Metabolic networks and metabolites underlie associations between maternal glucose during pregnancy and newborn size at birth

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ABSTRACT

Maternal metabolites and metabolic networks underlying associations between maternal glucose during pregnancy and newborn birth weight and adiposity demand fuller characterization. We performed targeted and non-targeted gas-chromatography/mass-spectrometry metabolomics on maternal serum collected at fasting and 1-hour following Trutol consumption during an oral glucose tolerance test for 400 Northern European mothers at ~28 weeks gestation in the Hyperglycemia and Adverse Pregnancy Outcome Study. Amino acids, fatty acids, acylcarnitines and products of lipid metabolism decreased and triglycerides increased following glucose ingestion during the OGTT. Analyses of individual metabolites indicated limited maternal glucose associations at fasting, but broader associations including amino acids, fatty acids, carbohydrates and lipids at 1-hour. Network analyses modeling metabolite correlations provided context for individual metabolite associations and elucidated collective associations of multiple classes of metabolic fuels with newborn size and adiposity, including acylcarnitines, fatty acids, carbohydrates and organic acids. Random forest analyses indicated improved ability to predict newborn size outcomes using maternal metabolomics data beyond traditional risk factors including maternal glucose. Broad scale association of fuel metabolites with maternal glucose is evident during pregnancy, with unique maternal metabolites potentially contributing specifically to newborn birth weight and adiposity.
Offspring of mothers with pre-existing or gestational diabetes mellitus (GDM) are at risk for higher birth weight (BW) and adiposity as well as childhood metabolic disorders including obesity, impaired glucose tolerance, and dyslipidemia (1-3). Mechanisms underlying these risks are not well defined but likely relate to fetal overnutrition in the setting of available maternal fuels (2; 3).

One fuel present in increased supply in GDM is glucose. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study, a population-based study of >23,000 women conducted from 2000-2006, and others demonstrated a linear relationship between maternal glucose and offspring BW and fatness (2; 4). This is likely mediated through glucose-stimulated insulin secretion in the fetus. Additional fuels also likely contribute (5). For example, pregnant women with GDM have increased circulating triglycerides in the third trimester. Maternal free fatty acids, which are derived from triglycerides, cross the placenta, serve as substrates for triglyceride synthesis, and contribute to fetal growth (2; 3; 6). Amino acids in maternal fasting plasma have been correlated with BW among women with diet-controlled GDM (7). The role of these and other fuels in risks associated with maternal hyperglycemia is unknown.

To address possible metabolic linkages between maternal hyperglycemia and offspring phenotypes, we performed targeted and non-targeted metabolomics together with pathway, network and random forest analyses on 400 European ancestry HAPO mothers.

**RESEARCH DESIGN AND METHODS**

**Participants**
We studied 400 HAPO Caucasian mother-offspring dyads of Northern European ancestry from Belfast, UK, Brisbane and Newcastle, Australia field centers (Table 1). Dyads were sampled so that maternal glucose and BMI, newborn BW and sum of skinfolds (SS), spanned the range observed in HAPO (4).

**Data and Sample Collection**

HAPO (conducted 2000-2006) methods were described previously (4). Eligible women underwent a 75-g OGTT at 24-32 weeks’ gestation. Fasting (FPG) and 1-hr plasma glucose were measured. Serum samples collected during the HAPO OGTT were stored at -80°C until the present metabolomics assays. Maternal height and weight and newborn BW and SS were measured using standard procedures and calibrated equipment. Gestational age was determined as previously described (4). Demographic and lifestyle characteristics were collected via questionnaire. Participants, caregivers and HAPO staff remained blinded to glucose values unless glucose exceeded predefined levels. “Unblinded” participants were excluded.

**Conventional Metabolite and Targeted Amino Acid and Acylcarnitine Analyses**

Conventional metabolites (lactate, triglycerides, β-hydroxybutyrate, glycerol) were measured as previously described (8) with the addition of non-esterified fatty acids (NEFA) using reagents from Wako (Mountain View, CA). Targeted assays of amino acids and acylcarnitines (ACs) were performed using stable-isotope-labeled internal standards on an Acquity TQD Triple Quadrupole system (Waters Corporation, Milford, MA) (8). In total, 63 conventional and targeted metabolites were analyzed to complement non-targeted GC/MS analyses to the fullest extent possible.
Non-targeted GC/MS Analyses

Non-targeted assays, which were designed to analyze the full range of metabolites present in serum, were performed using gas-chromatography (GC)/mass-spectrometry (MS). Methanol, the extraction solvent, was spiked with a retention-time-lock (RTL) internal standard of perdeuterated myristic acid. Extracts were dried, prepared by methoximation and trimethylsilylation (8; 9), and run on a 6890N GC-5975 Inert MS (Agilent Technologies, Santa Clara, CA). Programmed-temperature vaporization in the inlet and post-run, mid-column, hot back-flushing of the GC column minimized analyte decomposition, carryover, and fouling of GC and MS. Peaks were deconvoluted with AMDIS freeware (10), and parsed against the Fiehn RTL spectral library (9) with additions from our laboratory. Detected peak areas were log$_2$-transformed for analysis. Manual curation included identification of co-eluting groups of isomeric metabolites and selection of reliable peaks (8). Some fatty acids are methylated during sample preparation, and the methylated fatty acid was used in analyses. alpha- and beta-Monopalmitin likely represent both endogenous metabolite and contaminant from sample preparation. In total, 84 GC/MS metabolites not assayed using targeted approaches were used for data analysis.

Batches of fasting and 1-hr maternal serum sample pairs were constructed to balance field center, maternal glucose and BMI and newborn outcomes across batches. Quality control (QC) pools were prepared with equal volumes from all maternal samples and prepared for analysis as described above. QCs were injected as first, middle and last samples of each GC/MS run. Batches of equal size were run over 50 consecutive days.
Data Analysis

GC/MS data were normalized to control technical variability attributable to batch and run order using a mixture model approach in the metabomxtr R package (11). The mixture model can be viewed as a combination of a linear and logistic regression models with the linear portion modeling quantifiable metabolite abundance and the logistic portion modeling detectability or lack thereof for a metabolite in a given sample. Given batch-specific detection thresholds, this approach is uniquely suited to GC/MS data. We specified separate mixture models for each metabolite using QC data with metabolite level as the outcome, categorical batch variables in the logistic and linear model components and log-transformed run order in the linear component. Variations in metabolite levels due to batch and run order were identified using the QC data and then subtracted from analytical sample data to control technical noise for each metabolite.

Differences between maternal fasting and 1-hr metabolite levels were evaluated using paired t-tests for metabolites with ≥90% observed data. Associations between maternal fasting and 1-hr glucose with metabolites at corresponding time points were evaluated using separate linear models with metabolites as outcomes and glucose as predictors. For GC/MS metabolites undetectable in >10% of samples, mixture models were used (11), similar to normalization. Linear models were also used to investigate associations between maternal metabolite predictors and newborn SS and BW outcomes. GC/MS metabolites undetectable in >10% of samples were treated as four categories, one for undetected values and three for tertiles of normalized log2-peak areas. To model longitudinal associations, for glucose and metabolites with detected values at both timepoints in ≥90% of mothers, we computed ‘percent changes’ by dividing the
difference between 1-hr and fasting levels by the fasting level and repeated linear model analyses. Models were also examined for absolute differences between 1-hr and fasting. All models included adjustment for field center, gestational age, maternal age, BMI and mean arterial pressure at OGTT, newborn sex and sample storage time. Adaptive Benjamini-Hochberg false discovery rate (FDR) correction was applied separately for targeted and non-targeted data (12).

Pathway analyses were conducted using MetaboAnalyst 3.0 [http://www.ncbi.nlm.nih.gov/pubmed/25897128] and its included set of human KEGG pathways (13). Quantitative enrichment ‘globaltest’ analysis (14) was used to identify maternal metabolites that are members of the same pathways and also demonstrate collective associations with maternal fasting or 1-hr glucose.

