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Associations of cord blood metabolites with perinatal characteristics, newborn anthropometry, and cord blood hormones in Project Viva

Wei Perng[a], **Sheryl L. Rifas-Shiman**[b], **Scott McCulloch**[c], **Leda Chatzi**[d,e,f], **Christos Mantzoros**[g], **Marie-France Hivert**[b], and **Emily Oken**[b,h]

[a]Department of Nutritional Sciences, Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI [b]Division of Chronic Disease Research Across the Lifecourse, Department of Population Medicine, Harvard Medical School/Harvard Pilgrim Health Care Institute, Boston, MA [c]Metabolon, Inc., Durham, NC, USA [d]Department of Social Medicine, Faculty of Medicine University of Crete, Heraklion, Greece [e]Department of Preventive Medicine, Keck School of Medicine, University of South California, Los Angeles, CA [f]Department of Genetics & Cell Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands [g]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA [h]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

Abstract

Context—Metabolomics has emerged as a powerful tool to characterize biomarkers and elucidate physiological processes underlying adverse health outcomes. Little is known of these relationships during gestation and infancy, which are critical period for development of metabolic disease risk.

Objectives—To identify cord blood metabolite patterns associated with birth size; and to investigate relations of the birth size-associated metabolite patterns, and a branched chain amino acid (BCAA) metabolite pattern with a range of newborn and perinatal characteristics.

Methods—Using untargeted mass-spectrometry, we quantified metabolites in cord blood of 126 mother-child pairs. After excluding 103 xenobiotics, we used principal components analysis (PCA) to consolidate the remaining 606 metabolites into principal components (“factors”). Next,

Corresponding author: Wei Perng (perngwei@umich.edu) 1415 Washington Heights, Room 1860 SPH1 Ann Arbor, MI 48109-2029.

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we identified factors associated with gestational age-and sex-standardized birthweight z-score (BW/GA) and examined associations of the BW/GA-associated pattern(s) and the BCAA pattern with cord blood insulin, leptin, adiponectin, insulin-like growth factor (IGF)-1, IGF-2, and IGF binding protein 3 (IGFBP-3) using multivariable linear regression. Finally, we examined associations of maternal/perinatal characteristics with the cord blood metabolite patterns

Results—Mean BW/GA z-score was 0.27 ± 0.98 units. About half of the infants were male (52.4%) and white (57.1%). Of the 6 factors identified from PCA, one was associated with higher BW/GA: Factor 5, which comprised metabolites involved in energy production (malate, succinate, fumarate) and nucleotide turnover (inosine 5-monophosphate, adenosine 5-monophosphate, cytidine 5-monophosphate) pathways. In multivariable analysis, Factor 5 was related to higher cord blood leptin (1.64 [95% CI: 0.42, 2.87] ng/mL) and IGF-1 even after adjusting for IGFBP-3 (3.35 [0.25, 6.44] ng/mL). The BCAA pattern was associated with higher BW/GA (0.20 [0.03, 0.36] z-scores) and IGFBP-3 (106.5 [44.7, 168.2] ng/mL). No maternal characteristics were associated with either metabolite pattern; however, infants born via Cesarean delivery exhibited a higher score for Factor 5, and gestation length was inversely associated with the BCAA pattern.

Conclusions—Metabolites in energy production and DNA/RNA turnover pathways in cord blood are associated with larger size at birth, and higher leptin and IGF-1. Similarly, the BCAA pattern was associated with larger birth size and IGFBP-3.

Keywords

birth size; birthweight-for-gestational age z-score; IGF-1; IGF-2; IGFBP-3; cord blood leptin; metabolomics; branched-chain amino acids; neonatal adiposity; DNA/RNA; cord blood hormones

1. INTRODUCTION

In the field of developmental origins of health and disease, researchers have traditionally used size at birth as an indicator of short- and long-term health risk. For example, lower birthweight is related to risk of diabetes (1–3) and heart disease (4), while higher birthweight predicts future obesity (5). In recent years, researchers have assessed gestational age- and sex-specific birthweight as a more accurate gauge of fetal growth. Specifically, infants weighing less than the 10th percentile of gestational age- and sex-specific birthweight are categorized as small-for-gestational age (SGA), while those who fall above the 90th percentile are considered large-for-gestational age (LGA). Both ends of the birth size spectrum have been associated with a suboptimal gestational milieu (e.g., gestational diabetes with LGA (6); maternal smoking with SGA (7, 8)) and both are risk factors for poor cardiovascular and metabolic health later in life (1–4). However, birth size is only a crude indicator of the infant's health status and response to the intrauterine environment, given that there is considerable variability in long-term health outcomes across the continuum of birth size (9).

Metabolomics, the systematic study of low-molecular-weight compounds in biological tissues and fluids, has emerged as a powerful tool for elucidating aberrations in metabolism that predispose individuals for disease. Metabolomics analyses in children have found that higher circulating branched chain amino acids (BCAAs) are associated with obesity status

(10, 11), as well as subclinical risk factors for metabolic disease like insulin resistance (10, 12). Studies in adults have shown that elevations in serum branched chain amino acids (BCAAs) are detectable several years prior to development of insulin resistance and incident type 2 diabetes (13, 14), even among individuals of similar weight status (13). These findings shed light on biochemical pathways of diabetes pathogenesis that operate independently of body size, and also point toward BCAAs as potential early biomarkers of worsening glycemia. Accordingly, metabolomics analyses of cord blood may improve assessment of neonatal metabolic status beyond current knowledge of the implications of newborn size, and possibly may enhance assessment of future metabolic disease risk. To date, the literature includes only a few case-control studies comparing cord blood metabolite profiles of SGA (15) low birthweight (16), and very low birthweight (17–19) newborn vs. their AGA or normal birthweight counterparts, as well as a few studies exploring associations of maternal urinary metabolites and with fetal growth restriction (20, 21). In general, these analyses revealed associations of low birthweight and poor fetal growth with alterations in amino acid and lipid metabolites (15, 16, 18, 19) indicative of impaired nutrient transfer to the fetus during development, as well as compounds on energy and polyamine metabolism pathways (17) that are upregulated during period of rapid cell turnover. However, given that size at birth is associated with long-term health outcomes across the continuum of birthweight (9), studies exploring the relation of biomarkers/mediators and fetal growth should also evaluate the full distribution of birth size.

