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Original article

Metabolomics signatures associated with an oral glucose challenge in pregnant women

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ABSTRACT

Aim. – The oral glucose tolerance test (OGTT), widely used as a gold standard for gestational diabetes mellitus (GDM) diagnosis, provides a broad view of glucose pathophysiology in response to a glucose challenge. We conducted the present study to evaluate metabolite changes before and after an oral glucose challenge in pregnancy; and to examine the extent to which metabolites may serve to predict GDM diagnosis in pregnant women.

Methods. – Peruvian pregnant women ($n = 100$) attending prenatal clinics (mean gestation 25 weeks) participated in the study with 23% of them having GDM diagnosis. Serum samples were collected immediately prior to and 2-hours after administration of a 75-g OGTT. Targeted metabolic profiling was performed using a LC-MS based metabolomics platform. Changes in metabolite levels were evaluated using paired Student's *t*-tests and the change patterns were examined at the level of pathways. Multivariate regression procedures were used to examine metabolite pairwise differences associated with subsequent GDM diagnosis.

Results. – Of the 306 metabolites detected, the relative concentration of 127 metabolites were statistically significantly increased or decreased 2-hours after the oral glucose load (false discovery rate [FDR] corrected P-value < 0.001). We identified relative decreases in metabolites in acylcarnitines, fatty acids, and diacylglycerols while relative increases were noted among bile acids. In addition, we found that C58:10 triacylglycerol ($\beta = -0.08$, SE = 0.04), C58:9 triacylglycerol ($\beta = -0.07$, SE = 0.03), adenosine ($\beta = 0.70$, SE = 0.32), methionine sulfoxide ($\beta = 0.36$, SE = 0.13) were significantly associated with GDM diagnosis even after adjusting for age and body mass index.

Conclusions. – We identified alterations in maternal serum metabolites, representing distinct cellular and metabolic pathways including fatty acid metabolism, in response to an oral glucose challenge. These findings offer novel perspectives on the pathophysiological mechanisms underlying GDM.

Introduction

Gestational diabetes mellitus (GDM), or impaired glucose intolerance first diagnosed during pregnancy, is one of the most common medical complications of pregnancy [1]. Annually, it is estimated to affect approximately 8% of pregnancies in the US [2] and 5% globally [3,4]. GDM is associated with increased risk of caesarean and operative vaginal delivery, macrosomia, intrauterine growth retardation, shoulder dystocia, neonatal hypoglycaemia, hypocalcaemia, and hyperbilirubinemia [5–7]. Recently, the International Diabetes Federation reported that approximately 16% of live births were complicated by hyperglycaemia during pregnancy and the frequency of GDM affected pregnancies is most likely to increase concurrently with the increase in risk factors like obesity and physical inactivity [3].

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In uncomplicated pregnancies, maternal tissues have been shown to progressively become insensitive to insulin and insulin-mediated whole-body glucose disposal decreases by 50% and insulin secretion by 200–250%, in order to maintain a euglycaemic state [8]. In pregnancies complicated by GDM, however, the increased demand for insulin is not met due to inadequate pancreatic β-cell function leading to inadequate compensation for the body's insulin needs [9]. Of note, a subgroup of women with a history of GDM is at increased risk of developing type 2 diabetes mellitus (T2DM) postpartum [10]. However, the underlying metabolic pathophysiology of GDM is not well understood [11].

Emerging metabolomics technologies that enable systemic analysis of small molecules in biological specimens have been successfully used to provide a novel and deeper insight in the etiopathogenesis of diseases [11]. Metabolomics provides an integrated profile of biological status by identifying biochemical changes before the onset of overt clinical disease [12–16]. Relatively few studies have been conducted to assess the underlying metabolic pathophysiology of GDM and no adequate predictive methods are available for GDM thus posing a clear need to develop non-invasive and objective diagnostic methods [11,12,17,18].

