z-score	0.19 (1.22)	-0.19 (0.75)	0.21
Weight for length, Z-score	1.52 (1.00)	0.74 (0.60)	0.002
Change in weight for age Z-score 0-6 mo	1.64 (0.66)	0.24 (0.41)	<0.0001
Anthropometry at mid-childhood			
Weight, kg	40.6 (12.8)	26.4 (3.4)	<0.0001
Height, cm	132.2 (10.1)	128.5 (6.6)	0.12
BMI, percentile	95.5 (4.7)	51.5 (15.5)	<0.0001
Total fat mass (DEXA), kg	13.2 (4.7)	6.0 (1.2)	<0.0001
Total lean mass (DEXA), kg	16.6 (5.4)	20.4 (3.1)	<0.0001

Table 2
Top-ranking cord blood metabolites altered in cases vs. controls

A. Log2-transformed metabolite levels (missing values replaced with 0.5 * minimum value detected) in cord blood from 26 cases of accelerated early postnatal weight gain and 26 controls with normal growth trajectories from Project Viva. Shown are the 16 metabolites with unadjusted $P < 0.05$, out of the 415 metabolites detected. Fold-change was calculated as $2^{(\log \text{ mean Cases}) / [2^{(\log \text{ mean Controls})}]}$.

	Cases		Controls		Fold-change	P-value		FDR
BIOCHEMICAL	N	Mean (SD)	N	Mean (SD)	Case/Control	Unadjusted **	Adjusted ***	Q-value
Homostachydrine	22	14.33 (0.82)	16	13.71 (0.78)	1.54	0.007	0.02	0.97
4-vinylphenol sulfate	26	15.8 (1.39)	26	16.81 (1.27)	0.5	0.009	0.04	0.97
Dimethylglycine	26	18.88 (0.45)	26	19.17 (0.34)	0.82	0.01	0.14	0.97
N-acetylmethionine	26	15.06 (0.36)	26	15.32 (0.36)	0.84	0.01	0.01	0.97
Threonine	26	18.50 (0.30)	26	18.31 (0.25)	1.14	0.02	0.23	0.97
Serotonin (5HT)	12 *	14.08 (1.31)	20	14.96 (1.32)	0.54	0.02	0.03	0.97
Pyridoxate	26	17.26 (1.51)	26	18.08 (0.91)	0.57	0.02	0.11	0.97
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	26	17.40 (1.51)	26	18.27 (1.15)	0.55	0.02	0.45	0.97
Tryptophylleucine	25	15.43 (0.79)	26	15.87 (0.58)	0.74	0.03	0.02	0.97
Tryptophan betaine	25	18.42 (1.96)	26	19.37 (0.86)	0.52	0.03	0.22	0.97
Sphingosine	26	17.56 (0.94)	26	18.13 (0.94)	0.68	0.03	0.08	0.97
Maltose	22	18.83 (1.56)	26	19.67 (1.22)	0.56	0.03	0.12	0.97
N-methyl proline	11 *	13.80 (1.60)	19	14.70 (1.53)	0.54	0.04	0.74	0.97
Alanylalanine	10	11.40 (1.63)	17	12.35 (1.71)	0.52	0.04	0.28	0.97
2-docosahexaenoyl-glycerophosphocholine	26	18.31 (0.36)	26	18.53 (0.41)	0.86	0.04	0.06	0.97
1-stearoyl-glycerophosphoethanolamine	25	18.35 (0.84)	26	18.79 (0.72)	0.73	0.05	0.04	0.97

B. Log-2 transformed mean metabolite levels in cord blood from 26 cases of accelerated early postnatal weight gain and 26 controls with normal growth trajectories from Project Viva, adjusted for maternal BMI, maternal age, and infant breastfeeding duration. Shown are the 17 metabolites with adjusted $P < 0.05$, out of the 415 metabolites detected. Fold-change was calculated as $2^{(\log \text{ mean Cases}) / [2^{(\log \text{ mean Controls})}]}$.

	Cases		Control		Fold Change	P-value		FDR
BIOCHEMICAL	N	Mean (SD)	N	Mean (SD)	Case/control	Unadjusted **	Adjusted ***	Q-value
Alpha-hydroxyisovalerate	26	20.30 (0.52)	26	20.54 (0.34)	0.85	0.06	0.005	0.97

B. Log-2 transformed mean metabolite levels in cord blood from 26 cases of accelerated early postnatal weight gain and 26 controls with normal growth trajectories from Project Viva, adjusted for maternal BMI, maternal age, and infant breastfeeding duration. Shown are the 17 metabolites with adjusted $P < 0.05$, out of the 415 metabolites detected. Fold-change was calculated as $2^{(\log \text{mean Cases})/(\log \text{mean Controls})}$.

