Maternal BMI and Glycemia Impact the Fetal Metabolome

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EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

OBJECTIVE

We used targeted metabolomics to determine associations of maternal BMI and glucose levels with cord blood metabolites and associations of cord blood metabolites with newborn birth weight and adiposity in mother-offspring dyads.

RESEARCH DESIGN AND METHODS

Targeted metabolomic assays were performed on cord blood plasma samples from European ancestry, Afro-Caribbean, Thai, and Mexican American newborns (400 from each ancestry group) whose mothers participated in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study and who had anthropometric measurements at birth.

RESULTS

Meta-analysis across the four cohorts demonstrated significant correlation of all cord blood metabolites analyzed with maternal fasting levels of the same metabolites at ~28 weeks' gestation except for triglycerides, asparagine/aspartate, arginine, and the acylcarnitine C14-OH/C12-DC. Meta-analyses also demonstrated that maternal BMI with or without adjustment for maternal glucose was associated with cord blood metabolites including the branched-chain amino acids and their metabolites as well as phenylalanine. One-hour but not fasting glucose was associated with cord blood 3-hydroxybutyrate and its carnitine ester, a medium-chain acylcarnitine, and glycerol. A number of cord blood metabolites were associated with newborn birth weight and sum of skinfolds, including a negative association of triglycerides and positive association of 3-hydroxybutyrate, its carnitine ester, and serine with both newborn outcomes.

CONCLUSIONS

Maternal BMI and glycemia are associated with different components of the newborn metabolome, consistent with their independent effects on newborn size at birth. Maternal BMI is associated with a newborn metabolic signature characteristic of insulin resistance and risk of type 2 diabetes in adults.

Maternal obesity and hyperglycemia have well-described associations with both short- and long-term adverse outcomes in offspring (1–3), including obesity, dyslipidemia, hypertension, and type 2 diabetes (4,5). Mechanisms underlying these associations are still poorly understood. However, it is widely assumed that changes in the intrauterine environment related to these maternal phenotypes underlie the observed associations.

One reflection of the intrauterine environment is the fetal metabolome. The fetal metabolome is generated primarily from two sources: transplacental transfer of metabolites and fetal synthesis and metabolism of circulating metabolites. Studies of the impact of maternal BMI and glucose levels on the fetal metabolome and association of

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the fetal metabolome with newborn outcomes are limited. However, it is likely that both maternal phenotypes affect the availability of metabolites for transplacental transfer to the fetus as well as fetal metabolism of metabolites. We and others have shown previously that maternal glycemia and BMI are associated with the maternal metabolome (6–10). Given the above, the current study was designed to determine the associations of maternal BMI and glycemia with the fetal metabolome in a large, multiethnic cohort of newborns as well as the associations of fetal metabolites with newborn outcomes.

RESEARCH DESIGN AND METHODS

Data and Sample Collection

The study included mother-offspring dyads who participated in the population-based Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study conducted 2000–2006 at 15 international field centers (11). Participating mothers underwent a 75-g oral glucose tolerance test (OGTT) between 24 and 32 weeks' gestation (as close to 28 weeks as possible), and fasting, 1-h, and 2-h glucose and fasting and 1-h C-peptide levels were measured. Maternal insulin sensitivity was estimated as described by Scholtens et al. (7) using glucose and C-peptide measurements from the OGTT. Cord blood samples were obtained within 5 min of delivery and prior to delivery of the placenta. Maternal samples collected at the OGTT and cord samples were stored at −70°C prior to metabolomics assays.

Maternal anthropometric measurements, including height, weight, and mean arterial pressure, were measured by trained personnel using standardized procedures and calibrated equipment at the OGTT and used to calculate maternal BMI. Gestational age was calculated as described previously (11), and self-identified ancestry, parity, and other demographic data were ascertained via questionnaire. Newborn anthropometric measurements including birth weight and sum of skinfolds (SSF) (sum of flank, triceps, and subscapular skinfolds) were recorded within 72 h of birth using calibrated equipment and standardized methods across field centers. Mode of delivery was obtained from the medical record.

Metabolic profiling was performed on a total of 1,600 cord plasma samples from offspring of Afro-Caribbean, Mexican American, Northern European, and Thai ancestry (400 from each ancestry group) as well as 1,600 maternal fasting samples. Mothers of the offspring were sampled to span the range of maternal glucose and BMI observed in HAPO (11).

Conventional Metabolites and Targeted Metabolomics Assays

Conventional clinical metabolite and targeted metabolomics assays on maternal fasting serum and cord blood plasma samples were conducted at the Duke Molecular Physiology Institute Metabolomics Laboratory as previously described (7,12). Briefly, conventional metabolites (lactate, triglycerides, β-hydroxybutyrate, nonesterified fatty acids, and glycerol) were measured on a Beckman-Coulter Unicell DxC 600 clinical analyzer using reagents approved for use in clinical diagnosis. Reagents for triglycerides and lactate were from Beckman (Brea, CA) and for 3-hydroxybutyrate and nonesterified fatty acids from Wako Chemicals USA (Richmond, VA). For free glycerol, we modified reagents for glycerol-blanked triglycerides (Roche, Indianapolis, IN). Intra- and interday coefficients of variation (CVs) were consistently <5% for all assays. Samples from each mother-newborn dyad (i.e., maternal fasting, maternal 1-h, and neonatal cord blood samples) were run together.