Since metabolites are not independent of each other, but rather act in coordinated fashion, we conducted network analyses to complement individual metabolite analyses by simultaneously modeling metabolite correlations and phenotype associations. First, we constructed separate correlation networks for metabolites at fasting and 1-hr. Nodes represented metabolites and edges represented partial correlation of metabolite pairs at each time point with magnitude >0.25 after adjustment for all covariates used in regression analyses described above. Once correlation networks were constructed using all metabolites, we then identified subnetworks comprised of metabolites demonstrating joint association with our phenotypes. To do this, we applied a subnetwork identification algorithm that incorporates node ‘scores’ and edge ‘weights’ based on phenotype associations to find the optimally scoring subnetwork for that phenotype (15).
We first identified subnetworks of maternal metabolites associated with maternal glucose at fasting and 1-hr. Given the p-value (p) for association between the metabolite and glucose, node score was defined \( S = \log(-\log(p)) + \log(0.10) \). This assigns high positive scores for \( p < 0.10 \), modest positive scores for \( p \) close to but < 0.10 and increasingly negative scores for \( p > 0.10 \). Edge weights were assigned using algorithm defaults, giving higher weight to edges whose adjacent nodes have negative scores and low degree. Subnetworks associated with phenotype were determined by identifying connected sets of positively scoring nodes and evaluating whether uniting positive sets via negatively scoring node(s) results in a positive sum of node scores. If so, the lowest-weight edge path was used to connect nodes. To characterize local connectivity in networks, we applied spinglass community detection using the igraph R package (16; 17). Maternal glucose networks can be interpreted as sets of correlated metabolites that are all associated with fasting or 1-hr glucose, with highest correlation evident within spinglass communities.

Networks for maternal metabolites associated with both maternal glucose and newborn outcomes were identified using the same approach with a modified node score. Given p-values \( p_m \) and \( p_n \) for a metabolite’s association with maternal glucose and newborn outcome, respectively, we calculated an aggregate p-value \( p_a \) for the maximum of \( p_m \) and \( p_n \) based on two random draws from a uniform distribution (15). We then set \( S = -\log(p_a) + \log(0.10) \).

Random forest analyses were conducted using the ‘party’ R package to identify maternal metabolites that improved prediction of newborn BW and SS beyond known risk factors,
maternal glucose and BMI. Random forests are data-driven learning methods designed for prediction (18), in this case applied to continuous newborn BW and SS outcomes. Overall model accuracy is measured as ‘percent variation explained’ and contributions of individual predictors are measured by ‘variable importance’. Given known high correlations among metabolites, we used conditional permutations to evaluate variable importance (19). This approach evaluates predictor contributions independent of other correlated predictors. Variable importance scores >0 indicate higher percent variation explained when a predictor is included in the model. Variables with importance scores <0 may decrease prediction accuracy. We examined random forest models as follows: M0=maternal BMI at OGTT, gestational age at delivery, field center and sample storage time; M1=M0 + maternal glucose; M2=M1 + highest scoring metabolite within N spinglass communities for the network; M3=M1 + N metabolites with lowest aggregate p-values ($p_a$); M4= M1 + metabolites with variable importance scores >0 after running a model including all metabolites with $p_a$<.10.

RESULTS

Study Population

Study population characteristics are shown in Table 1. Mothers spanned the range of maternal BMI and glucose observed in HAPO. Roughly equal numbers of males and females were represented among offspring.

Maternal Metabolites During the OGTT

Changes in multiple metabolites between fasting and 1-hr were observed (Fig. 1, Supplementary Table 1). All targeted amino acids, several long- and medium-chain fatty acids and multiple
products of lipid metabolism, including acylcarnitines, glycerol and β-hydroxybutyrate, decreased following glucose ingestion. In contrast, triglycerides, carbohydrates, and metabolic intermediates, including pyruvate and citrate/isocitrate, increased.

Maternal Metabolites Associated with Maternal Glucose

A limited number of fasting metabolites were positively associated with FPG after FDR adjustment, including gluconeogenic substrates alanine and lactate as well as hexitols and fructose. Lauric acid, a medium-chain fatty acid, and palmitoleic acid and its acylcarnitine were negatively associated with FPG (Fig. 2, Supplementary Table 2).

At 1-hr, more associations between metabolites and glucose were significant (Fig. 2, Supplementary Table 2). Similar to fasting, positive associations were observed for 1-hr alanine, lactate, and fructose. Positive associations were also observed for NEFA, β-hydroxybutyrate, triglycerides, glycerol, asparagine/aspartate, glutamine/glutamate, leucine/isoleucine, ornithine, phenylalanine, proline and serine, and multiple acylcarnitines and fatty acids. Also distinct were positive associations of gluconic acid, a marker of oxidative stress, and 2-hydroxybutyrate, a previously reported biomarker for insulin resistance (20), and negative association of 1-hr 1,5-anhydroglucitol.

Longitudinal analyses identified similar associations observed at individual time points with a few additions (Fig. 2, Supplementary Table 2). Percent changes in citrulline and threonine exhibited significant associations with percent change in glucose. Analyses of absolute
differences from fasting to 1-hr were consistent with percent change associations (data not shown).

Pathway Analyses

Pathway analyses identified multiple pathways related to amino acid, triglyceride and sugar and carbohydrate metabolism whose metabolite members were jointly associated with FPG or 1-hr glucose at corresponding time points (Table 2). There was substantial overlap in the pathways associated with glucose at the two time points, with a greater number of pathways demonstrating association at 1-hr, including the tricarboxylic acid cycle and metabolism of ketone bodies.

Maternal Metabolite Associations with Newborn Outcomes

Although p-values were not significant after FDR adjustment, several association trends between maternal metabolites and both newborn SS and BW were evident (Fig. 3, Supplementary Table 3). Maternal fasting and 1-hr triglycerides, fasting AC C4-OH, 1-hr fructose, gluconic acid and hexitols were positively associated. Maternal fasting 1,5-anhydroglucitol, lysine, and pentonic acids were negatively associated.

Other metabolites demonstrated unique association trends with either outcome. Fasting maternal tyrosine, glycerol 1-phosphate, linoleic and stearic acids, 1-hr hypoxanthine and malonic acid and both fasting and 1-hr AC C8, AC C8:1-OH/C6:1-DC and glucuronic acid were negatively associated with SS. Fasting β-hydroxybutyrate was positively associated with SS. Other acylcarnitines demonstrated several associations in both directions with SS at either time point or longitudinally. For BW, positive associations included 1-hr dihydroxybutanoic acid, threonine
and citric acid. Fasting palmitic acid was negatively associated with BW. Percent changes in AC C16:2, glycerol 1-phosphate, threonine, urea, aminomalonic acid, fructose and several fatty acids were positively associated with BW.

**Maternal Glucose Networks**

Network analyses were conducted to contextualize individual metabolite associations and describe joint associations on behalf of correlated metabolites. Several metabolites demonstrating associations in separate models were part of identified networks, while others were excluded since they do not correlate with other metabolites. Some metabolites were included that did not reach individual statistical significance. The network associated with glucose at fasting includes three spinglass communities of carbohydrates and organic acids, amino acids, and acylcarnitines and fatty acids. The 1-hr network is substantially larger, and again sorted primarily into communities of carbohydrates and organic acids, amino acids and acylcarnitines (Fig. 4).

**Maternal Glucose and Newborn Outcome Networks**

Networks for metabolites associated with maternal glucose and newborn outcomes were smaller with more granular spinglass communities at both fasting and 1-hr (Fig. 5). Unlike the networks associated with maternal glucose alone, amino acids are largely absent from networks incorporating associations with newborn outcomes. Also striking is the transition of acylcarnitines from primarily negative associations with fasting glucose to largely positive associations with 1-hr glucose and the larger size and stronger associations of this community with SS compared to BW (Fig. 5).
Random Forest Analyses

To determine if metabolites associated with maternal glucose contribute to the prediction of newborn BW and SS beyond traditional risk factors including glucose, random forest analyses were performed (Table 3, Supplementary Fig. 1). Model 1 (M1), which includes maternal glucose at either fasting or 1-hr, explains higher overall percent variation for both newborn outcomes than Model 0 (M0) which includes maternal BMI and other baseline covariates. This reflects the well-substantiated role of maternal glucose in newborn size outcomes. As seen in Supplementary Fig. 1, the relative contribution of glucose as a predictor of newborn outcomes (measured by conditional variable importance) decreases in M2, M3 and M4 compared to M1 as additional metabolites are included in the models. This decrease suggests that maternal metabolites that correlate with glucose account in part for the effect of glucose on these newborn outcomes. Importantly, in models M2, M3 and M4, when metabolites associated with both glucose and newborn outcomes are included as predictors, the percent variation explained is consistently higher than M1 models for both BW and SS. Taken together, the decrease in variable importance for glucose in M2, M3 and M4 and the increase in percent overall variation explained by these models indicate that metabolites which are part of the broad-scale changes associated with maternal glycemia are independent contributors to newborn size outcomes.