In this study of 126 mother-infant dyads in Project Viva, we aimed to: (1) identify metabolite patterns associated with newborn anthropometry using an agnostic (e.g., data-driven) approach, and to examine associations of a branched chain amino acid (BCAA) metabolite pattern previously associated with excess adiposity and poor metabolic health in this population during mid-childhood; (2) to examine associations of these metabolite patterns with cord blood hormones implicated in growth and development, and (3) to identify maternal and perinatal predictors of these metabolite patterns.

2. MATERIALS AND METHODS

2.1 Study population

This study included participants of Project Viva, an ongoing pre-birth cohort study recruited from a multi-specialty group practice in eastern Massachusetts. Details on study design and recruitment are reported elsewhere (22). In brief, we recruited pregnant women during the first trimester from 8 obstetric offices of Atrius Harvard Vanguard Medical Associates in Boston, MA between 1999 and 2002. Eligibility criteria included singleton pregnancy, ability to answer questions in English, and having had an initial prenatal visit before 22 weeks gestation. Upon enrollment (~20 weeks gestation), we collected information from the women on self-reported race/ethnicity, age, education, and parity; household income; lifestyle characteristics, including smoking habits during pregnancy; and partner's weight and height. The Institutional Review Board of Harvard Pilgrim Health Care approved all study protocols. All mothers provided written informed consent.

2.2 Cord blood collection and assays

At delivery, obstetricians and midwives collected blood from the umbilical cord vein in EDTA tubes. We collected umbilical cord blood from approximately half of births (those who delivered at 1 of 2 delivery hospitals). All samples were refrigerated immediately, processed within 24 hours, and stored at −80°C until time of analysis.

Cord blood hormones—We quantified the following hormones in cord blood hormones using commercial assays: leptin and adiponectin (radioimmunoassay; Linco Research Inc., St. Charles, MO)(23); insulin (competitive electrochemiluminescence immunoassay; Roche Diagnostics, Indianapolis, IN); insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-3 (ELISA, R&D Systems, Minneapolis, MN); and IGF-2 (ELISA, Alpco Diagnostics, Salem, NH). Day-to-day variabilities for each of these assays were <10%.

Cord blood metabolomics—In collaboration with Metabolon R (Durham, NC), we carried out untargeted metabolomics profiling in cord blood plasma via a multi-platform mass spectroscopy (MS)-based technique (24–26). We prepared samples using the automated MicroLab STARR liquid handling machine from Hamilton Robotics, which employs an aqueous methanol extraction (with recovery standards to monitor extraction efficiency) to precipitate proteins fraction while allowing maximum recovery of small molecules. We then divided the extract into four fractions: one each for analysis on four different columns on ultrahigh performance liquid chromatography (UPLC)/MS/MS (2 for positive ions, 2 for negative ions) and mixed the samples for 5 minutes on a Geno/Grinder 2000 (Glen Mills, Inc.), followed by brief placement on a TurboVapR (Zymark) to remove the organic solvent.

Next, we carried out sample extraction and ultrahigh performance liquid chromatography (UPLC) as described previously (26). The liquid chromatography (LC)/MS of the platform was based on a Waters ACQUITY UPLC and a Thermo-Finnigan linear trap quadrupole mass spectrometer, which consisted of an electrospray ionization source and linear ion-trap mass analyzer. We reconstituted the sample extract in acidic or basic LC-compatible solvents, each of which contained 8 or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot each was analyzed using a reverse-phase positive ion method for polar compounds, a reverse-phase positive ion method for lipid compounds, a reverse-phase negative ion method, and a negative ion method for hydrophilic compounds. The MS analysis alternated between MS and data-dependent MS/MS scans using dynamic exclusion. Raw data files are archived and extracted as described below.

For quality assurance/quality control (QA/QC) purposes, we included additional samples, including extracts of a pool created from a small aliquot of the experimental samples and process blanks, with each day's analysis. We spaced the QC samples evenly among the injections and all experimental samples, and randomly distributed them throughout the run. We added a selection of QC compounds to every sample for chromatographic alignment, including those under test. These compounds were carefully chosen so as not to interfere with the measurement of the endogenous compounds.

Finally, we extracted the raw data and identified compound peaks using Metabolon's hardware and software. We identified compounds via comparison to library entries of purified standards or recurrent unknown entities. More than 4000 commercially available purified standard compounds have been acquired and registered into Laboratory Information Management System (LIMS) for distribution to both the LC and GC platforms for determination of their analytical characteristics.

2.3 Newborn anthropometry and blood pressure

Newborn anthropometric measurements of interest include sex-standardized birthweight-for-gestational age (BW/GA) z-score, body mass index (BMI) z-score, birth length z-score, and head circumference. We obtained information on birthweight, measured by clinicians after birth to the nearest 1 g, from hospital medical records. Trained research assistants measured length and head circumference to the nearest 1 mm within the 12 hours of delivery. We determined gestational age at birth in weeks by subtracting the date of the self-reported last menstrual period (LMP) from the date of delivery. For subjects whose ultrasound pregnancy dating estimate differed by more than 10 days from LMP, we used the dating obtained from the 2nd trimester ultrasound to determine gestational age at birth. Using these data, we calculated BW/GA z-score as well as percentiles based on a U.S. natality reference (27), We defined SGA as BW/GA <10th percentile, and (LGA as BW/GA ≥90th percentile. Additionally, we calculated (BMI)-for-age z-score and length z-score using the World Health Organization (WHO) growth standard for infants 0 – 2 years (28, 29).

For each newborn, we obtained 5 measurements of blood pressure (BP) with a Dinamap Pro 100 automated oscillometric recorder (GE Medical Services, Tampa, FL), each taken 1 minute apart. For the present analysis, we used the average of the 5 measurements and focused on systolic (SBP) rather than diastolic BP (DBP) because of the validity of its measurement and superior prediction of long-term health outcomes (30).

2.4 Maternal, perinatal, and lifestyle characteristics during pregnancy

2.4.1. Maternal and perinatal characteristics

Pre-pregnancy BMI: At enrollment, women reported their pre-pregnancy weight. We used these values in conjunction with measured height to determine pre-pregnancy BMI (31).

Gestational weight gain: We determined gestational weight gain as the difference between the pre-pregnancy weight and the last clinically-measured weight within 4 weeks prior to delivery. We evaluated this variable continuously, as well as in categories of "inadequate," "adequate," and "excessive" (32).