The oral glucose tolerance test (OGTT) is widely used as a gold standard for GDM diagnosis and provides a broad view of glucose pathophysiology in response to a glucose challenge [19]. Therefore, we designed a study to systematically characterize acute maternal metabolic changes subsequent to a 75 g oral glucose challenge in late pregnancy. We reasoned that the application of metabolomics methods in the context of clinical obstetrics will yield insights into metabolic alternations underlying GDM pathogenesis and lead to the discovery of additional diagnostic or prognostic biomarkers [20].

Methods

Participants and study setting

This analysis used data initially gathered for the Screening, Treatment and Effective Management of Gestational Diabetes Mellitus (STEM-GDM) study, a cohort study designed to evaluate the prevalence of GDM using the new diagnostic criteria proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) among Peruvian women attending perinatal care at Instituto Nacional Materno Perinatal (INMP) in Lima, Peru [21,22]. The INMP, overseen by the Peruvian Ministry of Health, is the primary referral hospital for maternal and perinatal care. Recruitment period was between February 2013 and February 2014. Women who initiated prenatal care before 28 weeks' gestation were eligible to participate. Women were ineligible if they were younger than 18 years of age, did not speak and read Spanish, did not plan to carry the pregnancy to term or deliver at INMP, and/or were past 28 weeks' gestation.

Enrolled subjects were asked to participate in a structured interview that gathered information regarding sociodemographic, lifestyle, medical, and reproductive characteristics between 24–28 weeks gestation (25 weeks gestation, on average). After an 8-hour, overnight fast, participants underwent a 75 g, 2-hour OGTT. Fasting and 2-hour blood samples were collected, processed, and stored at −80 °C until analyzed. Following the blood sample collection, a brief physical examination was administered by a trained research nurse who took anthropometric measures including standing height and weight. All participants provided informed consent and the research protocol was approved by the Institutional Review Boards of the INMP, Lima, Peru and the Harvard T. H. Chan School of Public Health Office of Human Research Administration, Boston, MA, USA.

Analytical population

The analytical population was derived from participants who enrolled in the STEM-GDM Cohort between February 2013 and February 2014. During this period, a total of 1,032 women participated in the study. A total of 100 women were randomly selected for the present metabolomics analysis. The 100 randomly selected women for this analysis did not differ in regards to sociodemographic and lifestyle characteristics as compared with the total cohort.