DISCUSSION

Maternal metabolism during pregnancy differs from the pregravid state due to metabolic adaptations to meet the mother’s and growing fetus’s energy needs (21-23). Pregnancy has been described as accelerated starvation during fasting to meet fetal demands for glucose, amino acids
and other nutrients and facilitated anabolism following nutrient ingestion to allow repletion of maternal reserves (21). For example, women in the third trimester of pregnancy exhibit a larger decrease in total free fatty acids and increase in triglycerides compared to the non-gravid state following glucose ingestion (5; 21). The present study characterized the maternal metabolome in women with glucose levels across the range observed in a population-based study of women who underwent an OGTT at ~28 weeks gestation (4). To date, studies characterizing the metabolome of pregnant women have largely been limited in size and focused on fasting women with GDM compared to healthy pregnant women (24). To our knowledge, this is the largest study of pregnant women and first to examine two OGTT time points to identify maternal metabolites associated with glucose as a quantitative trait.

Prior studies examining the effect of glucose ingestion on the metabolome have been limited to non-pregnant populations (25-31). Similar to those studies, we demonstrated a glucose-induced decrease in multiple metabolites, including glycerol, β-hydroxybutyrate, NEFA, medium- and long-chain fatty acids, acylcarnitines, and amino acids. Pyruvate increased as did lactate following glucose ingestion; the latter has been previously reported. Previous findings with Krebs cycle intermediates have varied (25; 31). We observed an increase in circulating citrate/isocitrate but no change in lactate, fumarate and malate. Thus, despite pregnancy-induced insulin resistance and attendant changes in maternal metabolism, glucose-stimulated insulin secretion in pregnancy inhibits lipolysis, proteolysis and ketogenesis and stimulates glycolysis, similar to non-pregnant populations.
To date, metabolomic studies during pregnancy have focused largely on women with GDM, a state of relative insulin insufficiency due to inadequate maternal beta cell compensation for pregnancy-induced insulin resistance (32). Differences between the metabolomes of women without and with GDM have been reported, but few metabolites have demonstrated consistent changes across studies, likely due to small sample sizes and different technologies and study designs (24). Total and individual free fatty acids have been reported as either higher or both higher and lower during the second or third trimester in women with GDM and/or an impaired glucose challenge test (24; 33-35). Previous studies of amino acid levels in women with GDM have been inconsistent (reviewed in (24)). We identified many metabolites and metabolic pathways associated with maternal glucose at 28 weeks gestation independent of maternal BMI. In the fasting state, a limited number of metabolites were positively associated with maternal glucose, most notably lactate and alanine. A potential explanation for the higher levels of these gluconeogenic substrates in the setting of maternal hyperglycemia is inefficient glucose utilization and relative mitochondrial inefficiency with diversion of glucose to alanine and lactate as opposed to entering the TCA cycle. This finding is consistent with the observation that alanine, lactate, and organic acids are higher early in pregnancy (~16 weeks gestation) in women subsequently diagnosed with GDM (36). Post-glucose ingestion, many more metabolites were associated with maternal glucose, including a positive association of many amino acids and acylcarnitines. This is consistent with blunted insulin-induced inhibition of proteolysis and lipolysis in women with higher glucose and suggests that metabolic changes during pregnancy are not limited to GDM.
A unique aspect of this study was the availability of fetal phenotypes in addition to maternal phenotype and metabolomic data. Association of maternal glucose and triglyceride levels with BW and fetal adiposity has been demonstrated (3; 4; 37-41). Maternal fatty acids increase during pregnancy, are transported across the placenta, and contribute to fetal growth (6), while levels of amino acids, which are important for fetal protein accretion and growth, fall (22; 42). Maternal fatty acids and glycerol have been shown to be associated with BW and fat mass in mothers with GDM but not controls (38; 43). Findings with amino acids have been inconsistent; some found no association of maternal amino acids late in gestation with BW, whereas others showed positive associations of maternal serine, lysine, proline, ornithine and arginine and negative association of methionine (44; 45). Others found positive associations of aspartate, alanine, ornithine, and arginine levels at 25 weeks gestation with BW (46). In the present study, individual maternal metabolites failed to demonstrate FDR-adjusted associations with either BW or SS. Fasting and 1-hr triglycerides were nominally positively associated with BW and SS and fasting levels of tyrosine and several fatty acids were nominally negatively associated with SS or BW. The reasons for the difference between our results and earlier studies are not known, but the earlier studies were small with different study designs.

Recognizing that metabolites likely act in concert rather than individually, we performed network analyses to identify correlated metabolites associated with both maternal and fetal phenotypes. These network studies identified interrelated groups of maternal metabolites that collectively demonstrate consistent, often subtle, associations with both maternal glucose and newborn outcomes. At fasting, clusters of fatty acids and the carnitine esters of long- and medium-chain fatty acids were associated with maternal fasting glucose and newborn SS and/or
BW. At 1-hr, a cluster of acylcarnitines of long- and medium-chain fatty acids together with the ketone body β-hydroxybutyrate demonstrated association with maternal 1-hr glucose and both SS and BW. Different from the fasting state, clusters of sugars and metabolic intermediates at 1-hr were also associated with maternal and newborn phenotypes. These include potential products of the polyol pathway, fructose and hexitols. The polyol pathway increases susceptibility to oxidative stress and contributes to diabetic complications (47). Of interest, amino acid clusters were associated with maternal fasting or 1-hr glucose alone but not newborn phenotypes.

The Pedersen hypothesis stated that higher transplacental transport of glucose and resulting fetal insulin secretion in the setting of maternal hyperglycemia contribute to macrosomia (5). The HAPO Study confirmed this hypothesis (4). Freinkel modified the hypothesis to suggest that nutrients in addition to glucose also contribute to fetal growth and fat accretion in the setting of maternal hyperglycemia (5). We used random forest analysis to confirm Freinkel’s modification of the Pedersen hypothesis by identifying multiple metabolites associated with maternal glucose that contribute to BW and SS independent of maternal glucose and BMI. As seen in Supplementary Figure 1, a broad array of maternal metabolites make some contribution to BW and/or SS. In the fasting state this includes a number of lipid-related metabolites (fatty acids, triglycerides, glycerol-1-phosphate, acylcarnitines of medium chain fatty acids), uric acid, and sugars (hexitols, myoinositol). Interestingly, lysine is the only amino acid in the fasting state which contributes to newborn size in these analyses. Previously, lysine levels helped explain variability in the development of GDM (48), and higher levels of lysine have been demonstrated in some studies of women with GDM (44; 45; 49). At 1 hr, a similar pattern was evident with
lipid-related metabolites (β-hydroxybutyrate among others), sugars (fructose, hexitols, disaccharides, maltose), products of carbohydrate metabolism (citrate/isocitrate, lactate), and amino acids (methionine, phenylalanine) all contributing to newborn outcomes. Together, these analyses demonstrate that, beyond glucose, additional metabolites associated with glucose are independent contributors to newborn size at birth and, in aggregate, may have the potential to help predict fetal size at birth.

Our study had several strengths. It is the largest to date examining association of maternal metabolic traits with maternal metabolites and first in pregnant women to examine associations across the full range of glucose and associations of metabolites with glucose at both fasting and 1-hr following a glucose load. As pregnancy has been described as a state of accelerated starvation and facilitated anabolism, it is important to examine metabolites in both the fasting and “post-prandial” state. This is also the first metabolomic study to include mother-newborn dyads, allowing examination of associations between the maternal metabolome and newborn outcomes and identification of metabolites associated with glucose important for newborn size at birth. One limitation is the use of non-targeted assays which are not strictly quantitative. These assays allow unbiased examination of metabolite-phenotype associations, but findings of interest will ultimately require development of targeted assays for confirmation. Moreover, the network and random forest analyses will require replication in independent studies.

In conclusion, we demonstrated broad-scale association of metabolites with either maternal fasting or 1-hr glucose using population-based data and provided new insight into metabolic changes characteristic of maternal hyperglycemia. We also found evidence for a role of maternal
triglycerides, fatty acids and their metabolites, together with sugars and metabolic intermediates in newborn outcomes, while random forest analyses suggested that these and other metabolites were independent contributors to newborn BW and SS. Further studies relating these findings to the fetal metabolome will provide additional insight into mechanisms underlying fetal size at birth.
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D.M.S. contributed to study design, data analysis, interpretation of findings and primary manuscript drafting. J.R.B. and M.J.M were responsible for conventional metabolite assays and non-targeted metabolomics, interpretation of findings, and manuscript writing. A.C.R. and M.N. contributed to data analysis. R.D.S. and O.I. developed and performed targeted metabolomics assays. L.P.L. contributed to study design. B.E.M. and C.B.N. contributed to study design and interpretation of findings. W.L.L. conceived the hypothesis and contributed to study design, interpretation of findings, and primary drafting of the manuscript.