Gestational glucose tolerance: We used medical record results from formal obstetric screening for gestational diabetes at 26–28 weeks of gestation to categorize women as having gestational diabetes mellitus (GDM), gestational impaired glucose tolerance, isolated hyperglycemia, or normoglycemia (33).

Hypertensive disorders of pregnancy: We reviewed outpatient charts for blood pressure and urine protein results and created a 4-level variable consisting of normotensive, chronic hypertension, gestational hypertension, and preeclampsia (34).

Delivery characteristics: We examined gestational age at delivery as a continuous variable as only 4 participants were born preterm (<37 gestational weeks). We obtained information on route of delivery from the medical record.

2.4.2. Lifestyle during pregnancy

Diet: Women completed a semi-quantitative 166-item food-frequency questionnaire (FFQ) administered at enrollment (~20 weeks gestation) and at 26–28 weeks gestation (35, 36). For the analysis, we took the average of frequency of intake between the two FFQs in order to derive the Western (comprising red meat, processed meat, refined grains, snacks, sweets and desserts, French fries, and pizza), and Prudent (comprising fruits, tomatoes, cabbages, green leafy vegetables, poultry, and fish) dietary patterns via principal components analysis (PCA) (37) and assessed adherence to each dietary pattern in quartiles.

Physical activity: At 26–28 weeks gestation, the women filled out a physical activity questionnaire modified from the physical activity/inactivity questionnaire (38). We parameterized physical activity during pregnancy as quartiles of total weekly hours of moderate-to-vigorous physical activity.

Smoking habits: At enrollment, we asked mothers whether they had ever smoked, and categorized those who had smoked >100 cigarettes in their lifetime as “ever smokers,” and those who smoked ≤100 cigarettes as “non-smokers.” Among ever-smokers, we categorized those who had smoked but quit before 3 months of pregnancy as “former smokers,” and those who smoked during the 3 months before pregnancy or at the delivery assessment as “early pregnancy smokers.”

2.5 Data analysis

The study sample for this analysis comprised 126 of 262 mother-child pairs from a published study on mid-childhood metabolite patterns, obesity, and metabolic risk (10). The only inclusion criteria for the present study was available cord blood for the metabolomics assays (≥50 uL; $n = 126$).

Step 1: Identification of relevant metabolite patterns—The metabolomics analysis yielded semi-quantitative concentrations expressed in quantion counts for 709 compounds. For purposes of this study, which focuses on identifying endogenous pathways, we excluded 103 metabolites on xenobiotic pathways (pharmaceuticals, food additives, or other exogenous substances). For the remaining 606 compounds, we replaced missing values with ½ the minimum detected value as we have previously done (10, 39). To prepare the data for statistical analyses, we converted all compounds into z-scores centered at the mean and scaled by 1 standard deviation (SD).

Next, we used PCA, an unsupervised multivariate technique suitable for reduction of high-dimensional data, to consolidate the 606 metabolites into groups of linearly independent components known as “factors,” which accounted for 60% of total variance in the original metabolites. We decided to retain 6 factors, which together accounted for 60% of total variance in the original metabolite dataset, based on the Scree plot and standard criterion of Eigenvalues >1. Each metabolite factor is depicted by a continuous and normally-distributed factor score, which is essentially a z-score that is calculated based on factor loadings of the component metabolites. The factor score for a given factor can be interpreted as the strength with which an individual’s metabolite profile followed the pattern captured by that particular cluster of metabolites.

To identify metabolite patterns of interest, we used linear regression models where each metabolite factor was the independent variable, mother’s race/ethnicity, and infant’s sex and gestational age at birth were covariates, and BW/GA z-score was the outcome. To limit the possibility of false-positive findings, we applied Bonferroni’s correction and considered a metabolite factor to be relevant to our analysis if the *P*-value associated with the beta estimate for the association between the factor and BW/GA z-score was < (0.05/6). Because the relationship between birth size and metabolic risk is likely not linear, we also used multivariable logistic regression to examine associations of each factor with odds of LGA (n = 23) vs. combined SGA (n = 2) and AGA (n = 101) due to the small number of SGA infants, also using Bonferroni-corrected *P*-values as criteria for statistical significance.

In addition to identifying metabolite patterns via the data-driven approach described above, we were interested in a branched chain amino acid (BCAA) metabolite pattern that was associated with excess adiposity and metabolic risk during mid-childhood in this cohort (10). To re-create this pattern in the present analysis, we identified the relevant compounds in cord blood (Table S1), weighted each compound by its factor loading value from the mid-childhood analysis, and calculated the BCAA factor score as the sum of the weighted compounds. To enhance comparability of the BCAA score to those derived from PCA, we standardized it as a z-score centered on the mean and scaled by 1 SD. After examining associations of the metabolite patterns with birth size, we also assessed relations of key metabolites within each pattern (e.g., those with factor loadings >|0.50|) with BW/GA z-score.

Step 2: Associations of metabolite patterns with newborn outcomes—We examined the extent to which the metabolite patterns of interest were associated with other measures of newborn anthropometry, including BMI z-score, length z-score, and head circumference; we also investigated blood pressure as well as cord blood insulin, leptin, adiponectin, insulin-like growth factor IGF-1, IGF-2, and IGFBP-3 using linear regression models where the metabolite factor was the independent variable and the newborn outcomes were assessed as continuous variables. We selected covariates for multivariable models based on bivariate associations and known predictors of birth size. The final models included maternal race/ethnicity, and child’s sex and gestational age at delivery. For models where IGF-1 and IGF-2 were the outcomes of interest, we also further adjusted for IGFBP-3 to examine associations with the two growth factors that are independent of the binding protein. While we present results for IGF-1 and IGF-2 with and without adjustment for

IGFBP-3, we focus on interpretation of the former as it represents the relationship between cord blood metabolites and unbound biologically active levels of the two hormones.

Step 3: Identify maternal characteristics associated with metabolite patterns— Finally, we investigated whether maternal, perinatal, and delivery characteristics known to be associated with BW/GA z-score were associated with the cord blood metabolite patterns. To do this, we used a separate linear regression model for each characteristic, we assessed its relation with the factor scores as outcomes. In the multivariable models, we accounted for maternal race/ethnicity, and child's sex and gestational age at delivery, with the exception of models for gestational age at delivery, which only included maternal race/ethnicity and child's sex as covariates.