Metabolic profiling

Fasting blood samples were collected at 25 weeks of gestation, on average, after an overnight fast, both immediately prior to and 2-hours after administration of 75-g OGTT. Samples were protected from ultraviolet light, kept on wet ice and centrifuged within 20 minutes of phlebotomy. Serum samples were stored at −80 °C until assayed. Four liquid chromatography-tandem mass spectrometry (LC-MS) methods were used to profile serum polar metabolites and lipids. Negative ion mode, targeted MS analyses of polar metabolites were conducted as described previously [23]. Briefly, LC-MS samples were prepared from serum (30 μL) via protein precipitation with the addition of four volumes of 80% methanol containing inosine-15N4, thymine-d4 and glycocholate-d4 internal standards (Cambridge Isotope Laboratories; Andover, MA). The samples were centrifuged (10 min, 9000 × g, 4 °C) and the supernatants were analysed using an ACQUITY UPLC (Waters, Milford MA) coupled to a 5500 QTRAP triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA). Extracts (10 μL) were injected directly onto a 150 × 2.0 mm Luna NH2 column (Phenomenex; Torrance, CA). The column was eluted at a flow rate of 400 μL/min with initial conditions of 10% mobile phase A (20 mM ammonium acetate and 20 mM ammonium hydroxide in water) and 90% mobile phase B (10 mM ammonium hydroxide in 75:25 v/v acetonitrile/methanol) followed by a 10 min linear gradient to 100% mobile phase. MS data were acquired using multiple reaction monitoring scans tuned for each compound using authentic reference standards. The ion spray voltage was −4.5 kV and the source temperature was 500 °C. Raw data were processed using MultiQuant 2.1 software (SCIEX, Framingham MA). Non-targeted, positive ion mode analyses of polar metabolites and lipids were conducted using two separate methods as described previously [24]. Data for both methods were acquired using a Nexera X2 U-HPLC system (Shimadzu Scientific Instruments; Marlborough, MA) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific; Waltham, MA). Polar metabolites were extracted from body fluids and tissue homogenates (10 μL) using addition of nine volumes of 74.9:24.9:0.2 v/v/v acetonitrile/methanol/formic acid containing stable isotope-labelled internal standards (valine-d8, Isotec; and phenylalanine-d8, Cambridge Isotope Laboratories; Andover, MA). The extracts were centrifuged (10 min, 9000 × g, 4 °C), and the supernatants were injected onto a 150 × 2 mm Atlantis HILIC column (Waters; Milford, MA). The column was eluted isocratically at a flow rate of 250 μL/min with 5% mobile phase A (10 mM ammonium formate and 0.1% formic acid in water) for 1 minute followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 minutes. Polar metabolite MS analyses were carried out using electrospray ionization in the positive ion mode, using full scan analysis over m/z 70–800 at 70,000 resolution and 3 Hz data acquisition rate. Additional MS settings were: ion spray voltage, 3.5 kV; capillary temperature, 350 °C; probe heater temperature, 300 °C; sheath gas, 40; auxiliary gas, 15; and S-lens RF level 40. Lipids were extracted from plasma and lung tissue homogenates (10 μL) using 190 μL of isopropanol containing 1-dodecanoyl-2-tridecanoyl-sn-

glycero-3-phosphocholine as an internal standard (Avanti Polar Lipids; Alabaster, AL). After centrifugation (10 min, 9,000 × g, ambient temperature), supernatants (2 μL) were injected directly onto a 100 × 2.1 mm ACQUITY BEH C8 column (1.7 μm; Waters; Milford, MA). The column was eluted at a flow rate of 450 μL/min isocratically for 1 minute at 80% mobile phase A (95:5:0.1 vol/vol/vol 10 mM ammonium acetate/methanol/acetic acid), followed by a linear gradient to 80% mobile-phase B (99.9:0.1 vol/vol methanol/acetic acid) over 2 minutes, a linear gradient to 100% mobile phase B over 7 minutes, and then 3 minutes at 100% mobile-phase B. MS analyses were carried out using electrospray ionization in the positive ion mode using full scan analysis over m/z 200–1100 at 70,000 resolution and 3 Hz data acquisition rate. Additional MS settings were: ion spray voltage, 3.0 kV; capillary temperature, 320 °C; probe heater temperature, 300 °C; sheath gas, 50; auxiliary gas, 15; and S-lens RF level 60.

Laboratory analytical procedures

Fasting blood samples were collected and processed in accordance with standard international procedures using the glucose oxidase method. The diagnosis of GDM was determined using the new IADPSG recommendations [25]. With this definition, the diagnosis of GDM was made when any of the following values from the 75-g OGTT is equaled or exceeded: fasting plasma glucose 5.1 mmol/L, 1-h plasma glucose 10.0 mmol/L, or 2-h plasma glucose 8.5 mmol/L.

Other covariates

Maternal body mass index (BMI) was calculated as weight in kilograms divided by height in square meters (kg/m^2). Categories of BMI were characterized as normal weight (< 25 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥ 30 kg/m^2). Maternal age was categorized as follows: < 19, 20–29, 30–34, ≥ 35 years. Other social and demographic variables were categorized as: maternal education (≤ 6, 7–12, and > 12 completed years of schooling), nulliparous (yes vs. no), marital status (married or living with partner vs. single or living alone/divorced), difficulties to pay for necessities like food (very hard/hard, somewhat hard, and not hard), and difficulties to access medical care (very hard/hard, somewhat hard, and not hard).