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Table 1: Demographics of mothers and their offspring

| Field center               | N (%)       |
|----------------------------|-------------|
| Belfast, UK                | 188 (47.0)  |
| Brisbane, Australia        | 136 (34.0)  |
| Newcastle, Australia       | 76 (19.0)   |

Maternal parity

| Maternal parity          | N (%)       |
|--------------------------|-------------|
| First child              | 203 (50.7)  |
| Second or third child    | 197 (49.2)  |

Newborn gender

| Newborn gender | N (%)       |
|----------------|-------------|
| Male           | 209 (52.2)  |
| Female         | 191 (47.8)  |

Maternal characteristics

| Maternal characteristics                      | Mean (SD) |
|-----------------------------------------------|-----------|
| Age at OGGT (yrs)                             | 29.4 (5.12) |
| BMI at OGGT (kg/m²)                           | 29.0 (4.89) |
| Mean arterial pressure (mmHg)                 | 83.1 (6.89) |
| Fasting plasma glucose (mg/dl)                | 82.1 (6.14) |
| One-hour plasma glucose (mg/dl)               | 131.7 (27.24) |
| Two-hour plasma glucose (mg/dl)               | 111.0 (20.34) |
| Sample storage time (yrs)                     | 9.9 (1.25)  |

Newborn characteristics
|                                |        |
|--------------------------------|--------|
| Gestational age at OGTT (wks)  | 28.6 (1.37) |
| Gestational age at delivery (wks) | 40.2 (1.15) |
| Cord C-peptide (ug/l)            | 1.1 (0.51)  |
| Birth weight (g)                 | 3667.6 (485.28) |
| Sum of skin folds (mm)           | 12.8 (2.74)  |
Table 2: MetaboAnalyst pathway analysis results with FDR adjusted p<0.05 for fasting and/or 1-hr maternal metabolites

| Pathway name                                      | Number of metabolites | Number of measured metabolites | FDR-adj p-value at fasting | FDR-adj p-value at 1-hr |
|--------------------------------------------------|-----------------------|-------------------------------|-----------------------------|-------------------------|
| Galactose metabolism                             | 41                    | 4                             | 4.5e-07                     | 1.6e-57                 |
| Selenoamino acid metabolism                      | 22                    | 1                             | 3.6e-05                     | 0.00084                 |
| Alanine, aspartate, glutamate metabolism          | 24                    | 5                             | 4.7e-05                     | 6.2e-08                 |
| Cysteine and methionine metabolism               | 56                    | 6                             | 0.0013                      | 0.00012                 |
| Arginine and proline metabolism                  | 77                    | 9                             | 0.0027                      | 1.6e-06                 |
| Aminoacyl-tRNA biosynthesis                      | 75                    | 15                            | 0.0044                      | 0.00013                 |
| D-Glutamine and D-glutamate metabolism           | 11                    | 1                             | 0.015                       | 1.2e-07                 |
| Nitrogen metabolism                              | 39                    | 6                             | 0.016                       | 3.7e-05                 |
| Histidine metabolism                             | 44                    | 3                             | 0.016                       | 7.3e-07                 |
| Phenylalanine metabolism                         | 45                    | 4                             | 0.016                       | 0.011                   |
| Lysine biosynthesis                              | 32                    | 3                             | 0.036                       | 0.00026                 |
| Phenylalanine, tyrosine, tryptophan biosynthesis  | 27                    | 2                             | 0.036                       | 0.026                   |
| Glycine, serine and threonine metabolism         | 48                    | 7                             | 0.058                       | 0.0041                  |
| D-Arginine and D-ornithine metabolism            | 8                     | 2                             | 0.079                       | 0.015                   |
| beta-Alanine metabolism                          | 28                    | 4                             | 0.12                        | 0.00053                 |
| Cyanoamino acid metabolism                       | 16                    | 3                             | 0.12                        | 0.0018                  |
| Sugar and Carbohydrate Metabolism                                      |       |   |         |   |
|---------------------------------------------------------------------|-------|---|---------|---|
| Glycolysis or Gluconeogenesis                                       | 31    | 3 | 4.5e-07 | 9.8e-51 |
| Fructose and mannose metabolism                                     | 48    | 1 | 4.5e-07 | 1.6e-20 |
| Pyruvate metabolism                                                 | 32    | 2 | 3.1e-05 | 7.3e-07 |
| Pentose phosphate pathway                                           | 32    | 4 | 9.2e-05 | 2.2e-62 |
| Starch and sucrose metabolism                                       | 50    | 3 | 0.0031  | 5.4e-45 |
| Amino sugar, nucleotide sugar metabolism                            | 88    | 3 | 0.0098  | 6.6e-42 |
| Citrate cycle (TCA cycle)                                           | 20    | 3 | 0.12    | 0.00027 |

| Lipid Metabolism                                                    |       |   |         |   |
|---------------------------------------------------------------------|-------|---|---------|---|
| Fatty acid biosynthesis                                             | 49    | 2 | 0.00015 | 0.0018 |
| Propanoate metabolism                                               | 35    | 5 | 0.0017  | 5.9e-08 |
| Butanoate metabolism                                                | 40    | 5 | 0.015   | 1.2e-12 |
| Glycerolipid metabolism                                             | 32    | 3 | 0.112   | 9.0e-05 |
| Fatty acid metabolism                                               | 50    | 2 | 0.22    | 0.00064 |
| Synthesis and degradation of ketone bodies                          | 6     | 2 | 0.33    | 7.2e-09 |
| Sphingolipid metabolism                                             | 25    | 1 | 0.50    | 0.012  |

| Vitamin Metabolism                                                  |       |   |         |   |
|---------------------------------------------------------------------|-------|---|---------|---|
| Nicotinate and nicotinamide metabolism                              | 44    | 2 | 0.0050  | 9.0e-05 |
| Pantothenate and CoA biosynthesis                                   | 27    | 3 | 0.0098  | 0.00081 |
| Vitamin B6 metabolism                                               | 32    | 1 | 0.025   | 0.086  |
| Biotin metabolism                                                   | 11    | 1 | 0.073   | 0.033  |
| Ascorbate and aldarate metabolism                                   | 45    | 5 | 0.12    | 0.024  |

| Other                                                               |       |   |         |   |
| Metabolism                                    | Count | Genes | p-value | q-value |
|----------------------------------------------|-------|-------|---------|---------|
| Taurine and hypotaurine metabolism           | 20    | 3     | 4.7e-05 | 0.0031  |
| Terpenoid backbone biosynthesis              | 33    | 1     | 0.025   | 0.086   |
| Glutathione metabolism                       | 38    | 4     | 0.050   | 2.8e-06 |
| Porphyrin and chlorophyll metabolism         | 104   | 3     | 0.05    | 2.7e-05 |
| Glyoxylate and dicarboxylate metabolism      | 50    | 4     | 0.11    | 0.00076 |
| Sulfur metabolism                            | 18    | 2     | 0.74    | 0.027   |
Table 3: Overall percent variation explained for random forest models predicting newborn size outcomes

|                                | Percent variation explained |
|--------------------------------|-----------------------------|
|                                | M0  | M1  | M2  | M3  | M4  |
| Maternal FPG and fasting       | 3.99| 6.03| 7.59| 8.13| 10.08|
| metabolites – Newborn SS       |     |     |     |     |     |
| Maternal FPG and fasting       | 3.02| 4.35| 8.01| 7.47| 7.16|
| metabolites – Newborn BW       |     |     |     |     |     |
| Maternal 1-hr PG and 1-hr      | 3.99| 5.35| 7.08| 6.98| 8.52|
| metabolites – Newborn SS       |     |     |     |     |     |
| Maternal 1-hr PG and 1-hr      | 3.02| 7.07| 8.49| 7.89| 9.22|
| metabolites – Newborn BW       |     |     |     |     |     |

M0 = baseline model including maternal BMI, gestational age at delivery, field center, sample storage time

M1 = M0 + maternal glucose at fasting or 1-hr

M2, M3, M4 = M1 + metabolites selected according to strategies described in Methods, individual metabolites are listed in Supplementary Fig. 1
FIGURE LEGENDS

Fig. 1. Volcano plot of maternal metabolites demonstrating differences from fasting to 1-hr with FDR-adjusted p<0.05. The y-axis represents the negative log10 transform of the nominal p-value from paired t-tests. The x-axis represents mean within-individual fold changes from fasting to 1-hr. Points are colored according to metabolite class.