In *post hoc* analyses, we further accounted for BW/GA z-score in models for gestational age at delivery and delivery model to assess the extent to which the association between these delivery characteristics and metabolite patterns were independent of birth size. We also carried out sensitivity analyses where we excluded the 4 cases of preterm birth. Exclusion of these 4 participants did not change the direction, magnitude, or precision of the estimates, thus the results we show are for all participants.

All models met standard assumptions for linear regression. The distribution of cord blood insulin was somewhat right skewed, but when we re-ran our analyses using the natural log-transformed version of the variable, the direction, magnitude, and significance of the estimates were very similar, thus we present the untransformed values for ease of interpretation and comparability across outcomes.

For all analyses, with the exception of our strategy for identification of relevant metabolite pattern(s) in Step 1, we focus on the direction, magnitude, and precision of the estimates rather than on statistical significance given that many of the outcomes are biologically and statistically correlated (Table S2).

We performed all analyses using Statistical Analyses System 9.3 software (SAS Institute Inc., Cary, NC).

4. RESULTS

Descriptive statistics

Mean ± SD BW/GA z-score was 0.27 ± 0.98; approximately half (52.4%) of the infants were male and 57.1% were white. Mean ± SD for all factors, as designed, were approximately 0.0 ± 1.0.

To gain a sense of the relations among the newborn characteristics, we calculated Spearman correlations (R) among the indicators of newborn size (BW/GA z-score, BMI z-score, length z-score, and head circumference), blood pressure, and cord blood hormones (leptin, adiponectin, IGF-1, IGF-2, and IGFBP-3) (Table S2). In general, the indicators of newborn size (BW/GA z-score, BMI z-score, length z-score, and head circumference) were highly correlated with each other (R >0.6), with the exception of BMI z-score with head

circumference (R = 0.49) and length z-score (R = 0.34); and moderately correlated with SBP and the cord blood hormones (R of 0.12 to 0.51).

Main analysis

We carried out the main analysis in four steps. First, to identify potential confounders to the relationship between the metabolites and newborn characteristics, we examined bivariate associations of perinatal and sociodemographic characteristics with BW/GA z-score. We found that married women and those with higher annual household income had infants with higher BW/GA z-score. Likewise, being heavier prior to pregnancy (e.g., overweight and obese prepregnancy weight status) and excessive weight gain during pregnancy were determinants of higher BW/GA z-score (Table 1).

Next, in addition to examining associations of the BCAA pattern (which was previously related to obesity and metabolic risk during mid-childhood in this population (10)), we sought to identify metabolite patterns associated with birth size via a data-driven approach. Using PCA, we consolidated the 606 compounds identified in laboratory analysis into principal components, and retained 6 for further investigation. Of them, Factor 5 (which accounted for 4.2% of the variance in the original metabolites) was the only principal component significantly associated with birth size after accounting for multiple comparisons using Bonferroni's correction (Table 2). Each 1-unit increment in Factor 5 was associated with 0.28 (95% CI: 0.12, 0.43) higher BW/GA z-score, and 1.81 (95% CI: 1.09, 3.01) greater odds of LGA vs. SGA+AGA. We also evaluated associations of the BCAA pattern with birth size. Each 1-unit increment in the BCAA pattern was associated with 0.20 (95% CI: 0.03, 0.36) units higher BW/GA Z-score. We observed a similar positive, but albeit non-significant, odds ratio of LGA vs. SGA+AGA.

To determine whether Factor 5 and the BCAA pattern were also associated with the other newborn characteristics (BMI z-score, length z-score, head circumference, blood pressure, and the cord blood hormones) we then used multivariable linear regression models that accounted for key confounders: maternal race/ethnicity, and child's sex and gestational age at delivery (Table 3). Each 1 unit increment in Factor 5 was associated with 0.28 (95% CI: 0.12, 0.43) units higher BW/GA z-score and 0.27 (95% CI: 0.07, 0.47) units higher BMI z-score, and each 1 unit increment in the BCAA pattern corresponded with 0.20 (95% CI: 0.03, 0.36) z-scores higher BW/GA. With respect to the cord blood hormones, Factor 5 was associated with higher leptin (1.64 [95% CI: 0.42, 2.87] ng/mL) and IGF-1 (6.60 [95% CI: 2.07, 11.13]) even after adjusting for IGFBP-3 (3.35 [95% CI: 0.25, 6.44] ng/mL). The BCAA pattern was related to higher IGF-1 (7.48 [95% CI: 2.86, 12.10] ng/mL) and IGF-2 (24.21 [95% CI: 7.01, 41.40] ng/mL), although both estimates were attenuated to the null after adjusting for the binding protein IGFBP-3. We also observed a strong positive association between the BCAA pattern and IGFBP-3 (106.45 [95% CI: 44.69, 168.21] ng/mL).

Finally, to gain insight into upstream etiological pathways underlying the relationship between the cord blood metabolites and newborn characteristics, we examined associations of several maternal and perinatal characteristics with Factor 5 and the BCAA pattern. Generally speaking, none of the maternal or perinatal characteristics were associated with

the metabolite patterns of interest (Table 4). However, we found an inverse relationship between gestational age at delivery and the BCAA pattern (−0.30 [95% CI: −0.47, −0.13] units lower BCAA score per 1.5 gestational weeks). We also found that a Cesarean delivery was associated with 0.49 (95% CI: 0.01, 0.97) units higher Factor 5, although this relationship was attenuated after accounting for BW/GA z-score: 0.36 (95% CI: −0.10, 0.83).

To aid in interpretation of Factor 5 and the BCAA pattern, we identified super-pathways and sub-pathways of key compounds within each pattern (e.g., those with factor loadings >|0.5|), and examined associations of these compounds with BW/GA z-score (Table S1 and Table S3). Compounds with the highest factor loadings and strongest associations with BW/GA z-score were those involved in energy production, amino acid, and lipid metabolism pathways.