Targeted metabolomics assays for acylcarnitine and amino acid panels were performed by tandem mass spectrometry on an Acquity TQD Triple Quadrupole system (Waters Corporation, Milford, MA). Quantitative measurement of amino acids and acylcarnitines was achieved by addition of known quantities of stable isotope-labeled internal standards to biological samples. For amino acids, every targeted analyte had its own cognate stable isotope-labeled standard; for acylcarnitines, representative standards for each size class were provided. Use of internal standards for each assay module allowed quantitative and reproducible measurement of metabolites. Assays were run in a 96-well plate format with a calibration curve and two quality-control (QC) samples (high and low) at the beginning and end of each plate. Each plate contained 72 unknown biological samples with samples from mother-newborn dyads run together. Calibrants and QC samples were prepared by adding known quantities of analytes to dialyzed FBS. Use of two independent QC samples in each plate enabled monitoring of inter- and intraday precision of the assay. Coefficient of variation for the acylcarnitine assay over a span of several years is <15% and for plasma amino acids <10%. In total, 65 conventional and targeted metabolites were analyzed.

Statistical Analysis

Per-Metabolite Analysis

Prior to formal statistical analysis, acylcarnitine and β-hydroxybutyrate levels were log transformed, and outlying metabolite values, defined as values >5 SDs from the mean, were excluded from analysis. Additionally, data were excluded from analysis for one Mexican American newborn with >10 outlying metabolites.

Partial correlations between maternal and cord metabolite levels were calculated, with adjustment for field center, neonatal sex, gestational age at delivery, cord blood storage time, mode of delivery, and maternal age, mean arterial pressure, and BMI at OGTT.

Associations between phenotypes and metabolites with <10% missing data within sample type were identified using linear regression within ancestry group. Analyses treating maternal phenotypes as predictors and offspring metabolites as outcomes were adjusted for baseline covariates, including field center, mode of delivery, maternal mean arterial pressure, and age, neonatal sex, sample storage time, and gestational age at delivery. Maternal phenotypes of interest included BMI (adjusted for baseline covariates with and without adjustment for glucose), fasting and 1-h glucose (adjusted for baseline covariates with and without maternal BMI), and insulin sensitivity (adjusted for baseline covariates with and without maternal BMI). Analyses treating cord blood metabolites as predictors and newborn phenotypes as outcomes were adjusted for field center, mean arterial pressure, maternal age, neonatal sex, sample storage time, mode of delivery, maternal BMI at the OGTT, and gestational age at delivery with and without adjustment for cord C-peptide. False discovery rate (FDR) correction (13) was applied to all ancestry-specific analyses; FDR-adjusted P values <0.05 were considered statistically significant.

Meta-analysis

β-Estimates from per-metabolite linear regression analyses and Fisher Z

transformations of maternal metabolite-cord metabolite partial correlation coefficients were combined across ancestry groups using random effects meta-analysis with inverse variance weights and restricted maximum likelihood estimation for heterogeneity using the metafor R package (14). Heterogeneity of β-estimates for per-metabolite analyses among ancestry groups was assessed descriptively with I^2 statistics and formally tested via Cochran *Q* tests (15,16). Benjamini-Hochberg FDR correction (13) was again applied to meta-analysis results. Cochran *Q* test *P* values and FDR-adjusted meta-analysis *P* values <0.05 were considered statistically significant. All statistical analyses were conducted using R (version 3.2.2).

RESULTS

Mother-offspring dyads from four ancestry groups were examined. The offspring were roughly equally distributed between males and females, and mothers of the offspring spanned the range of maternal glucose levels and BMI observed in HAPO (Table 1).

Correlation Between Cord and Maternal Metabolite Levels

The correlation between maternal fasting (at ~28 weeks' gestation) and cord blood metabolite levels was determined (Supplementary Table 1). Consistent with the transfer of many metabolites across the placenta, essentially all metabolites showed a significant correlation between maternal and cord blood levels. Four exceptions were triglycerides, which are not transported across the placenta; asparagine/aspartate; arginine; and C14-OH/C12-DC (which circulates at low concentrations and was detectable in only 2 ancestry groups). Correlations varied across ancestry groups, but for 30 of the 50 cord metabolites significantly correlated with maternal metabolite levels, the partial correlation estimate from meta-analysis ranged from 0.20 to 0.48. Cord glucose also showed a weak correlation with maternal fasting glucose (0.14; $P = 1.2 \times 10^{-4}$) in the meta-analysis.

Association of Maternal Phenotypes with Cord Blood Metabolites

The association of maternal BMI, fasting and 1-h glucose, and estimated insulin sensitivity at the time of the OGTT with cord blood metabolites was examined. As these phenotypes are interrelated, associations with and without adjusting for related phenotypes were examined to define the independent association of each phenotype with the cord blood metabolome.

In a meta-analysis across the four ancestry groups, maternal BMI was positively associated with a number of cord blood metabolites (Table 2 and Supplementary Table 2). Most notable was the association of maternal BMI with cord blood levels of the branched-chain amino acids (BCAAs) leucine/isoleucine and valine, as well as their metabolic byproducts, propionyl carnitine (AC C3), butyryl carnitine/isobutyryl carnitine (AC C4/C*i* 4), and isovaleryl carnitine (AC C5). Also positively associated with maternal BMI was the aromatic amino acid phenylalanine. Adjusting for maternal FPG attenuated these associations to varying degrees, but all of the above associations remained significant (Supplementary Table 2). No cord metabolites were significantly associated with maternal BMI in individual ancestry groups.