Fig. 2. Heat map showing positive (red) and negative (blue) associations of maternal glucose and metabolites at fasting (lane A) and 1-hr (lane B) during the OGTT, as well as fold change associations from fasting to 1-hr for maternal glucose and metabolites (lane C). AC refers to acylcarnitine. All metabolites with FDR-adjusted p<0.05 for at least one time point or for fold change analyses are shown.

Fig. 3. Heat map showing positive (red) and negative (blue) associations of maternal metabolites at fasting, 1-hr and fold change with newborn sum of skinfolds (lanes A, B and C, respectively) and birth weight (lanes D, E and F, respectively). AC refers to acylcarnitine. Glucose in this figure refers to the original HAPO Study OGTT maternal glucose measurements. All metabolites with a nominal p-value < 0.05 for at least one time point or in fold change analyses are shown.

Fig. 4. Subnetworks of maternal metabolites associated with maternal fasting and 1-hr glucose. Nodes represent metabolites and edges represent partial correlation >0.25 for metabolite pairs. Nodes are sized according to node score based p-values with larger nodes corresponding to higher scores. Nodes are colored according to metabolite class (AA=amino acid, AC=...
acylcarnitine, CHO=carbohydrate, FA=fatty acid, GC/TCA=glycolysis/tricarboxylic acid cycle). Gray shading is used to identify spinglass communities within the subnetworks. Direction of association is noted by color shading in the nodes. Positive associations are darker and negative associations are lighter. Panel A is the subnetwork of maternal fasting metabolites associated with maternal fasting plasma glucose. Panel B is the subnetwork of maternal 1-hr metabolites associated with maternal 1-hr plasma glucose.

**Fig. 5.** Subnetworks of maternal metabolites associated with maternal glucose and newborn outcomes. Nodes represent metabolites and edges represent partial correlation >0.25 for metabolite pairs. Nodes are sized according to node score based on aggregate p-values with larger nodes corresponding to higher scores. Nodes are colored according to metabolite class (AA=amino acid, AC=acylcarnitine, CHO=carbohydrate, FA=fatty acid, GC/TCA=glycolysis/tricarboxylic acid cycle). Gray shading is used to identify spinglass communities within the subnetworks. Direction of association for maternal glucose and newborn outcome is noted by color shading in the left and right sides of the nodes, respectively. Positive associations are darker and negative associations are lighter. Panel A is the subnetwork of maternal fasting metabolites associated with maternal fasting plasma glucose (FPG) and newborn sum of skin folds (SS). Panel B is the subnetwork of maternal fasting metabolites associated with maternal FPG and newborn birthweight (BW). Panel C is the subnetwork of maternal 1-hr metabolites associated with maternal 1-hr plasma glucose (1PG) and newborn SS. Panel D is the subnetwork of maternal 1-hr metabolites associated with maternal 1PG and newborn BW.
fold change

-0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2

-\log_{10}(\text{nominal } p)

amino acids
acyl carnitines
fatty acids
lipids
glycolysis/TCA
carbohydrates
A) Maternal FPG (BMI) - Newborn SS

B) Maternal FPG (BMI) - Newborn BW

C) Maternal 1PG (BMI) - Newborn SS

D) Maternal 1PG (BMI) - Newborn BW
### Supplementary Table 1: Differences in maternal metabolite levels at fasting and 1-hr into OGTT

| Metabolite                          | Fasting mean (sd) | 1-hr mean (sd) | Mean percent change | Nominal p*   |
|-------------------------------------|-------------------|----------------|---------------------|--------------|
| **Conventional Metabolites**        |                   |                |                     |              |
| β-Hydroxybutyrate (µmol/L)          | 3.85 (.68)        | 2.91 (.65)     | -.241               | 2.08E-119    |
| Glycerol (mg/dL)                    | .95 (.27)         | .77 (.26)      | -.168               | 3.73E-41     |
| NEFA (mmol/L)                       | 36.68 (12.44)     | 18.93 (8.38)   | -.32                | 5.23E-106    |
| Triglycerides (mg/dL)               | 190.89 (6.68)     | 202.63 (6.04)  | .073                | 9.64E-29     |
| **Amino acids (Targeted assay, µmol/L)** |                   |                |                     |              |
| Leucine/Isoleucine                  | 121.71 (15.44)    | 9.57 (14.87)   | -.258               | 2.42E-235    |
| Citrulline                          | 16.88 (3.04)      | 11.33 (2.1)    | -.326               | 4.15E-210    |
| Phenylalanine                       | 64.71 (1.01)      | 51.26 (9.97)   | -.21                | 6.58E-207    |
| Tyrosine                            | 42.05 (5.51)      | 34.17 (5.07)   | -.187               | 3.07E-197    |
| Valine                              | 158.25 (21.68)    | 133.28 (2.14)  | -.158               | 4.31E-195    |
| Methionine                          | 21.36 (2.44)      | 17.28 (2.25)   | -.19                | 1.83E-175    |
| Serine                              | 119.22 (19.53)    | 99.57 (18.29)  | -.165               | 3.17E-168    |
| Ornithine                           | 43.99 (8.5)       | 34.36 (7.15)   | -.218               | 4.06E-167    |
| Glycine                             | 19.3 (36.45)      | 157.97 (33.53) | -.171               | 2.96E-166    |
| Asparagine/Aspartic acid            | 48.4 (11.25)      | 38.95 (11.41)  | -.202               | 8.94E-166    |
| Proline                             | 116.33 (23.95)    | 102.75 (2.98)  | -.115               | 3.75E-137    |
| Glutamine/Glutamic acid             | 129.27 (29.23)    | 108.1 (28.67)  | -.165               | 5.81E-109    |
| Arginine                            | 91.08 (22.34)     | 83.98 (22.76)  | -.079               | 1.92E-45     |
| Alanine                             | 334.01 (54.69)    | 321.79 (51.11) | -.033               | 1.25E-18     |
| **Amino acids (Non-targeted GC/MS)** |                   |                |                     |              |
| Lysine                              | 18.73 (.66)       | 18.37 (.74)    | -.019               | 1.18E-21     |
| Threonine                           | 22.77 (.44)       | 22.58 (.41)    | -.008               | 1.75E-20     |
| Histidine                           | 55.96 (1.05)      | 53.62 (9.12)   | -.037               | 4.56E-18     |
| Homoserine/Diethanolamine           | 18.56 (.71)       | 18.29 (.57)    | -.014               | 2.19E-15     |
| Hydroxyprolines                     | 18.17 (.71)       | 17.95 (.59)    | -.011               | 5.25E-12     |
| 2-Aminobutanoic acid                | 15.79 (.65)       | 15.65 (.61)    | -.008               | 9.20E-10     |
| Pipecolic acid                      | 15.52 (.74)       | 15.42 (.66)    | -.006               | .000245      |
| Urea                                | 24.88 (.48)       | 24.82 (.5)     | -.002               | .00208       |
| 3-Indolelactic acid/Tryptophan      | 14.52 (.56)       | 14.46 (.6)     | -.004               | .00299       |
| 2-Hydroxyvaleric acid               | 15.72 (.62)       | 15.78 (.66)    | -.004               | .0137        |
| **Acylcarnitines (Targeted assay, µmol/L)** |             |                |                     |              |
| AC C2                               | 1.02 (.28)        | .67 (.27)      | -.358               | 7.26E-163    |
| AC C10                              | -2.35 (.46)       | -3.05 (.48)    | -.326               | 3.07E-145    |
| AC C8                               | -3.04 (.46)       | -3.7 (.49)     | -.229               | 7.69E-140    |
| AC C14:1                            | -3.59 (.49)       | -4.13 (.51)    | -.158               | 2.14E-132    |
| AC C12:1                            | -3.42 (.39)       | -3.96 (.46)    | -.161               | 1.07E-129    |
| AC C10:1                            | -3.08 (.4)        | -3.6 (.43)     | -.172               | 8.55E-127    |
| AC C14:2                            | -4.47 (.43)       | -5.13 (.52)    | -.15                | 1.50E-123    |
| AC C18:1                            | -2.77 (.26)       | -3 (.29)       | -.084               | 2.94E-115    |
| AC C18:2                            | -3.79 (.27)       | -4.07 (.3)     | -.073               | 6.77E-109    |
| AC C12                              | -3.56 (.42)       | -4 (.43)       | -.129               | 7.10E-107    |
| AC C16:1                            | -4.38 (.37)       | -4.72 (.36)    | -.08                | 7.72E-99     |
| AC C16                              | -2.95 (.21)       | -3.07 (.23)    | -.044               | 8.96E-65     |
| AC C14                              | -4.24 (.32)       | -4.46 (.33)    | -.052               | 4.53E-62     |
| AC C10-OH/C8-DC                     | -4.51 (.39)       | -4.82 (.45)    | -.073               | 1.85E-59     |
| Compound            | Retention Time (min) | Concentration (µg/mL) | RSD (%) | Detection Limit (µg/mL) |
|---------------------|----------------------|-----------------------|---------|-------------------------|
| AC C8:1-DC          | 24.5 (.4)            | 2.054                 | 2.59E244|
| AC C14:1-4OH        | 24.66 (.27)          | 2.043                 | 7.11E41 |
| AC C16:2            | 25.87 (.44)          | 2.043                 | 7.93E37 |
| AC C4:C4            | 25.68 (.36)          | 3.10E241              |         |
| AC C20:4            | 25.75 (.29)          | 3.72E13               |         |
| AC C18:1-4OH/C6:1-4DC| 25.82 (.45)  | 5.61E213              |         |
| AC C12:4OH/C10:4DC | 25.68 (.36)          | 5.81E210              |         |
| AC C10:2            | 24.62 (.44)          | 4.69E14               |         |
| AC C5               | 22.51 (.44)          | 4.73E13               |         |
| AC C6-DC/C8-OH      | 25.26 (.7)           | 7.11E13               |         |
| AC C3               | 27.22 (.28)          | 1.94E92               |         |
| AC C14:4OH/C12:4DC | 27.22 (.28)          | 1.94E92               |         |
| AC C8:1             | 27.22 (.28)          | 1.94E92               |         |
| AC C18:4OH/C16:4DC | 27.22 (.28)          | 1.94E92               |         |
| AC C5:1             | 27.22 (.28)          | 1.94E92               |         |
| AC C6-DC/C8-OH      | 27.22 (.28)          | 1.94E92               |         |
| Gluconic acid or similar sugar acid | 27.22 (.28) | 1.94E92               |         |