5. DISCUSSION

In this study of 126 Project Viva mother-infant pairs, we consolidated 606 endogenous metabolites in cord blood into 6 metabolite patterns and identified one that was associated with birth size: Factor 5, which comprised several compounds on cell growth and energy production pathways. This metabolite pattern was also related to higher newborn BMI z-score, cord blood leptin, and cord blood IGF-1. We also considered a branched chain amino acid (BCAA) metabolite pattern that was previously implicated in obesity and metabolic risk in this population during mid-childhood and that predicts worsening insulin resistance (40) and overt type 2 diabetes in other populations (13). The BCAA pattern in cord blood was associated with higher BW/GA z-score and cord blood IGFBP-3. When we examined associations of maternal and perinatal characteristics with the metabolite patterns, we found that a Cesarean delivery was associated with a higher score for Factor 5 – a relationship that was attenuated after accounting for BW/GA z-score. We also detected an inverse relationship between gestational age at delivery and the BCAA pattern.

Associations of the Factor 5 metabolite pattern cord blood with newborn characteristics

Factor 5 comprised several metabolites of energy production and cell proliferation pathways. Compounds that exhibited the highest factor loadings (i.e. those that were of greatest relative importance to the metabolite pattern) as well as the strongest associations with birth size were malate, succinate, and fumarate, which are intermediates of oxidative phosphorylation from the tricarboxylic acid (TCA) cycle (41), the series of reactions that generate adenosine triphosphate (ATP) in aerobic organisms.

This metabolite pattern also captured several biochemicals involved in DNA and RNA metabolism, including both pyrimidine (cytidine 5′-monophosphate [5′-CMP], cytidine, 5,6-dihydrouracil) and purine (inosine 5′-monophosphate [IMP], adenosine 5′-monophosphate [AMP], hypoxanthine, xanthine, xanthosine) pathways primarily involved in the synthesis and degradation nucleic acids; spermidine and creatine, which are generated by polyamine cycling pathways during periods of rapid DNA and RNA turnover (42); and 5-oxoproline and N-acetylmethionine sulfoxide, markers of oxidative stress associated with hepatic glutathione production(43), a process that modulates DNA synthesis (44). Other noteworthy compounds within Factor 5 that were also associated with birth size included the

lipid metabolites glycerophosphoglycerol, glycerophosphorylcholine (GPC), and 1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2), which are markers of synthesis and turnover of the phospholipid backbone in cell walls. Together, compounds in Factor 5 – particularly those on energetic, DNA/RNA, and cell proliferation pathways – likely reflect the rapid growth and development that occurs during gestation.

When we examined associations of Factor 5 with newborn characteristics, we found positive associations with BW/GA and BMI z-score, which makes sense in light of the fact that rapid cell growth and proliferation likely correspond with more rapid overall growth; and with the cord blood hormones leptin and IGF-1, both of which are positively correlated with fetal growth and neonatal adiposity (45–47). Although the exact metabolites in Factor 5 have not been previously studied with respect to birth size or cord blood hormones, several pathways within this metabolite pattern were paradoxically found to be higher in very low birthweight (VLBW) infants(17). Using a mass-spectrometry based untargeted platform, Alexandre-Gouabau et al. (17) compared metabolite profiles of blood from the umbilical cord vein, which provides information on blood going from the placenta to the fetus, and the umbilical cord artery, which represents blood of the fetus directed to the placenta, and maternal venous blood for 7 VLBW infants (<32 weeks and/or birthweight <1500 g) vs. 8 controls (>37 gestational weeks and AGA). The researchers found evidence of enhanced polyamine cycling, as indicated by higher levels of acetylated spermine and putrescine in cord venous vs. arterial blood; upregulated TCA cycle activity and glutamine utilization, based on a lower levels of the precursor amino acids serine, betaine, glutamate, and methionine in cord arterial vs. venous blood; and enhanced lipid oxidation according to higher levels of acylcarnitine metabolites of short- and medium-chain fatty acids in cord vs. maternal venous blood among VLBW infants. Although we are not able to make inference on metabolic flux in our study since we only analyzed cord venous blood, the overlap in altered DNA and RNA cycling and energy production pathways detected in our analysis of predominantly AGA and LGA newborns and those identified among VLBW neonates emphasizes their importance in fetal growth and development.

Associations of the BCAA metabolite pattern with newborn characteristics

The BCAA metabolite pattern, which we created to mimic a metabolite pattern previously identified in our published study of metabolomics profiles and obesity during mid-childhood (10) and in other published studies of obesity and diabetes risk, was composed of amino acids on leucine, isoleucine, and valine metabolic pathways. Key compounds within this pattern included the BCAAs valine, leucine, and isoleucine (although only valine was associated with birth size in our study); several downstream intermediates of BCAA metabolism including 3-methyl-2-oxovalerate, and 4-methyl-2-oxopentanoate; and kynurenine, a derivative of tryptophan, a large neutral amino acid that shares cellular transport mechanisms with BCAAs (48).

The positive association of the BCAA pattern with BW/GA z-score aligns with findings in school-age children that higher serum BCAA is related to obesity status (10, 11) as well as continuous measures of adiposity (10). The relationship between BCAA in cord blood and birth size could arise from a number of pathways, including maternal hyperglycemia during

pregnancy, which is associated with elevated BCAA in maternal serum (49), excess maternal adiposity, which we previously found to be predictive of higher offspring serum BCAA during mid-childhood – even after accounting for the child's BMI (10), or possibly maternal diet, although the current literature on maternal physiological determinants of cord blood metabolites is scant.

With respect to existing literature regarding cord blood metabolites and birth size, our findings complement those of a small case-control study which found lower levels of leucine and valine in cord arterial blood among 56 preterm infants with intrauterine growth restriction as compared to 56 gestational-age matched AGA controls (50). The authors posited that lower cord blood BCAA among IUGR fetuses could be explained by the fact that plasma concentrations of BCAA are reduced in instances of chronic malnutrition. Accordingly, it is possible that in Project Viva where the average BW/GA is approximately 0.3 SD higher than the U.S. natality reference (27), higher cord blood BCAA is a marker of fetal overnutrition. Interestingly, in another untargeted metabolomics study that sought to identify cord vein blood metabolites that discriminated between 22 intrauterine growth restricted (IUGR; BW/GA $<10^{th}$ percentile) and 21 AGA infants, Favretto et al. detected elevations in amino acids captured by the BCAA pattern – namely, phenylalanine and tryptophan – among IUGR neonates. The authors hypothesized that higher levels of these compounds were due to increased placental protein catabolism and defective feto-placental amino acid transfer among IUGR fetuses (51), ultimately resulting in accumulation of amino acid compounds in venous cord blood. Although the direction of association for the relationship between compounds in the BCAA pattern and birth size are not consistent in the literature, the fact that metabolites within this pattern are consistently identified in studies of cord blood metabolites and birth size indicates a role for BCAA in the pathophysiology of abnormal fetal growth and emphasize the need for additional studies exploring metabolite profiles associated with both ends of the birth size spectrum to corroborate current evidence.