Maternal FPG was not associated with any cord blood metabolites in the meta-analysis, although a positive ancestry-specific association with the carnitine ester of 3-hydroxybutyrate (AC C4-OH), with and without adjustment for maternal BMI, was observed in Mexican Americans. In contrast, maternal 1-h glucose was positively associated with a limited number of cord blood metabolites in the meta-analysis (Table 2). These included the

Table 1—Demographics of mothers and their offspring

	Afro-Caribbean	Northern European	Mexican American	Thai
Maternal parity, *N* (%)				
First child	189 (47.2)	203 (50.8)	85 (21.2)	211 (52.8)
Second child or more	211 (52.8)	197 (49.2)	315 (78.8)	189 (47.2)
Newborn sex, *N* (%)				
Male	208 (52.0)	209 (52.2)	180 (45.0)	201 (50.2)
Female	192 (48.0)	191 (47.8)	220 (55.0)	199 (49.8)
Mode of delivery, *N* (%)				
Vaginal	365 (91.2)	294 (73.5)	319 (79.8)	287 (71.8)
Prelabor cesarean section	22 (5.5)	64 (16.0)	58 (14.5)	70 (17.5)
In-labor cesarean section	13 (3.2)	42 (10.5)	23 (5.8)	43 (10.8)
Maternal characteristics, mean (SD)				
Age at OGTT (years)	25.7 (5.63)	29.4 (5.12)	29.0 (5.30)	28.0 (5.80)
BMI at OGTT (kg/m^2)	28.2 (6.26)	29.2 (5.27)	29.6 (5.19)	25.8 (3.66)
Mean arterial pressure (mmHg)	78.9 (7.51)	83.1 (6.89)	82.6 (7.32)	79.5 (7.13)
Fasting plasma glucose (mg/dL)	80.6 (6.99)	82.1 (6.14)	83.7 (7.20)	80.1 (6.50)
1-h plasma glucose (mg/dL)	123.2 (28.99)	131.7 (27.24)	135.6 (33.88)	148.0 (30.92)
2-h plasma glucose (mg/dL)	109.4 (22.51)	111.0 (20.34)	110.5 (23.03)	118.5 (23.92)
Insulin sensitivity	4.1 (1.73)	3.7 (1.44)	3.1 (1.20)	4.0 (1.45)
Newborn characteristics, mean (SD)				
Gestational age at OGTT (weeks)	27.1 (1.84)	28.6 (1.37)	26.9 (2.10)	28.1 (1.85)
Gestational age at delivery (weeks)	39.8 (1.21)	40.2 (1.15)	39.7 (1.14)	39.4 (1.26)
Cord C-peptide (μg/L)	1.0 (0.59)	1.1 (0.56)	1.1 (0.55)	1.0 (0.50)
Birth weight (g)	3,250.8 (440.34)	3,667.6 (485.28)	3,555.2 (444.09)	3,148.2 (373.52)
SSF (mm)	11.6 (1.82)	12.8 (2.74)	14.2 (3.06)	11.6 (2.34)
Sample storage time (years)	12.1 (1.19)	9.7 (1.26)	11.7 (1.60)	11.2 (0.93)