| Carbohydrates (Non-targeted GC/MS) |
|-------------------------------------|
| Glucosic acid                       | 19.44 (.61) | 20.72 (.66) | 0.67 | 1.07E103  |
| Hexitoles                           | 25.26 (.7) | 26.20 (.79) | 0.37 | 6.67E103  |
| Glucose and other aldohexoses       | 27.22 (.28) | 27.72 (.29) | 0.18 | 1.94E92   |
| Fructose or similar ketohexose      | 19.06 (.53) | 19.81 (.58) | 0.4  | 5.52E72   |
| Gluconic acid or similar sugar acid | 16.72 (1.06) | 17.64 (1.05) | 0.58 | 5.84E45   |
| Disaccharide                        | 17.78 (1.03) | 17.94 (.96) | 0.1  | 9.82E19   |
| Pentonic acids                      | 15.52 (.93) | 15.97 (.99) | 0.3  | 3.99E14   |
| Dehydroascorbic acid                | 23.99 (.69) | 24.21 (.67) | 0.1  | 4.83E08   |
| Aldopentoses                        | 17.45 (1.4) | 17.79 (1.4) | 0.023 | 1.46E05   |
| Ribitol and other pentose alcohols  | 16.29 (.75) | 16.46 (.67) | 0.11 | 0.00192   |
| Dihydroxybutanoic acid              | 16.65 (.79) | 16.58 (.77) | -0.04 | 0.00563   |

| Fatty acids (Non-targeted GC/MS) |
|-----------------------------------|
| Palmitoleic acid                  | 19.07 (1) | 16.98 (1.3) | -1.13 | 1.29E139  |
| Lauric acid                       | 16.18 (.87) | 15.31 (1.12) | -0.53 | 7.94E45   |
| Arachidonic acid (also Eicosapentaenoic acid) | 19.66 (.57) | 19.56 (.6) | -0.05 | 4.81E10   |
| 1-Hexadecanoyl/                   | 14.96 (.76) | 15.07 (.71) | 0.09  | 0.00258   |

| Glycolysis/Tricarboxylic Acids (Non-targeted GC/MS) |
|------------------------------------------------------|
| Pyruvic acid                                        | 17.82 (1.55) | 18.5 (1.5) | 0.42  | 5.16E-22  |
| Citric acid/Isocitric acid                          | 21.2 (.92) | 21.58 (.85) | 0.19  | 1.12E-20  |
| Glyceric acid                                       | 18.98 (.6) | 18.92 (.6) | -0.03 | 0.000564  |
| 2-Hydroxybutyric acid                               | 18.75 (.63) | 18.81 (.64) | 0.03  | 0.00172   |
| Succinic acid                                       | 16.75 (.62) | 16.67 (.69) | -0.04 | 0.0625    |

| Lipids (Non-targeted GC/MS)                          |
|------------------------------------------------------|
| Ethanolamine                                         | 15.65 (.49) | 15.46 (.45) | -0.11 | 1.28E-16  |
| Metabolite                  | Fasting Levels | 1 hr Levels | Mean Percent Change | p-value |
|----------------------------|----------------|-------------|---------------------|---------|
| Cholesterol                | 26.88 (.26)    | 26.85 (.28) | -0.001              | 0.00363 |
| Glycerol 1-phosphate       | 21.34 (1)      | 21.27 (1)   | -0.03              | 0.0358  |

**Other Metabolites (Non-targeted GC/MS)**

| Metabolite                  | Fasting Levels | 1 hr Levels | Mean Percent Change | p-value |
|----------------------------|----------------|-------------|---------------------|---------|
| O-Methylphosphate          | 2.13 (1.13)    | 2.29 (1.21) | 0.009               | 0.00239 |
| Phosphoric acid            | 24.52 (.58)    | 24.57 (.68) | 0.002               | 0.00345 |
| Malonic acid               | 16.36 (1.28)   | 16.24 (1.16) | 0.006              | 0.0123  |

*Nominal p-values are reported for paired t-tests of fasting and 1 hr maternal metabolite levels for metabolites with observed values for at least 90% of participants at both fasting and 1 hr. All tests listed here are significant at p<.05 after FDR correction. Mean percent changes are reported as descriptive statistics only. All non-targeted metabolites are measured as log2 transformed mass spectrometry peak areas.
### Supplementary Table 2: Associations between maternal metabolites and maternal glucose at fasting and 1-hr and associations between maternal metabolite and maternal glucose percent changes.