We also found that a higher score for the BCAA pattern corresponded with higher cord blood IGFBP-3. While there have not been any metabolomics studies investigating associations with this particular hormone, the positive association between the BCAA pattern and IGFBP-3 makes sense given that IGFBP-3 was positively correlated with birth size in this and other populations (52–54). Whether the relationship between BCAA and IGFBP-3 is a cause vs. consequence of the relationship between BCAAs and birth size deserves additional investigation in mechanistic studies.

Associations of maternal characteristics with cord blood metabolite patterns

The only maternal/perinatal variables that were associated with the metabolite patterns of interest were the delivery characteristics. Specifically, Cesarean delivery was associated with higher cord blood Factor 5, and gestation length was inversely related to BCAA metabolites cord blood. In *post hoc* analyses, we determined that the association between Cesarean delivery and Factor 5 was driven by larger size at birth.

While we did not find any significant associations between the other maternal or perinatal characteristics with the two metabolite patterns of interest, we noted that the BCAA score was higher in cord blood of women who were overweight (vs. normal weight) prior to

pregnancy, which is in line with literature on weight status and the BCAA pattern in serum of non-pregnant adults (55). On the other hand, a recent study by Hellmuth et al. reported no relationship between pre-pregnancy BMI and amino acids, including BCAAs, in serum of 167 pregnant women throughout pregnancy (56); a potential explanation for this discrepancy could be related to the type of fluid used for metabolomics analyses

Strengths & weaknesses

Strengths of this study include our relatively large sample size in comparison to other studies of cord blood metabolomics and birth size (n <50) (15–17, 19), the fact that we examined associations of cord blood metabolite patterns across a spectrum of birth size, and our ability to investigate associations of these patterns with additional neonatal anthropometric measurements, cord blood hormones, and maternal/perinatal characteristics.

Our study also has several weaknesses. First, assessment of venous cord blood metabolites at a single point in time precludes our ability to infer on upregulation vs. downregulation of specific pathways, or differences in metabolite concentrations across the maternal/fetal unit. Second, the cross-sectional assessment of metabolic profiles with newborn characteristics and cord blood hormones precludes temporal inference on potential mechanistic pathways. Third, the length of sample storage time may have caused degradation in specimen integrity. However, given that all samples were stored the same way across a similar length of time, we expect that inter-sample concentrations are preserved (57) and thus, the differences detected with respect to the exposures of interest are valid. Finally, because our study population was derived from a previous study focused on metabolite patterns associated with excess adiposity during mid-childhood, our results may not be generalizable to other populations.

6. CONCLUSIONS

In this sample of 126 Boston-area mother-child pairs, we found that metabolites of energy production and DNA/RNA turnover pathways in cord blood are associated with larger neonatal size, as well as higher leptin and IGF-1. Similarly, BCAA metabolites and compounds on related biochemical pathways (e.g., acylcarnitines, large neutral amino acids) were associated with larger birth size and IGFBP-3. Studies comparing metabolite concentrations in maternal blood, cord vein and cord artery blood are warranted to evaluate metabolic flux, and ascertain temporal ordering of metabolites vs. hormones in cord blood.

While the application of metabolomics to maternal-fetal medicine is still an embryonic science, the systematic and comprehensive nature of metabolomics analyses has potential to provide novel insights into the complex interactions between the mother and the developing fetus. Our results – if replicable in other populations – may serve as a foundation for mechanistic studies targeting specific biochemical pathways hypothesized to underlie excess infant adiposity, as well as prospective investigations of compounds within Factor 5 and the BCAA pattern with long-term metabolic health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BCAA	branched-chain amino acids
BW/GA	birthweight-for-gestational age z-score
BMI	body mass index
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
PCA	principal components analysis
SGA	small-for-gestational age
AGA	appropriate-for-gestational age
LGA	large-for-gestational age
MS	mass spectrometry
UPLC	ultrahigh performance liquid chromatography

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Table 1

Associations of maternal and perinatal characteristics with birthweight-for-gestational age (BW/GA) z-score of 126 infants in Project Viva

	N	Mean ± SD BW/GA z-score	*P*
Overall	126	0.27 ± 0.98	--
Maternal characteristics			
Age at enrollment			0.28
15–24 years	14	0.12 ± 0.88	
25–34 years	68	0.22 ± 1.01	
35–44 years	44	0.39 ± 0.96	
Marital status			0.01
Married/cohabiting	111	0.35 ± 0.97	
Single	15	−0.29 ± 0.84	
Race/ethnicity			0.06
Black	31	−0.03 ± 1.09	
Hispanic	11	−0.12 ± 0.58	
White	75	0.45 ± 0.93	
Other	9	0.31 ± 1.09	
Education			0.07
Less than college graduate	55	−0.02 ± 0.85	
College graduate	41	0.68 ± 0.99	
Graduate school	30	0.25 ± 1.00	
Annual household income			0.02
<$40,000	19	−0.25 ± 1.03	
$40,000 – $69,999	28	0.36 ± 1.04	
>$70,000	65	0.40 ± 0.91	
Pre-pregnancy BMI [b]			0.06
Normal weight (18.5–24.9 kg/m^2)	62	0.01 ± 0.87	
Overweight (25.0–29.9 kg/m ^{2})	37	0.72 ± 0.95	
Obese (≥30 kg/m ^{2})	27	0.26 ± 1.05	
Parity			0.30
0	47	0.05 ± 0.88	
1	48	0.52 ± 0.91	
≥2	31	0.22 ± 1.14	
Smoking habits during pregnancy			0.38
Never	81	0.21 ± 0.99	
Quit before pregnancy	30	0.49 ± 0.97	
Smoked in early pregnancy	15	0.18 ± 0.90	
Gestational weight gain[a]			0.007
Inadequate	14	−0.02 ± 0.93	
Adequate	43	0.01 ± 0.90	

	N	Mean ± SD BW/GA z-score	*P*
Excessive	69	0.50 ± 0.99	
Gestational glucose tolerance			0.94
Normoglycemic	95	0.26 ± 0.98	
Isolated hyperglycemia	14	0.23 ± 1.17	
Gestational impaired glucose tolerance	6	0.50 ± 1.05	
Gestational diabetes mellitus	11	0.30 ± 0.77	
Hypertensive disorders of pregnancy [c]			0.92
Yes	11	0.32 ± 0.77	
No	111	0.29 ± 1.00	
Delivery method			0.11
Cesarean	20	0.59 ± 0.97	
Vaginal	106	0.21 ± 0.97	
Preterm delivery (<37 gestational weeks)			0.51
Yes	4	−0.04 ± 1.01	
No	122	0.28 ± 0.98	
Infant sex			0.55
Male	66	0.32 ± 0.94	
Female	60	0.22 ± 1.02	

[a] According to the 2009 Institute of Medicine (IOM) guidelines (32).