Table 2—Associations of maternal phenotypes with newborn cord metabolites

Metabolite	Afro-Caribbean		Northern European		Mexican American		Thai		Meta-analysis			
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	*Q*	*Q* test *P*	I^2
Maternal BMI (kg/m^2)													
AC C4/C*i*4	0.0068 (0.0029)	1.88e-01	0.0094 (0.0036)	1.53e-01	0.0087 (0.0034)	6.70e-02	0.0092 (0.0043)	4.58e-01	0.0083 (0.0017)	7.87e-05	0.41	9.74e-01	0.00
AC C3	0.0097 (0.0033)	6.50e-02	0.0092 (0.0036)	1.53e-01	0.0102 (0.0036)	5.75e-02	0.0031 (0.0047)	8.31e-01	0.0087 (0.0019)	8.02e-05	1.74	9.74e-01	0.00
Valine	0.5220 (0.2879)	3.07e-01	1.1494 (0.2904)	4.85e-03	1.3943 (0.4481)	4.63e-02	1.5170 (0.4632)	3.10e-02	1.0638 (0.2356)	1.14e-04	5.04	9.74e-01	42.21
Phenylalanine	0.2088 (0.0913)	1.93e-01	0.1583 (0.1115)	6.37e-01	0.3906 (0.1292)	4.63e-02	0.2347 (0.1353)	6.45e-01	0.2350 (0.0563)	4.11e-04	2.00	9.74e-01	0.00
AC C5	0.0083 (0.0046)	3.07e-01	0.0102 (0.0049)	4.31e-01	0.0151 (0.0056)	6.25e-02	0.0026 (0.0072)	8.91e-01	0.0096 (0.0027)	3.42e-03	2.01	9.74e-01	0.00
Leucine/isoleucine	0.3236 (0.2351)	5.76e-01	0.6703 (0.2381)	1.38e-01	1.4162 (0.3587)	4.87e-03	1.2310 (0.3494)	2.58e-02	0.8534 (0.2504)	5.87e-03	8.75	5.92e-01	66.95
Maternal insulin sensitivity													
AC C4/C*i*4	−0.0345 (0.0100)	1.07e-02	−0.0189 (0.0133)	5.04e-01	−0.0319 (0.0140)	1.51e-01	−0.0192 (0.0108)	7.34e-01	−0.0266 (0.0058)	2.95e-04	1.56	8.52e-01	0.00
AC C3	−0.0417 (0.0116)	1.07e-02	−0.0223 (0.0135)	5.04e-01	−0.0360 (0.0148)	1.35e-01	−0.0122 (0.0117)	8.88e-01	−0.0277 (0.0073)	4.28e-03	3.66	8.11e-01	23.81
AC C5-DC	−0.0262 (0.0108)	8.69e-02	−0.0227 (0.0107)	4.92e-01	−0.0142 (0.0138)	7.45e-01	−0.0164 (0.0113)	7.34e-01	−0.0206 (0.0057)	5.98e-03	0.67	9.15e-01	0.00
Leucine/isoleucine	−1.8664 (0.8205)	1.20e-01	−0.9002 (0.8902)	5.28e-01	−4.7055 (1.4966)	9.32e-02	−1.1477 (0.8891)	7.34e-01	−1.7003 (0.5081)	1.10e-02	5.26	8.11e-01	11.45
AC C5	−0.0507 (0.0158)	1.77e-02	−0.0301 (0.0183)	5.04e-01	−0.0595 (0.0231)	1.27e-01	−0.0084 (0.0180)	9.32e-01	−0.0361 (0.0111)	1.27e-02	4.35	8.11e-01	30.77
Valine	−2.5728 (1.0051)	7.91e-02	−0.9247 (1.0969)	5.60e-01	−3.3397 (1.8725)	3.56e-01	−1.1046 (1.1772)	8.88e-01	−1.7911 (0.5947)	2.34e-02	2.25	8.11e-01	0.00
AC C2	−0.0208 (0.0108)	1.77e-01	−0.0163 (0.0114)	5.04e-01	−0.0374 (0.0129)	1.05e-01	0.0012 (0.0140)	9.55e-01	−0.0189 (0.0068)	4.10e-02	4.19	8.11e-01	20.04
AC C4-OH	−0.0469 (0.0235)	1.77e-01	−0.0351 (0.0272)	5.05e-01	−0.0765 (0.0304)	1.27e-01	0.0024 (0.0286)	9.55e-01	−0.0388 (0.0144)	4.90e-02	3.75	8.11e-01	11.75
Glycerol	−0.0311 (0.0088)	1.07e-02	−0.0096 (0.0104)	5.39e-01	0.0058 (0.0115)	8.56e-01	−0.0104 (0.0084)	7.34e-01	−0.0123 (0.0075)	2.11e-01	7.08	8.11e-01	57.81
Asparagine/aspartic acid	−0.5692 (0.1804)	1.77e-02	0.0927 (0.2923)	8.45e-01	0.0153 (0.3053)	9.79e-01	0.1517 (0.2173)	8.88e-01	−0.1104 (0.1928)	6.33e-01	8.26	8.11e-01	60.58
Maternal fasting glucose (mg/dL)													
AC C4-OH	0.0034 (0.0059)	9.42e-01	0.0063 (0.0064)	6.95e-01	0.0183 (0.0051)	1.93e-02	−0.0016 (0.0064)	8.62e-01	0.0071 (0.0045)	6.06e-01	7.05	7.75e-01	56.45
Maternal 1-h glucose (mg/dL)													
3-Hydroxybutyrate	0.0052 (0.0018)	1.14e-01	0.0043 (0.0018)	3.72e-01	0.0041 (0.0014)	1.13e-01	0.0029 (0.0017)	3.84e-01	0.0041 (0.0008)	4.10e-05	0.85	9.93e-01	0.00
AC C4-OH	0.0033 (0.0014)	4.25e-01	0.0022 (0.0014)	3.72e-01	0.0032 (0.0010)	1.13e-01	0.0023 (0.0013)	3.84e-01	0.0028 (0.0006)	2.85e-04	0.60	9.93e-01	0.00
AC C10-OH/C8-DC	0.0013 (0.0012)	7.46e-01	0.0017 (0.0009)	3.72e-01	0.0016 (0.0008)	5.11e-01	0.0016 (0.0008)	3.84e-01	0.0016 (0.0004)	5.59e-03	0.10	9.93e-01	0.00
Glycerol	0.0011 (0.0005)	6.28e-01	0.0006 (0.0005)	5.06e-01	0.0007 (0.0004)	5.88e-01	0.0007 (0.0004)	3.84e-01	0.0007 (0.0002)	1.04e-02	0.46	9.93e-01	0.00

All amino acids and acylcarnitines, as well as 3-hydroxybutyrate, are measured in μmol/L. Glycerol is measured in mg/dL. Both ancestry-specific and meta-analysis *P* values represent the *P* value after FDR adjustment.

ketone body 3-hydroxybutyrate and its carnitine ester (AC C4-OH) as well as glycerol and 3-hydroxy-decanoyl carnitine (AC C10-OH/C8-DC). These associations were present before and after adjustment for maternal BMI (Supplementary Table 2). No ancestry-specific associations were found.

Like maternal BMI, maternal insulin resistance was associated with cord blood levels of the BCAAs leucine/isoleucine and valine and their metabolic byproducts, propionyl carnitine (AC C3), butyryl carnitine/isobutyryl carnitine (AC C4/C*i* 4), isovaleryl carnitine (AC C5), and glutaryl carnitine (AC C5-DC), in the meta-analysis (Table 2). Maternal insulin resistance was also associated with 3-hydroxybutyryl carnitine (AC C4-OH) and acetylcarnitine (AC C2). All of these associations were attenuated by adjustment for maternal BMI (Supplementary Table 2). In ancestry-specific analyses, maternal insulin resistance was associated with glycerol and asparagine/aspartate in cord blood from Afro-Caribbean newborns. These associations were again attenuated by adjustment for maternal BMI (Supplementary Table 2).

Given the previously described association of BCAAs and phenylalanine with insulin resistance in adult cohorts (17–20), we examined the association of cord blood BCAAs and their metabolic byproducts with the level of cord blood C-peptide, a peptide derived from fetal insulin processing and secretion (Table 3). As cord blood C-peptide levels are impacted by cord glucose levels, analyses were adjusted for cord glucose levels. A significant association of cord blood leucine/isoleucine but not valine with C-peptide levels was observed; however, the association was negative not positive; i.e., higher leucine/isoleucine levels were associated with lower C-peptide levels. Isovaleryl carnitine (AC C5), a product of BCAA metabolism, was positively associated with C-peptide levels. Cord blood levels of butyryl/isobutyryl carnitine (AC C4/C*i*4) and propionyl carnitine (AC C3), products of BCAA metabolism, as well as the aromatic amino acid phenylalanine were not associated with cord blood C-peptide levels.