|                     | Fasting association | 1-hr association | Percent change association |
|---------------------|---------------------|------------------|---------------------------|
|                     | Beta    | SE     | p     | r   | Beta    | SE     | p     | r   | Beta    | SE     | p     | r   |
| **Conventional Metabolites** |          |        |      |    |          |        |      |    |          |        |      |    |
| β2Hydroxybutyrate (µmol/L) | -0.0093 | 0.0061 | .13  | -.077 | 0.0072 | 0.0011 | 5.1e-10* | .31 | 0.034  | 0.022  | .12  | .079 |
| Glycerol (mg/dL)     | -0.0003 | 0.0022 | .89  | -.0070 | 0.0018 | 0.00050 | 1.8e-4*  | .19 | -0.058 | 0.038  | .13  | -.077 |
| Lactate (mmol/L)     | 0.23    | 0.048  | 1.4e-6* | .24 | 0.062   | 0.010  | 5.4e-9*  | .29 | 0.10    | 0.029  | 4.3e-4* | .18 |
| NEFA (mmol/L)        | -0.28   | 0.11   | .015^  | -.12 | 0.096   | 0.015  | 2.1e-10* | .32 | 0.030   | 0.24   | .90  | .0062 |
| Triglycerides (mg/dL)| 1.30    | 0.55   | .021^  | .12 | 0.49    | 0.12   | 3.8e-5*  | .21 | 0.026   | 0.020  | .20  | .067 |
| **Amino acids (Targeted assay, µmol/L)** |          |        |      |    |          |        |      |    |          |        |      |    |
| Alanine             | 2.3     | 0.49   | 3.9e-6* | .23 | .34     | 0.096  | 4.7e-4*  | .18 | 0.039   | 0.012  | .0019* | .16 |
| Arginine            | 0.35    | 0.18   | 0.50^  | .10 | 0.075   | 0.036  | 0.037*  | .11 | 0.0075  | 0.013  | .57  | .029 |
| Asparagine/Aspartate| 0.24    | 0.093  | 0.11^  | .13 | 0.081   | 0.019  | 3.4e-5*  | .21 | 0.040   | 0.014  | 0.0029* | .15 |
| Citrulline          | 0.0098  | 0.028  | .72   | .018 | -.00800 | 0.0040 | .85    | -.0097 | -.054   | 0.011  | 5.3e-7* | -.25 |
| Glutamine/Glutamate | 0.59    | 0.21   | 0.0055 | .14 | 0.23    | 0.040  | 1.9e-8*  | .28 | 0.035   | 0.015  | 0.017^ | .12 |
| Leucine/Isoleucine  | 0.057   | 0.14   | .68   | .021 | 0.066   | 0.026  | 0.011*  | .13 | 0.023   | 0.0098 | 0.019^ | .12 |
| Ornithine           | 0.12    | 0.079  | .13   | .078 | 0.030   | 0.013  | 0.027*  | .11 | 0.028   | 0.012  | 0.016^ | .12 |
| Phenylalanine       | 0.22    | 0.084  | 0.0080 | .14 | 0.044   | 0.017  | 0.0078* | .14 | 0.0082  | 0.010  | .41  | .042 |
| Proline             | 0.61    | 0.22   | 0.0059 | .14 | 0.083   | 0.040  | 0.038*  | .11 | 0.029   | 0.0080 | 3.5e-4* | .18 |
| Serine              | 0.12    | 0.17   | 0.49   | .036 | 0.089   | 0.032  | 0.0057* | .14 | 0.019   | 0.010  | 0.067  | .093 |
| **Acylcarnitines (Targeted assay, µmol/L)** |          |        |      |    |          |        |      |    |          |        |      |    |
| AC C10              | -0.0057 | 0.0042 | .17   | -.069 | 0.0038 | 0.0009 | 1.1e-5* | .22 | 0.0015  | 0.033  | .96  | .0023 |
| AC C10-OH/C8-DC     | -0.0070 | 0.0035 | 0.044^ | -.10 | 0.0028 | 0.0008 | 7.0e-4* | .17 | 0.0054  | 0.012  | .64  | .0024 |
| AC C10:1            | -0.0010 | 0.0036 | .78   | -.014 | 0.0027 | 0.0008 | 4.1e-4* | .18 | 0.0004  | 0.017  | .98  | .0012 |
| AC C12              | -0.0050 | 0.0039 | .19   | -.066 | 0.0032 | 0.0008 | 3.20-5* | .21 | -.0086  | 0.014  | .55  | -.031 |
| AC C12-OH/C10-DC    | -0.0064 | 0.0041 | .12   | -.078 | 0.0024 | 0.0008 | 0.0040* | .15 | 0.0056  | 0.013  | .67  | .0022 |
| AC C12:1            | -0.0076 | 0.0035 | 0.028^ | -.11 | 0.0032 | 0.0008 | 3.7e-5* | .21 | 0.0019  | 0.014  | .89  | .0068 |
| AC C14              | -0.0053 | 0.0029 | .066  | -.093 | 0.0027 | 0.0006 | 5.4e-6* | .23 | 0.0013  | 0.0084 | .88  | .0076 |
| AC C14:1            | -0.012  | 0.0045 | 0.0085^ | -.13 | 0.0054 | 0.0009 | 3.8e-9* | .29 | 0.0043  | 0.015  | .78  | .015 |
| AC C14:2            | -0.0063 | 0.0040 | .11   | -.081 | 0.0038 | 0.0010 | 6.1e-5* | .20 | 0.0056  | 0.014  | .68  | -.021 |
| AC C16              | -0.0031 | 0.0018 | 0.084  | -.088 | 0.0014 | 0.0004 | 6.2e-4* | .17 | 0.0052  | 0.0068 | .45  | .0039 |
| AC C16-OH/C14-DC    | -0.0008 | 0.0038 | .812  | -.012 | 0.0015 | 0.0007 | 0.028*  | .11 | 0.015   | 0.011  | .16  | -.071 |
### Carbohydrates (Non-targeted GC/MS)

| Compound                        | 15-A   | 5.4e-5* | 19e-20* | 2.3e-75* | 3.5e-47* | 1.2e-21* | 2.1e-8* | 2.0e-9* | 0.93 | 0.0089* | 2.1e-32* | 0.011^ | 0.12 | 0.0020* |
|--------------------------------|--------|---------|---------|----------|----------|----------|---------|---------|-------|---------|----------|---------|-------|---------|
| 1,5-Anhydroglucitol            | -      | .34     | -       | +        | +        | +        | +       | +       | +     | +       | +        | .34     | +     | .37     |
| Fructose or similar ketohexose | +      | 5.4e-5* | +       | 1.9e-20* | +        | 1.1e-41* | +       | +       | +     | +       | +        | 1.8e-14* | +     | +       |
| Gluconic acid                  | +      | .011^   | +       | 2.3e-75* | +        | +        | +       | +       | +     | +       | +        | +       | +     | +       |
| Gluconic acid or similar sugar acid | +  | .12     | +       | .056     | +        | +        | +       | +       | +     | +       | +        | .0017*  | +     | +       |
| Glucose or other aldohexoses   | +      | 2.2e-4* | +       | 3.5e-47* | +        | 1.2e-20* | +       | +       | +     | +       | +        | 1.2e-20* | +     | +       |
| Hexitols                       | +      | 2.1e-8* | +       | 1.2e-21* | +        | 2.6e-32* | +       | +       | +     | +       | +        | 2.6e-32* | +     | +       |
| Maltose                         | +      | .93     | +       | .0020*   | +        | +        | +       | +       | +     | +       | +        | 2.6e-32* | +     | +       |

### Glycolysis/Tricarboxylic Acids (Non-targeted GC/MS)

| Compound                        | 3.1e-7* | 2.8e-5* | .19    | .0099* | 9.3e-4* | .71e-3* |
|--------------------------------|---------|---------|--------|--------|---------|---------|
| 2-Hydroxybutyric acid           | +       | .45     | +      | +      | +       | +       |
| Citric acid/Isocitric acid      | -       | .46     | +      | +      | +       | +       |
| Pyruvic acid                    | +       | .017^   | +      | +      | +       | +       |

### Fatty Acids (Non-targeted GC/MS)

| Compound                  | 0.97   | .34    |        |        |        |
|---------------------------|--------|--------|--------|--------|--------|
| Arachidic acid            | +      | .87    | +      | +      | +      |
| Lauric acid               | -      | .016^  | +      | +      | +      |
| Palmitoleic acid          | -      | 3.2e-5*| +      | 9.3e-4*| +      |

### Amino Acids (Non-targeted GC/MS)

| Compound         | 7.1e-3* |
|------------------|---------|
| Pipecolic acid   | +       |

Diabetes
| Metabolite                | +  | .29 | -  | .93 | +  | .0030* |
|--------------------------|----|-----|----|-----|----|--------|
| **Other Metabolites (Non-targeted GC/MS)** |    |     |    |     |    |        |
| Hypoxanthine             | -  | .63 | +  |     | +  | 3.5e-4*|
| O-Methylphosphate        | +  | .078| +  |     | +  | .0028* |
| Purine riboside          | +  | .66 | +  |     | +  | .0022* |

For targeted metabolites, beta and standard error (SE) estimates from linear regression models are reported along with p-values. Partial correlation coefficients (r) are also reported after adjustment for model covariates. For non-targeted metabolites, only the direction of association and p-values are reported. All non-targeted metabolites are measured as log2 transformed mass spectrometry peak areas.