[b] The “Normal weight” category includes 4 women classified as “Underweight” according to the W.H.O. Adult Weight Status categories.

[c] Excludes 2 participants with chronic hypertension.

Table 2

Associations of birthweight-for-gestational age z-score (BW/GA) and birth size categories with cord blood metabolomics factor scores

	β (95% CI)[a] in BW/GA z-score per 1 unit factor score		OR (95% CI)[b] of LGA vs. SGA+AGA	
	BW/GA z-score	*P*	LGA *n* = 23	*P*
Factor 1	0.06 (−0.12, 0.23)	0.53	1.21 (0.71, 2.07)	0.49
Factor 2	0.00 (−0.17, 0.17)	0.98	0.87 (0.53, 1.42)	0.57
Factor 3	0.11 (−0.06, 0.27)	0.21	1.39 (0.86, 2.23)	0.18
Factor 4	0.06 (−0.11, 0.23)	0.46	0.96 (0.60, 1.54)	0.87
Factor 5	**0.28 (0.12, 0.43)**	**0.0004**	1.81 (1.09, 3.01)	0.02
Factor 6	−0.09 (−0.27, 0.09)	0.33	0.93 (0.55, 1.57)	0.79
BCAA[c]	0.20 (0.03, 0.36)	0.02	1.51 (0.92, 2.47)	0.10

[a] Estimates are from a linear regression model with the factor score as the exposure, BW/GA as the outcome, and maternal race/ethnicity, infant sex, and gestational age at delivery as covariates. Bolded estimates indicate statistical significance after accounting for multiple comparisons using Bonferroni's correction ($P < 0.05/6$).

[b] Estimates are from a logistic regression model with the factor score as the exposure, LGA ($n = 34$) vs. SGA ($n = 2$) + AGA ($n = 101$) as the outcome, and includes the same covariates as footnote (a).

[c] A weighted metabolite pattern score based on a previously-characterized metabolite pattern implicated in excess adiposity and metabolic risk among Project Viva participants during mid-childhood (10).

Table 3

Associations of cord blood metabolomics factor scores with newborn characteristics

	N	Mean ± SD	β (95% CI) for newborn characteristics or hormone per 1 unit factor score[a]	
			Factor 5 0.00 ± 1.03	**BCAA 0.00 ± 1.00**
Characteristics at birth				
BW/GA z-score[b]	126	0.27 ± 0.98	**0.28 (0.12, 0.43)**	**0.20 (0.03, 0.36)**
BMI z-score [c]	73	0.55 ± 0.98	**0.27 (0.06, 0.47)**	0.11 (−0.10, 0.32)
Length z-score[c]	73	0.36 ± 1.15	0.05 (−0.15, 0.26)	0.17 (−0.03, 0.37)
Head circumference (cm)	76	34.2 ± 1.2	0.08 (−0.13, 0.30)	0.13 (−0.09, 0.34)
SBP (mmHg)	74	72.4 ± 9.6	0.43 (−1.72, 2.57)	0.54 (−1.57, 2.65)
DBP (mmHg)	74	43.8 ± 6.5	−0.02 (−1.53, 1.49)	−0.26 (−1.74, 1.23)
Cord blood hormones				
Insulin (μU/mL)	122	6.6 ± 5.6	0.29 (−0.66, 1.24)	0.95 (−0.01, 1.92)
Leptin (ng/mL)	111	8.6 ± 6.6	**1.64 (0.42, 2.87)**	1.03 (−0.12, 2.19)
Adiponectin (μg/mL)	113	29.0 ± 6.3	0.13 (−1.09, 1.34)	0.28 (−0.85, 1.41)
IGF-1 (ng/mL)	122	58.9 ± 26.9	**6.60 (2.07, 11.13)**	**7.48 (2.86, 12.10)**
Adjusted for IGFBP-3			**3.35 (0.25, 6.44)**	1.70 (−1.62, 5.02)
IGF-2 (ng/mL)	122	424.3 ± 99.9	**20.59 (3.72, 37.45)**	**24.21 (7.01, 41.40)**
Adjusted for IGFBP-3			7.61 (−2.80, 18.02)	0.90 (−10.21, 12.01)
IGFBP-3 (ng/mL)	122	1140.4 ± 364.2	60.09 (−1.88, 122.07)	**106.45 (44.69, 168.21)**

[a] Estimates are adjusted for mother's race/ethnicity; and child's sex and gestational age at delivery.

[b] According to a U.S. natality reference (27).

[c] According to the W.H.O. growth standard for children 0–2 years of age (25, 26).

Table 4

Associations of maternal perinatal characteristics with cord blood metabolomics factor scores