Association of Cord Blood Metabolites With Newborn Outcomes

Subsequent analyses examined the association of cord blood metabolites with two newborn outcomes: birth weight and SSF

Table 3—Association of cord blood metabolites with cord C-peptide (μg/L)

	Afro-Caribbean		Northern European		Mexican American		Thai		Meta-analysis			
Metabolite	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	*Q*	*Q* test *P*	I^2
Leucine/isoleucine	−0.0009 (0.0011)	4.05e-01	−0.0044 (0.0011)	1.28e-04	−0.0017 (0.0008)	3.29e-02	−0.0026 (0.0010)	7.21e-03	−0.0023 (0.0007)	8.78e-04	5.92	1.15e-01	50.71
AC C5	0.1383 (0.0551)	1.25e-02	0.0549 (0.0572)	3.37e-01	0.0643 (0.0516)	2.14e-01	0.0596 (0.0477)	2.12e-01	0.0777 (0.0263)	3.09e-03	1.58	6.64e-01	0.00
AC C3	0.1427 (0.0772)	6.51e-02	0.1134 (0.0773)	1.43e-01	0.0149 (0.0811)	8.54e-01	0.0300 (0.0742)	6.87e-01	0.0757 (0.0387)	5.02e-02	1.94	5.86e-01	0.00
Valine	−0.0002 (0.0009)	7.84e-01	−0.0001 (0.0010)	8.96e-01	−0.0015 (0.0006)	1.65e-02	−0.0003 (0.0007)	6.42e-01	−0.0007 (0.0004)	7.70e-02	2.56	4.65e-01	7.47
AC C4/C*i* 4	0.1134 (0.0876)	1.96e-01	0.0492 (0.0777)	5.27e-01	0.0045 (0.0844)	9.58e-01	−0.0245 (0.0796)	7.58e-01	0.0331 (0.0410)	4.19e-01	1.52	6.77e-01	0.00
Phenylalanine	0.0037 (0.0028)	1.83e-01	−0.0022 (0.0025)	3.91e-01	−0.0030 (0.0022)	1.69e-01	−0.0005 (0.0025)	8.50e-01	−0.0008 (0.0014)	5.81e-01	3.98	2.64e-01	22.32

Values are adjusted for cord glucose. All amino acids and acylcarnitines are measured in μmol/L.

(Table 4 and Supplementary Tables 3 and 4). In a meta-analysis across the four cohorts, multiple cord blood metabolites were positively associated with newborn birth weight (Table 4), including the amino acids serine, proline, glutamine/glutamate, and glycine, as well as 3-hydroxybutyrate and its carnitine ester. The medium-chain carnitine esters C12-OH/C10-DC, C10-OH/C8-DC, C8:1-DC, C6-DC/C8-OH, and C8:1-OH/C6:1-DC were also positively associated with birth weight. Cord blood triglyceride levels were negatively associated with birth weight. In ancestry-specific analyses, a long-chain carnitine ester (3-hydroxyeicosanoyl carnitine/octadecanedioyl carnitine [AC C20-OH/C18-DC]) was negatively associated with birth weight in Thai newborns. Because fetal insulin is a major determinant of newborn size at birth (21), we performed similar analyses adjusting for cord C-peptide to determine which of the above associations were independent of fetal insulin (Supplementary Table 3). All of the previously reported associations were robust to adjustment for C-peptide. However, after adjustment for C-peptide, additional amino acids, including leucine/isoleucine, arginine, ornithine, and citrulline, were positively associated with birth weight, while a by-product of BCAA metabolism, AC C4/C*i* 4, was negatively associated with birth weight. The ancestry-specific, negative association of AC C20-OH/C18-DC in Thais also remained after adjustment for C-peptide.

A more limited number of metabolites were positively associated with SSF in the meta-analysis (Table 4), including proline, serine, 3-hydroxybutyrate and its carnitine ester (AC C4-OH), and the medium-chain carnitine esters AC C10-OH/C8-DC and C8:1-DC. In ancestry-specific analyses, AC C20-OH/C18-DC and triglycerides were negatively associated with SSF in Thais. All of the significant associations in the meta-analysis, except for AC C8:1-DC, were robust to adjustment for cord C-peptide (Supplementary Table 4), while new positive associations of glycine and ornithine were observed after adjustment for cord C-peptide. The ancestry-specific, negative associations of triglycerides and AC C20-OH/C18-DC remained in Thais after adjustment for C-peptide with new positive association of octenoyl carnitine (AC C8:1) and decatrienoyl carnitine (AC C10:3) in Afro-Caribbeans.

CONCLUSIONS

Previous studies in the HAPO cohort and other cohorts have demonstrated that the combined effect of maternal obesity and gestational diabetes mellitus (GDM) on newborn size at birth is greater than either obesity or GDM alone, suggesting that their effects on newborn outcomes are, in part, independent and additive (22–24). A recent study examined the impact of maternal obesity or overweight on the fetal metabolome but failed to show an association of these maternal phenotypes with cord blood metabolites (25). However, in contrast to our study, this previous study was small and may have been limited by low statistical power.

Consistent with the observation that maternal BMI and glycemia have independent effects on newborn size at birth, the current study demonstrated that maternal BMI and glucose levels were associated with different sets of cord blood metabolites. Maternal FPG was not associated with any cord blood metabolites in the meta-analysis, but the maternal response to a glucose load, as reflected by maternal 1-h glucose, was associated with 3-hydroxybutyrate and its carnitine ester, glycerol, and a medium-chain carnitine ester. Maternal BMI, on the other hand, was associated with the aromatic amino acid phenylalanine as well as the BCAAs and their metabolic by-products. Thus, one potential explanation for the independent effects of maternal glycemia and BMI on newborn outcomes would be their association with different metabolites in the fetal metabolome.