Statistical analysis

After excluding 19 metabolites with more than 20% missing or undetectable values, a total of 306 metabolites were analyzed. Metabolites were transformed on the natural logarithmic scale due to skewed distribution. Paired Student's *t*-tests were performed to examine the difference in metabolite levels between the two-time points (fasting and 2-hours post-glucose challenge). To account for multiple testing, a false discovery rate (FDR) procedure was applied [26]. For each metabolite, fold change was determined by taking the mean of the ratios of the log-transformed values between the two-time points for everyone. Then, we used two approaches to compute risk scores of metabolites associated with GDM. In the first approach, we used principal component analysis (PCA) and evaluated the association between the first principal component and GDM status using logistic regression, as the first principal component is the one that explains the maximum amount of variance possible in the dataset. The risk score was defined as the product of the estimated regression coefficient and the value of the first principal component. For the second approach, we used logistic regression models to examine the relation between each metabolite within pathways and GDM status. Scores were computed by multiplying the delta value of each metabolite with its corresponding effect size then summing these products across all metabolites. A leave-one-out cross-validation procedure was implemented to protect against model over-fitting. Thus, successively one observation was left out from the sample (n), the remaining observations (n-1) constituted the training set and the left-out one the validation set. Each time, and for both approaches, regression coefficients were estimated on the training set. This process was repeated n times until all observations were validated. Scores were calculated for the validation set using estimated regression coefficients from the training set. Once all scores were computed a logistic regression model was fit to examine the association of the scores with GDM status. All statistical analyses were conducted using R [27].

Results

The socio-demographic characteristics of the participants are presented in Table 1. A total of 100 pregnant women between the ages of 18 and 45 years (mean age = 28.6 years, standard deviation = 6.6 years) with mean gestational age of 25.6 weeks (SD = 1.2 weeks) participated in the study. Most participants were married or living with their partner (84.0%) while 28.5% were employed during pregnancy. Approximately 20% of participants reported smoking before pregnancy while 29% reported alcohol consumption before index pregnancy. Only 1% reported smoking during index pregnancy while 4% reported alcohol consumption during pregnancy. Gestational diabetes (defined according to the international association for diabetes and pregnancy study group with fasting glucose ≥ 92 mg/dL) was present in 23% of study participants.

Out of the 306 metabolites that were detected, the relative abundance of 127 metabolites had a statistically significantly increase or decrease after OGTT (FDR-corrected P-value < 0.001). Most metabolites decreased in response to OGTT (Table 2a and Supplemental Figure 1a; see supplementary materials associated with this article online) while a few increased in response to OGTT (Table 2b and Supplemental Figure 1b; see supplementary materials associated with this article online). The largest relative

Table 1
Characteristics of Study Population ($n = 100$).

Characteristics	*n*
Gestational age (weeks)[a]	25.6 ± 1.2
Maternal age (years)	28.7 ± 6.6
18–19	
20–29	54
30–34	19
≥ 35	22
Marital status	
Married/living with partner	84
Other	16
Employed during pregnancy	28
Smoked before pregnancy	20
Smoked during pregnancy	1
Alcohol consumption before pregnancy	29
Alcohol consumption during pregnancy	4
Nulliparous	34
Body mass index (kg/m^2)[a]	28.2 ± 3.9
Body mass index (kg/m^2)	
< 18.5	23
18.5–24.9	48
25.0–29.9	23
≥ 30.0	6
Diagnosed gestational diabetes[b]	23

[a] Data in mean ± SD or number (%).
[b] Gestational diabetes (GDM) defined according to the International Association for Diabetes and Pregnancy Study Group with fasting glucose ≥ 92 mg/dL [25]. BMI at the time of testing.

Table 2a
Metabolites that decreased after glucose challenge (FDR adjusted paired t-test *P*-value < 0.001).