	β (95% CI) in factor score according to each maternal characteristic[a]	
	Factor 5	**BCAA**
Maternal pre- and perinatal condition		
Pre-pregnancy BMI		
Normal weight (*n* = 62)	0.00 (Reference)	0.00 (Reference)
Overweight (*n* = 37)	0.30 (−0.10, 0.71)	0.40 (0.02, 0.79)
Obese (*n* = 27)	−0.11 (−0.58, 0.35)	0.08 (−0.36, 0.53)
P-difference (Type 3 test)	0.21	0.12
Per 1 SD (5.7 kg/m²)	0.02 (−0.16, 0.21)	−0.02 (−0.19, 0.16)
Gestational weight gain		
Inadequate (*n* = 14)	0.33 (−0.27, 0.93)	−0.06 (−0.64, 0.51)
Adequate (*n* = 43)	0.00 (Reference)	0.00 (Reference)
Excessive (*n* = 69)	0.33 (−0.05, 0.72)	0.30 (−0.07, 0.67)
P-difference (Type 3 test)	0.22	0.20
Per 1 SD (6.6 kg)	0.08 (−0.11, 0.29)	0.09 (−0.10, 0.29)
Gestational glucose tolerance		
Normoglycemic (*n* = 95)	0.00 (Reference)	0.00 (Reference)
Isolated hyperglycemia (*n* = 14)	−0.26 (−0.84, 0.32)	0.31 (−0.25, 0.88)
Gestational impaired glucose tolerance (*n* = 6)	0.77 (−0.05, 1.58)	−0.07 (−0.86, 0.73)
Gestational diabetes (*n* = 11)	−0.36 (−0.97, 0.25)	0.00 (−0.59, 0.59)
P-difference (Type 3 test)	0.12	0.73
Hypertensive disorders of pregnancy[c]		
No (*n* = 111)	0.00 (Reference)	0.00 (Reference)
Yes (*n* = 13)	0.31 (−0.27, 0.90)	0.26 (−0.29, 0.82)
Maternal lifestyle characteristics during pregnancy		
Prudent dietary pattern		
Q1 (lowest adherence)	0.00 (Reference)	0.00 (Reference)
Q2	0.26 (−0.26, 0.78)	0.04 (−0.47, 0.55)
Q3	−0.03 (−0.54, 0.49)	0.00 (−0.51, 0.50)
Q4 (highest adherence)	0.34 (−0.17, 0.86)	0.15 (−0.36, 0.65)
P-trend	0.37	0.92
Western dietary pattern		
Q1 (lowest adherence)	0.00 (Reference)	0.00 (Reference)
Q2	−0.51 (−1.09, 0.07)	−0.16 (−0.73, 0.41)
Q3	−0.36 (−0.82, 0.10)	−0.35 (−0.81, 0.10)
Q4 (highest adherence)	0.01 (−0.48, 0.49)	−0.23 (−0.70, 0.25)
P-trend	0.15	0.50
Physical activity		
Q1 (lowest level)	0.00 (Reference)	0.00 (Reference)

	β (95% CI) in factor score according to each maternal characteristic[a]	
	Factor 5	**BCAA**
Q2	0.17 (−0.43, 0.77)	0.12 (−0.45, 0.70)
Q3	0.15 (−0.47, 0.77)	0.09 (−0.49, 0.68)
Q4 (highest level)	0.43 (−0.20, 1.06)	−0.15 (−0.75, 0.45)
P-trend	0.58	0.74
Smoking habits during pregnancy		
Never (*n* = 81)	0.00 (Reference)	0.00 (Reference)
Former (*n* = 30)	−0.19 (−0.62, 0.23)	0.24 (−0.16, 0.65)
Current (*n* = 15)	0.13 (−0.42, 0.69)	0.30 (−0.23, 0.83)
P-difference (Type 3 test)	0.55	0.33
Delivery characteristics		
Gestational age at delivery		
Per 1 SD (1.5 weeks)	−0.17 (−0.35, 0.01)	**−0.30 (−0.47, −0.13)**
Delivery mode		
Vaginal (*n* = 106)	0.00 (Reference)	0.00 (Reference)
Cesarean (*n* = 20)	**0.49 (0.01, 0.97)**	0.04 (−0.42, 0.50)

[a] Estimates are adjusted for maternal race/ethnicity, and child's sex and gestational age at delivery. Estimates for continuous gestational weight gain is further adjusted for pre-pregnancy BMI, and estimates for gestational age at delivery are only adjusted for maternal race/ethnicity and child's sex.

[b] The "Normal weight" category includes 4 women classified as "Underweight" according to the W.H.O. Adult Weight Status Classification categories.

[c] Hypertensive disorders include chronic hypertension (*n*=2), gestational hypertension (*n*=8), and preeclampsia (*n*=3)

Table 5

Associations (β [95% CI]) of Factor 5 and the BCAA pattern with adiposity and metabolic risk during mid-childhood (age 6–10 y).

	Factor 5	BCAA
Mid-childhood outcomes		
Adiposity		
BMI z-score	0.12 (−0.07, 0.30)	0.01 (−0.17, 0.19)
Waist circumference (cm)	0.58 (−1.01, 2.18)	1.27 (−0.34, 2.88)
DXA total fat (kg)	0.25 (−0.48, 0.99)	0.50 (−0.22, 1.22)
DXA trunk fat (kg)	0.12 (−0.22, 0.46)	0.21 (−0.12, 0.54)
Metabolic risk		
Glycemia		
Fasting insulin (uU/mL)	0.36 (−0.58, 1.30)	**1.01 (0.09, 1.93)**
Fasting glucose (mg/dL)	−0.23 (−3.03, 2.57)	−1.37 (−4.18, 1.44)
HOMA-IR[b]	−0.09 (−0.36, 0.19)	0.06 (−0.22, 0.34)
Lipid profile		
Triglycerides (mg/dL)	−2.83 (−6.57, 0.92)	−2.00 (−5.82, 1.82)
Total cholesterol (mg/dL)	−2.83 (−7.59, 1.93)	2.01 (−2.84, 6.85)
HDL (mg/dL)	−0.12 (−2.39, 2.15)	0.84 (−1.46, 3.15)
LDL (mg/dL)	−2.14 (−6.32, 2.03)	1.56 (−2.68, 5.81)
Adipocytokines		
Leptin (ng/mL)	1.20 (−0.42, 2.81)	**1.73 (0.03, 3.44)**
Adiponectin (ug/mL)	0.05 (−1.51, 1.61)	1.40 (−0.25, 3.04)
Blood pressure		
SBP (mmHg)	0.45 (−1.02, 1.91)	0.76 (−0.72, 2.24)
DBP (mmHg)	0.12 (−0.78, 1.03)	0.11 (−0.80, 1.03)
MetRisk z-score[d]	−0.03 (−0.12, 0.07)	0.01 (−0.09, 0.11)

[a] Estimates are adjusted for child's race, age at the mid-childhood visit, and sex.

[b] Calculated as [fasting insulin × fasting serum glucose]/405

[c] Calculated as the average of 5 internally-standardized z-scores for waist circumference, fasting insulin, fasting glucose, triglycerides/HDL, and (SBP+DBP)/2.