Newborn size at birth is a function of both lean body and fat mass (26). SSF reflects fetal adiposity, while birth weight reflects both lean body and fat mass. As might be expected, all of the metabolites associated with SSF were also associated with birth weight, while a broader array of metabolites were associated with birth weight but not SSF. This suggests that the subset of metabolites associated with SSF contributes to fetal fat accretion while the remaining metabolites associated with birth weight likely contribute primarily to lean body mass.

Fetal metabolites in cord blood are derived largely from transplacental transfer and/or fetal synthesis of metabolites. Our analyses demonstrated a significant correlation between fasting serum maternal metabolites at ~28 weeks' gestation and

cord blood metabolites. This suggests that the cord blood metabolome may reflect, in part, the fetal metabolome over the third trimester of pregnancy. With the exception of asparagine/aspartate and arginine, amino acids in the maternal and fetal metabolome were significantly correlated. Amino acids are transported across the placenta via a series of transporters (27), although transplacental transport may not fully account for the correlations; e.g., glycine, which is poorly transported across the placenta and is derived in significant quantities from fetal serine, was significantly correlated with maternal glycine levels. Another major group of metabolites is the acylcarnitines, which, with the exception of AC C14-OH/C12-DC, demonstrated a significant correlation between maternal and cord blood levels. Less is known about the placental transport of acylcarnitines. A previous study using in vitro perfused human placenta demonstrated transplacental transfer of acetylcarnitine (C2) but not palmitoylcarnitine (C16), a long-chain acylcarnitine (28). The transport of short- and medium-chain acylcarnitines was not examined. Acylcarnitines are generated by mitochondrial metabolism of amino acids and fatty acids. The extent to which fetal levels are generated by fetal synthesis versus placental transport is not known, but the results of the previous study noted above suggest that, at a minimum, fetal long-chain acylcarnitines are derived largely from fetal synthesis as opposed to transplacental transfer.

The association of maternal BMI with cord blood BCAAs and their metabolic by-products as well as phenylalanine is of particular interest. In previous cross-sectional and longitudinal studies, we and others have observed that the BCAAs and phenylalanine (together with tyrosine) are associated with obesity and insulin resistance and predict onset of and adverse outcomes in type 2 diabetes and cardiovascular disease (17–20). While association of the BCAAs, their metabolites, and aromatic amino acids with metabolic phenotypes is well described in adolescents and adults (17–20), studies in newborns are limited, and longitudinal studies of BCAAs and aromatic amino acid metabolism during development have not been performed. One recent small study reported that cord blood levels of leucine and two BCAA metabolites, α-hydroxyisovalerate