Metabolite	Pathway group	Mean change	SD	Fold change[a]
C8 carnitine	Acylcarnitines	−1.45	0.95	1.20
C14:2 carnitine	Acylcarnitines	−1.15	0.53	1.13
C12 carnitine	Acylcarnitines	−1.07	0.56	1.13
C4-OH carnitine	Acylcarnitines	−0.95	0.64	1.13
C14:1 carnitine	Acylcarnitines	−1.09	0.53	1.13
C10 carnitine	Acylcarnitines	−1.04	0.57	1.11
5-HETE	Fatty acid	−1.00	0.96	1.10
C6 carnitine	Acylcarnitines	−0.76	0.40	1.09
C12:1 carnitine	Acylcarnitines	−1.07	0.55	1.08
C10:2 carnitine	Acylcarnitines	−0.60	0.38	1.08
C14 carnitine	Acylcarnitines	−0.57	0.47	1.07
C30:0 DAG	Diacylglycerols	−0.61	0.59	1.07
Palmitoleic acid	Fatty acid	−1.26	0.43	1.07
Gamma-Linolenic acid	Fatty acid	−1.18	0.47	1.06
C32:2 DAG	Diacylglycerols	−0.56	0.69	1.06
Aspartate	Amino acid	−0.54	0.40	1.06
Methionine sulfoxide	Amino acid	−0.44	0.56	1.06
Glycine	Amino acid	−0.34	0.34	1.06
Alpha-glycerophosphocholine	Glycerophospholipids	−0.70	0.35	1.06
Allantoin	Purine	−0.43	0.61	1.05
Docosapentaenoic acid	Fatty acid	−0.84	0.38	1.05
C18:2 carnitine	Acylcarnitines	−0.46	0.38	1.05
Adrenic acid	Fatty acid	−0.79	0.34	1.05
C9 carnitine	Acylcarnitines	−0.60	0.54	1.05
Eicosapentaenoic acid	Fatty acid	−0.77	0.36	1.05
C18:1 carnitine	Acylcarnitines	−0.47	0.34	1.05
Glutamate	Amino acid	−0.48	0.32	1.05
Leucine	Amino acid	−0.56	0.26	1.04
Isoleucine	Amino acid	−0.52	0.22	1.04
8.11.14-Eicosatrienoic acid	Fatty acid	−0.67	0.30	1.04
Methionine	Amino acids	−0.42	0.23	1.04
Citrulline	Amino acid	−0.38	0.20	1.04
Serine	Amino acid	−0.37	0.22	1.04
Linoleic acid	Amino acid	−0.70	0.28	1.04
Phenylalanine	Amino acid	−0.47	0.27	1.04
Oleic acid	Fatty acid	−0.67	0.26	1.03
Thiamine	Amino acids	−0.46	0.37	1.03
C18:0 LPE	Lysophosphatidylethanolamines	−0.33	0.33	1.03
Tyrosine	Amino acid	−0.39	0.23	1.03
Docosahexaenoic acid	Fatty acid	−0.58	0.30	1.03
C2 carnitine	Acylcarnitines	−0.43	0.30	1.03
Asparagine	Amino acid	−0.29	0.19	1.03
Niacinamide	Amino acid	−0.28	0.42	1.03
Homocysteine	Amino acid	−0.29	0.64	1.03
C16 carnitine	Acylcarnitines	−0.26	0.21	1.03
Palmitic acid	Fatty acid	−0.51	0.18	1.03
Taurine	Biogenic amines	−0.33	0.43	1.03
Valine	Amino acids	−0.30	0.17	1.03
Arachidonic acid	Fatty acid	−0.45	0.36	1.02
C32:1 DAG	Diacylglycerols	−0.29	0.21	1.02
Hydroxyproline	Amino Acid	−0.25	0.18	1.02
C16:0 LPE	Lysophosphatidylethanolamines	−0.24	0.33	1.02
C42:0 TAG	Triacylglycerol	−0.25	0.36	1.02
Ornithine	Biogenic amines	−0.23	0.25	1.02
C18:1 LPE	Lysophosphatidylethanolamines	−0.20	0.41	1.02
Kynurenic acid	Amino Acid	−0.19	0.35	1.02
Arginine	Amino acid	−0.31	0.20	1.02
C32:0 DAG	Diacylglycerols	−0.25	0.19	1.02
C34:0 DAG	Diacylglycerols	−0.23	0.17	1.02
Myristic acid	Fatty acid	−0.38	0.16	1.02
Stearic acid	Fatty acid	−0.37	0.18	1.02
C18:0 LPC	Lysophosphatidylcholines	−0.28	0.24	1.02
Threonine	Amino acids	−0.19	0.15	1.02
C34:3 DAG	Diacylglycerols	−0.18	0.20	1.02
Choline	Amino Acid	−0.22	0.24	1.02