^Nominal p<0.05, *FDR-adjusted p<0.05
### Supplementary Table 3: Associations between fasting, 1-hr and percent changes in maternal metabolites with newborn outcomes

| Sum of skinfolds | Birth weight |
|------------------|--------------|
|                  | Fasting     | 1 hr       | Percent change | Fasting     | 1 hr       | Percent change |
|                  | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) |
| Fast Metabolites  | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) | Beta sign (nominal p) |
| Glucose (mg/dL)  | + (8.48e-05) * | + (2.84e-02) * | + (3.77e-01) | + (3.71e-04) * | + (9.68e-04) * | + (3.63e-02) * |
| Conventional metabolites |
| Triglycerides (mg/dL) | + (2.82e-02) * | + (4.82e-02) * | - (5.06e-01) | + (3.24e-02) * | + (4.83e-02) * | - (8.74e-01) |
| β-Hydroxybutyrate (µmol/L) | + (4.19e-02) * | + (1.95e-01) | - (4.73e-01) | + (2.97e-01) | + (2.45e-01) | - (9.64e-01) |
| Amino acids (Targeted assay, µmol/L) |
| Tyrosine | - (4.10e-02) * | - (1.00e-01) | + (7.96e-01) | - (1.29e-01) | - (7.25e-01) | + (7.59e-02) |
| Acylcarnitines (Targeted assay, µmol/L) |
| AC C18-OH/C16-DC | + (8.69e-02) |
| AC C4-OH | + (1.54e-03) | + (3.78e-01) | - (3.47e-02) | + (6.47e-03) | + (5.51e-01) | - (6.97e-02) |
| AC C5-DC | - (3.60e-01) | - (2.98e-02) | - (8.36e-02) | - (5.78e-01) | - (1.91e-01) | - (3.00e-01) |
| AC C8 | - (1.07e-02) | - (2.16e-02) | + (1.93e-01) | - (6.07e-02) | - (1.20e-01) | + (3.90e-01) |
| AC C8:1-DC | - (4.19e-02) | - (5.12e-01) | + (7.35e-02) | - (6.71e-01) | + (9.57e-01) | + (5.09e-01) |
| AC C8:1-OH/C6:1-DC | - (1.93e-02) | - (1.83e-02) | + (8.66e-01) | - (9.87e-01) | - (9.84e-01) | - (9.65e-01) |
| AC C16:2 | - (9.95e-01) | + (7.07e-01) | + (6.54e-01) | - (5.24e-01) | + (1.35e-01) | + (1.85e-02) |
| Carbohydrates (Non-targeted GC/MS) |
| 1,5-Anhydroglucitol | - (2.48e-02) | - (1.53e-01) | + (9.94e-02) | - (6.74e-03) | - (9.51e-02) | + (6.92e-02) |
| Fructose or similar ketohexose | - (5.90e-01) | + (4.52e-03) | + (4.70e-03) | + (6.92e-01) | + (4.44e-03) | + (3.91e-02) |
| Glucuronic acid | + (7.35e-01) | + (3.02e-02) | + (1.64e-01) | + (8.07e-01) | + (4.88e-03) | + (5.63e-02) |
| Gluconic acid | - (4.73e-02) | - (1.72e-02) | - (8.71e-01) | - (7.74e-02) | - (4.49e-01) | + (2.07e-01) |
| Hexitols | + (3.26e-01) | + (2.35e-02) | + (9.43e-02) | + (2.73e-01) | + (2.04e-02) | + (1.07e-01) |
| Pentonic acids | - (4.07e-02) | - (3.62e-01) | + (2.84e-01) | - (4.96e-03) | - (6.27e-01) | + (9.39e-02) |
| Dihydroxybutanoic acid | + (8.73e-01) | + (2.16e-01) | + (1.19e-01) | + (3.26e-01) | + (1.01e-02) | + (2.53e-02) |
| Glycolysis/Tricarboxylic Acids (Non-targeted GC/MS) |
Citric acid/Isocitric acid

| Metabolite         | Value     | Value     | Value     | Value     | Value     |
|--------------------|-----------|-----------|-----------|-----------|-----------|
|                    | - (4.23e-01) | + (1.55e-01) | + (1.28e-02)^ | + (6.93e-01) | + (3.01e-02)^ |
| Fatty Acids (Non-targeted GC/MS) |           |           |           |           |           |
| Linoleic acid      | - (2.69e-02)^ | - (2.64e-01) | + (1.19e-01) | - (5.55e-02) | - (4.67e-01) | + (8.08e-02) |
| Oleic acid         | - (5.03e-02) | - (5.87e-01) | + (3.71e-02)^ | - (1.37e-01) | - (9.88e-01) | + (2.84e-02)^ |
| Palmitic acid      | - (5.30e-02) | - (4.88e-01) | + (9.17e-02) | - (3.99e-02)^ | - (6.08e-01) | + (3.31e-02)^ |
| Stearic acid       | - (2.36e-02)^ | - (4.90e-01) | + (4.24e-02)^ | - (1.49e-01) | + (7.39e-01) | + (1.79e-02)^ |
| Lipids (Non-targeted GC/MS) |           |           |           |           |           |
| Glycerol 1-phosphate | - (2.52e-02)^ | - (1.98e-01) | + (1.72e-01) | - (7.59e-02) | - (9.63e-01) | + (1.16e-02)^ |
| Amino Acids (Non-targeted GC/MS) |           |           |           |           |           |
| Aminomalonic acid  | - (1.36e-01) | - (7.36e-01) | + (1.76e-01) | - (2.10e-01) | + (4.56e-01) | + (3.69e-02)^ |
| 3-Indolelactic acid/Tryptophan | - (2.77e-02)^ | - (4.42e-01) | + (1.79e-02)^ | - (8.51e-02) | - (9.71e-01) | + (2.99e-02)^ |
| Lysine             | - (2.88e-02)^ | - (1.77e-01) | + (5.18e-01) | - (4.69e-02)^ | - (5.63e-01) | + (2.28e-01) |
| Threonine          | - (6.99e-01) | + (3.04e-01) | + (1.20e-01) | - (9.12e-01) | + (2.89e-02)^ | + (1.47e-02)^ |
| Urea               | - (3.31e-01) | + (6.51e-01) | + (1.02e-01) | - (2.90e-01) | + (3.57e-01) | + (2.20e-02)^ |
| Other Metabolites (Non-targeted GC/MS) |           |           |           |           |           |
| Hypoxanthine       | - (8.56e-01) | - (3.93e-02)^ |
| Malonic acid       | - (2.68e-01) | - (4.38e-02)^ | - (4.63e-01) | - (4.06e-01) | - (5.90e-01) | + (4.73e-01) |
| Uric acid          | - (6.40e-03)^ | - (3.51e-01) | + (4.14e-01) | - (2.82e-02)^ | - (5.65e-01) | + (3.60e-01) |

^Nominal p<0.05

Cells are left blank when metabolites were treated as categorical variables and direction of associations were inconsistent across categories. All non-targeted metabolites are measured as log2 transformed mass spectrometry peak areas.
Supplementary Fig. 1: Conditional variable importance for all predictors in random forest analyses

A) Maternal FPG and fasting metabolites – Newborn SS

B) Maternal FPG and fasting metabolites – Newborn BW

C) Maternal 1PG and 1-hour metabolites – Newborn SS

D) Maternal 1PG and 1-hour metabolites – Newborn BW
Supplementary Fig. 1 Plots of variable importance under a conditional permutation scheme for random forest analyses predicting newborn BW and SS. Conditional variable importance is plotted for: Model 0 (M0) = maternal BMI at OGTT, gestational age at delivery, field center and sample storage time; Model 1 (M1) = M0 + maternal glucose at fasting or 1-hr; Model 2 (M2) = M1 + highest scoring metabolite within N spinglass communities for the network (N=3 for SS and N=4 for BW); Model 3 (M3) = M1 + N metabolites (N=3 for SS and N=4 for BW) with lowest aggregate p-values (p_a) measuring joint association with maternal glucose and newborn outcome; Model 4 (M4) = M1 + metabolites with positive conditional variable importance scores after running a model including all metabolites with p_a < 0.10. Conditional variable importance scores reflect the relative contribution of a predictor to the model. For example, Panel A reports results for maternal glucose and metabolites at fasting and newborn SS. In the baseline M0 model, maternal BMI has highest conditional variable importance. When maternal fasting glucose is included in M1, conditional variable importance for maternal BMI decreases and maternal fasting glucose has high conditional variable importance. This indicates that maternal fasting glucose explains in part the effect of BMI on newborn outcomes, but is also an important independent contributor to these outcomes. Metabolites included in M2, M3 and M4 demonstrate conditional variable importance that is comparable to maternal glucose levels. A decrease in variable importance for maternal glucose in M2, M3 and M4 is also evident. This decrease suggests that these maternal metabolites account in part for the effect of glucose on newborn outcomes. Overall percent variation explained for models M0-M4 is reported in the legend for each panel. Importantly, overall variance explained is higher for M2, M3 and M4 compared to M1. Panel B reports results for maternal glucose and metabolites at fasting and newborn BW. Panel C reports results for maternal glucose and metabolites at 1-hr and newborn SS. Panel D reports results for maternal glucose and metabolites at 1-hr and newborn BW. Results in all panels can be interpreted similarly.