Table 4—Associations of newborn cord metabolites with birth weight and SSF

	Afro-Caribbean		Northern European		Mexican American		Thai		Meta-analysis			
Metabolite	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	*Q*	*Q* test *P*	I^2
Birth weight (g)													
3-Hydroxybutyrate	71.09 (22.67)	3.37e-02	77.44 (24.46)	4.50e-02	53.34 (21.43)	2.29e-01	35.87 (16.87)	1.92e-01	54.85 (10.43)	7.85e-06	2.64	9.69e-01	1.11
AC C10-OH/C8-DC	106.09 (38.36)	6.88e-02	105.83 (47.93)	3.00e-01	91.89 (41.63)	3.32e-01	71.19 (35.94)	2.18e-01	91.76 (20.14)	1.40e-04	0.55	9.92e-01	0.00
AC C4-OH	77.28 (28.37)	6.88e-02	67.20 (32.15)	3.35e-01	51.98 (29.20)	4.75e-01	45.67 (21.66)	1.92e-01	57.91 (13.47)	3.07e-04	0.91	9.88e-01	0.00
Proline	2.37 (0.99)	1.09e-01	1.46 (1.00)	3.78e-01	1.27 (0.85)	5.92e-01	1.76 (0.78)	1.73e-01	1.69 (0.45)	1.61e-03	0.78	9.92e-01	0.00
Triglycerides	−2.27 (1.77)	4.64e-01	−4.91 (1.49)	4.50e-02	−2.59 (1.64)	5.92e-01	−7.36 (1.77)	2.15e-03	−4.27 (1.13)	1.61e-03	5.56	9.69e-01	45.61
AC C6-DC/C8-OH	57.08 (41.17)	4.47e-01	73.07 (45.81)	3.78e-01	106.10 (39.03)	2.29e-01	56.91 (33.88)	3.26e-01	72.37 (19.63)	1.76e-03	1.09	9.88e-01	0.00
AC C8:1-DC	108.26 (54.59)	2.04e-01	102.97 (63.74)	3.78e-01	83.64 (57.48)	5.92e-01	98.04 (43.75)	1.73e-01	98.24 (26.66)	1.76e-03	0.10	9.98e-01	0.00
Serine	4.66 (1.50)	3.37e-02	1.52 (1.36)	5.54e-01	1.17 (0.96)	6.64e-01	2.57 (1.02)	1.26e-01	2.25 (0.66)	4.61e-03	4.24	9.69e-01	22.29
Glutamine/glutamic acid	0.50 (0.57)	6.05e-01	0.70 (0.70)	5.80e-01	0.25 (0.72)	9.53e-01	1.39 (0.49)	9.32e-02	0.82 (0.30)	3.60e-02	2.28	9.69e-01	0.00
AC C12-OH/C10-DC	31.17 (36.33)	6.05e-01	44.80 (44.93)	5.80e-01	20.11 (31.55)	8.98e-01	73.18 (27.97)	1.25e-01	45.12 (16.82)	3.94e-02	1.78	9.69e-01	0.00
Glycine	2.61 (0.73)	1.98e-02	1.28 (0.72)	3.78e-01	0.32 (0.48)	8.98e-01	1.08 (0.54)	2.18e-01	1.22 (0.46)	4.14e-02	6.96	9.69e-01	57.35
AC C8:1-OH/C6:1-DC	68.87 (58.03)	5.23e-01	34.76 (57.20)	7.69e-01	72.38 (55.60)	6.64e-01	78.09 (41.96)	2.64e-01	66.16 (25.87)	4.75e-02	0.40	9.96e-01	0.00
AC C20-OH/C18-DC	−36.09 (62.03)	6.97e-01	−16.75 (65.72)	8.96e-01	−28.13 (50.24)	8.98e-01	−155.62 (46.25)	2.28e-02	−66.11 (36.43)	1.56e-01	5.07	9.69e-01	42.57
SSF (mm)													
3-Hydroxybutyrate	0.1701 (0.0903)	2.51e-01	0.4307 (0.1398)	1.19e-01	0.1454 (0.1550)	8.36e-01	0.2611 (0.1077)	2.84e-01	0.2369 (0.0576)	2.09e-03	2.87	8.31e-01	0.02
Serine	0.0133 (0.0059)	1.88e-01	0.0126 (0.0078)	6.47e-01	0.0110 (0.0069)	6.99e-01	0.0125 (0.0065)	4.73e-01	0.0124 (0.0033)	5.34e-03	0.07	9.96e-01	0.00
AC C4-OH	0.1992 (0.1128)	2.51e-01	0.3173 (0.1839)	6.47e-01	0.2002 (0.2101)	8.36e-01	0.2631 (0.1382)	4.73e-01	0.2366 (0.0739)	2.12e-02	0.37	9.96e-01	0.00
AC C10-OH/C8-DC	0.3586 (0.1529)	1.66e-01	0.3605 (0.2766)	6.82e-01	0.3522 (0.2990)	7.33e-01	0.2800 (0.2289)	7.99e-01	0.3406 (0.1077)	2.12e-02	0.09	9.96e-01	0.00
Proline	0.0085 (0.0039)	1.96e-01	0.0027 (0.0057)	8.49e-01	0.0052 (0.0061)	8.36e-01	0.0091 (0.0050)	4.73e-01	0.0070 (0.0025)	4.41e-02	0.99	9.57e-01	0.00
AC C8:1-DC	0.5139 (0.2165)	1.66e-01	0.3805 (0.3651)	7.31e-01	0.7178 (0.4122)	6.99e-01	0.1050 (0.2802)	9.36e-01	0.4083 (0.1451)	4.41e-02	1.98	8.63e-01	0.00
Triglycerides	−0.0113 (0.0070)	2.51e-01	−0.0207 (0.0086)	4.37e-01	−0.0195 (0.0119)	6.99e-01	−0.0566 (0.0112)	3.46e-05	−0.0261 (0.0098)	5.83e-02	11.97	4.05e-01	76.80
AC C20-OH/C18-DC	−0.2296 (0.2447)	5.81e-01	−0.3050 (0.3751)	8.06e-01	−0.3386 (0.3592)	8.36e-01	−1.1683 (0.2952)	2.43e-03	−0.5179 (0.2345)	1.33e-01	6.81	8.31e-01	55.41

All amino acids and acylcarnitines, as well as 3-hydroxybutyrate, are measured in μmol/L. Triglycerides are measured in mg/dL. Both ancestry-specific and meta-analysis *P* values represent the *P* value after FDR adjustment.

and α-hydroxyisocaproate, improved models for prediction of rapid postnatal weight gain, a risk factor for childhood obesity (29).

The mechanism underlying the well-defined association between the BCAAs, their metabolites, and insulin resistance has not been fully defined, but the association of the BCAAs and by-products of their catabolism, including AC C4/C*i*4, C3, and C5, with insulin resistance suggests that flux through the BCAA catabolic pathway may contribute (30). Indeed, there is evidence to suggest that BCAA metabolites, as well as the BCAAs themselves, contribute to insulin resistance and metabolic dysfunction (18,20,30,31). As noted, the current study demonstrated association of higher maternal BMI with higher cord blood levels of the BCAAs and AC C4/C*i*4, C3, and C5. As the BCAAs are essential amino acids, their presence in the fetal metabolome likely reflects placental transfer; however, whether their metabolic by-products are transported across the placenta or the higher levels reflect fetal BCAA catabolism is not known. Together, the above findings suggest that offspring of mothers with higher BMI are exposed in utero to higher levels of BCAA with increased flux through BCAA catabolic pathways. The long-term metabolic consequences of this exposure and its contribution to maternal obesity-induced intrauterine programming are not known, although our studies did not demonstrate a consistent association of cord blood BCAAs and their metabolic by-products with higher cord C-peptide levels, as a marker of fetal insulin resistance.

In the current study, we also examined the association of cord blood metabolites with newborn outcomes. To date, studies examining the association of cord blood metabolites with newborn size at birth have focused primarily on intrauterine growth retardation (IUGR) and low–birth weight newborns compared with appropriate-for-gestational-age (AGA) newborns (32–34). Some studies have reported higher levels of phenylalanine and BCAAs in cord blood from offspring with IUGR or low birth weight compared with AGA newborns (32,33), while others demonstrated lower levels of BCAAs in IUGR compared with AGA infants (35). In our much larger multiethnic study, we observed a positive association of cord blood leucine/isoleucine but not valine and negative association of a metabolic by-product of isoleucine, butyryl/isobutyryl carnitine (C4/C*i*4), with birth weight but not SSF after adjustment for cord C-peptide levels. Consistent with our findings, Ivorra et al. (33) demonstrated higher cord blood levels of proline and glutamine in AGA compared with low–birth weight newborns; however, in contrast to our findings, they observed a higher level of citrulline in low–birth weight infants. A second study reported lower levels of proline, isoleucine, glutamate, and phenylalanine in AGA compared with IUGR newborns (32). These findings stand in contrast to our results in a much larger multiethnic cohort of newborns, which spanned the spectrum of birth weight, although a U-shaped curve of association for these metabolites cannot be excluded.