	Cases		Control		Fold Change	P-value		FDR
BIOCHEMICAL	N	Mean (SD)	N	Mean (SD)	Case/control	Unadjusted **	Adjusted ***	Q-value
N-acetylmethionine	26	15.06 (0.36)	26	15.32 (0.36)	0.84	0.01	0.01	0.97
Homostachydrine	22	14.33 (0.82)	16	13.71 (0.78)	1.54	0.01	0.02	0.97
Erythro-sphingosine-1-phosphate	25	16.43 (0.84)	26	16.75 (0.74)	0.8	0.15	0.02	0.97
Tryptophyllucine	25	15.43 (0.79)	26	15.87 (0.58)	0.74	0.03	0.02	0.97
Serotonin (5HT)	12 *	14.08 (1.31)	20	14.96 (1.32)	0.54	0.02	0.03	0.97
Beta-alanine	19 *	14.76 (1.35)	25	15.39 (0.84)	0.65	0.05	0.03	0.97
Tryptophan	26	24.22 (0.26)	26	24.32 (0.24)	0.94	0.17	0.03	0.97
2-aminobutyrate	26	18.59 (0.42)	26	18.64 (0.27)	0.97	0.65	0.03	0.97
Alpha-hydroxyisocaproate	26	13.72 (0.81)	26	13.90 (0.84)	0.88	0.44	0.04	0.97
Nicotinamide	26	16.75 (0.82)	26	17.08 (0.53)	0.8	0.09	0.04	0.97
Leucine	26	24.18 (0.28)	26	24.23 (0.21)	0.97	0.47	0.04	0.97
4-vinylphenol sulfate	20	15.80 (1.39)	26	16.81 (1.27)	0.5	0.01	0.01	0.97
N-acetyltryptophan	19	12.31 (1.16)	24	12.47 (0.74)	0.89	0.54	0.04	0.97
1-stearoyl-glycerophosphoethanolamine	25	18.35 (0.84)	26	18.79 (0.72)	0.73	0.05	0.04	0.97
Caprylate (8:0)	26	17.39 (0.41)	26	17.57 (0.40)	0.88	0.11	0.05	0.97
Indolelactate	26	19.89 (0.40)	26	19.96 (0.33)	0.95	0.46	0.05	0.97

* refers to metabolites for which the number of detectable samples (N) differed between cases and controls (Fisher's exact test).

** Unadjusted P-value refers to T-test in cases vs. controls.

*** Adjusted P-value derived from linear regression, adjusted for maternal age, BMI and child breastfeeding duration.

Table 3
Associations of maternal and infant characteristics with odds of case vs. control status

A. Bivariate logistic regression models of parental and perinatal covariates associated with odds of case vs. control status. B. Multivariable logistic regression models incorporating clinical and metabolite data.

A: Bivariate logistic regression		
Covariate	OR (95% CI)	P
Breastfeeding duration, months	0.74 (0.62-0.89)	0.001
Mat age at enrollment, years	0.81 (0.71-0.93)	0.003
Maternal pre-pregnancy BMI, kg/m ²	1.19 (1.04-1.36)	0.01
Father's BMI, kg/m ²	1.21 (1.01-1.45)	0.04
Birth weight, grams	1 (1.00-1.00)	0.08
Non-white race/ethnicity	2.71 (0.85-8.64)	0.09
Birth weight for gestational age and sex, Z-score	0.56 (0.26-1.23)	0.15
Leptin, ng/ml, cord blood	0.91 (0.79-1.05)	0.19
Gestational age at delivery, wks	0.80 (0.56-1.14)	0.21
T4, microg/dl, newborn	1.04 (0.88-1.23)	0.64
Glucose, maternal	0.99 (0.97-1.02)	0.66
Gestational weight gain, kg	0.99 (0.89-1.10)	0.81
Adiponectin, microg/ml, cord blood	1.01 (0.93-1.09)	0.82
Newborn weight for length, Z-score	0.91 (0.39-2.15)	0.84

B: Multivariate logistic regression		
Model	OR (95% CI)	P (chi-square)
Model A: R ² = 0.39, P<0.0001		
Maternal age at enrollment, years	0.83 (0.68-0.98)	0.02
Maternal pre-pregnancy BMI, kg/m ²	1.21 (1.04-1.49)	0.01
Breastfeeding duration, months	0.73 (0.57-0.89)	0.001
Model A + homostachydrine: R ² = 0.46, P<0.0001		
Homostachydrine	3.72 (1.20-15.7)	0.04
Model A + N-acetylmethionine: R ² = 0.54, P<0.0001		
N-acetylmethionine	0.006 (5.3×10 ⁻⁵ -0.17)	0.01
Model A + tryptophyl leucine: R ² = 0.47, P<0.0001		
Tryptophyl leucine	0.18 (0.03-0.76)	0.04
Model A + 1-stearoyl-GPE: R ² = 0.49, P<0.0001		
1-stearoyl-GPE	0.19 (0.03-0.66)	0.02
Model A + alpha-hydroxyisovalerate: R ² = 0.53, P<0.0001		
Alpha-hydroxyisovalerate	0.02 (0.0002-0.26)	0.02
Model A + tryptophan: R ² = 0.45, P<0.0001		
Tryptophan	0.03 (0.0004-0.71)	0.047

B: Multivariate logistic regression		
Model	OR (95% CI)	P (chi-square)
Model A + 2-aminobutyrate: $R^2 = 0.47$, $P < 0.0001$ 2-aminobutyrate	0.04 (0.002 – 0.59)	0.03
Model A + alpha-hydroxyisocaproate: $R^2 = 0.46$, $P < 0.0001$ Alpha-hydroxyisocaproate	0.28 (0.07-0.87)	0.04
Model A + nicotinamide: $R^2 = 0.46$, $P < 0.0001$ Nicotinamide	0.26 (0.07-0.81)	0.03
Model A + leucine: $R^2 = 0.44$, $P < 0.0001$ Leucine	0.04 (0.001-0.91)	0.046
Model A + indolelactate: $R^2 = 0.45$, $P < 0.0001$ Indolelactate	0.09 (0.007-0.81)	0.046