Also of interest in our studies was the association of cord blood serine and glycine with birth weight. Glycine provides methyl groups needed for nucleotide synthesis and cell division and is important for the synthesis of proteins, especially collagen (36,37). As noted, glycine is poorly transported across the placenta, and a major source of fetal glycine is fetal conversion of serine (38). A by-product of this conversion is 5,10-methylenetetrahydrofolate, which is used in folate-dependent one-carbon metabolism (36). In addition to birth weight, serine was also associated with newborn SSF. A role for serine in adipogenesis has not been described, but serine does play a role in phospho- and sphingolipid biosynthesis (36).

The impact of maternal glycemia on cord blood metabolites has been restricted largely to studies of offspring of mothers with treated GDM. Two small studies reported differences in a number of amino acids in offspring of mothers with GDM. Both studies reported higher levels of valine, methionine, and alanine in cord blood of offspring of mothers with GDM (39,40), while one also reported higher levels of histidine, arginine, lysine, and α-ketoisovaleric acid and the other higher levels of leucine, isoleucine, ornithine, glutamate, and proline and lower levels of glutamate in cord blood from offspring of mothers with GDM. One caveat of these studies is that the mothers were treated and there was no difference in birth weight between the GDM and normoglycemic offspring. In our much larger study, we did not observe association of maternal glycemia with cord blood amino acids; rather, association with a limited number of lipid-derived metabolites was observed.

Among the cord blood metabolites associated with maternal 1-h glucose was 3-hydroxybutyrate and its carnitine ester. Cord blood 3-hydroxybutyrate was also associated with newborn SSF and birth weight. In a related study in the HAPO cohort, we demonstrated that maternal 1-h glucose levels were positively associated with maternal 3-hydroxybutyrate levels, and random forest analyses suggested that 3-hydroxybutyrate contributed to the effect of maternal glycemia on newborn adiposity (6). Human fetuses are not thought to produce significant ketones of their own, but 3-hdroxybutyrate crosses the placenta efficiently in a concentration-dependent manner and is used by the fetus as both an oxidative fuel and substrate for lipid biosynthesis (41). It is not known whether transplacental transfer of 3-hydroxybutyrate contributes to fetal fat accretion, but this is one possible mechanism by which maternal glycemia impacts fetal adiposity. Alternatively, the association of cord blood 3-hydroxybutyrate with maternal 1-h glucose and newborn SSF could reflect the activity of other metabolic pathways that affect fetal adiposity. Maternal 1-h glucose was also associated with cord blood 3-hydroxy-decanoyl carnitine (AC C10-OH/C8-DC), and, similar to 3-hydroxybutyrate, maternal 1-h glucose was associated with maternal serum levels of AC C10-OH/C8-DC (6), while cord blood levels of AC C10-OH/C8-DC were associated with newborn birth weight and SSF. These data are consistent with transplacental transfer of this medium-chain acylcarnitine in the setting of maternal hyperglycemia, but whether this occurs and the mechanism by which it contributes to newborn size at birth are not known.

One paradoxical finding was the negative association of cord blood triglyceride levels with birth weight and SSF. We and others have demonstrated a positive association of maternal triglycerides with newborn size at birth (6,42,43). Triglycerides are not transported across the placenta but can be hydrolyzed with subsequent transport of fatty acids across the placenta. These transported fatty acids can undergo β-oxidation for energy production or be used for triglyceride synthesis. Previous studies have demonstrated

higher cord blood triglyceride levels in small-for-gestational-age babies (44,45). We have now demonstrated a negative association across the range of birth weight as well as a negative association with SSF in Thai newborns. The mechanism for this paradoxical finding is not clear but may relate to impaired incorporation of triglycerides into adipose tissue in lower–birth weight newborns and/or more rapid accumulation of triglycerides into adipose tissue in higher–birth weight newborns.

Our study had several strengths. First, it is the largest study to date of cord blood metabolites and their association with newborn outcomes. Second, the design of the study allowed for identification of metabolite associations common across multiple environments and ancestry groups, ensuring that results are applicable to diverse populations. Third, the availability of maternal phenotype and metabolite data allowed for examination of their relationship with the fetal metabolome. One limitation is the observational nature of the study, which complicates causal interpretation of the associations between maternal phenotypes, the newborn metabolome, and newborn outcomes. A second limitation is that targeted metabolomics measures only a defined subset of metabolites as opposed to a broader analysis of all metabolites. Finally, the findings await replication in other cohorts.

In summary, a broad range of cord blood metabolites are associated with newborn birth weight, while a more limited subset, most notably 3-hydroxybutyrate and its carnitine ester, a medium-chain acylcarnitine as well as serine and proline, is associated with SSF. Further definition of the contribution of these metabolites to newborn adiposity awaits further studies. We have also demonstrated that higher maternal BMI and to a lesser extent maternal hyperglycemia are associated with metabolic signatures in the newborn metabolome, which, in the case of maternal BMI, included aromatic amino acids and BCAAs and their metabolites, a metabolic signature associated with insulin resistance and risk of type 2 diabetes in adults. A remaining question is the role of this metabolic signature in intrauterine programming.

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