[a] Fold change represents mean of log-pre-OGTT/log-post-OGTT value.

decrease after OGTT was seen in C8 carnitine which decreased by 20%, followed by C14:2 carnitine, C14:1 carnitine, C12 carnitine, and C4-OH carnitine which decreased by 13% each. The largest relative increases after OGTT were seen in adenosine and taurolithocholic acid, which increased by 25% and 12% respectively. Fig. 1 shows a heat map of partial Pearson correlation coefficients of metabolite concentrations between before and after responses to OGTT, adjusted for gestational age and grouped by pathways. We found that baseline concentrations of metabolites in common biological pathways were correlated after OGTT. Higher positive correlations were noted for carnitines while higher negative correlations were noted for triacylglycerols.

Table 2b
Metabolites that increased after glucose challenge (FDR adjusted paired t-test *P*-value < 0.001).

Metabolite	Pathway group	Mean change	SD	Fold change[a]
Adenosine	Purine	1.64	1.32	1.25
Taurolithocholic acid	Bile acid	1.28	1.14	1.12
Glycolithocholic acid	Bile acid	0.69	0.88	1.06
Glycochenodeoxycholic acid	Bile acid	0.87	0.85	1.06
Taurochenodeoxycholic acid	Bile acid	0.73	0.93	1.05
Taurodeoxycholic acid	Bile acid	0.68	0.89	1.05
Glycoursodeoxycholic acid	Bile Acid	0.56	0.96	1.05
Glycodeoxycholic acid	Bile acid	0.69	0.75	1.05
Taurocholic acid	Bile acid	0.53	0.95	1.04
Glycocholic acid	Bile Acid	0.50	0.97	1.04
Phosphocholine	Organonitrogen	0.42	0.70	1.03

[a] Fold change represents mean of log-post-OGTT/log- pre-OGTT value.

Fig. 2 shows changes in metabolite levels in response to OGTT in groups of metabolic pathways. For example, major decreases in metabolite levels were noted in acylcarnitine, fatty acid and diacylglycerol pathways where more than a 10% decrease in relative concentration was noted in the following metabolites: carnitines C12:2, C14:1, C12, C10:2 and C8. A major increase in concentration was noted in metabolites of bile acid pathway where adenosines increased by more than 20% and taurolithocholic acid increased by more than 10%.

In analyses using linear regression procedures on each metabolite versus GDM status, we found that C58:10 triacylglycerol, C58:9 triacylglycerol, adenosine, and methionine sulfoxide were significantly associated with GDM status even after adjusting for age and BMI (Table 3a).

Next, to identify a linear combination of metabolites that might be associated with of GDM, we calculated risk scores using two approaches. In the first approach, we performed a PCA on normalized data (Table 3b). The association between the first principal component (representing 88% of variability) and GDM status was examined using a logistic regression model. The corresponding risk score computed by multiplying the first principal component by its effect size was found to be significantly associated with GDM risk (coefficient = −29.37, SE = 8.6, $P < 0.001$). The distribution of the scores by GDM status are shown in Supplemental Figures 2a and b (see supplementary materials associated with this article online). Overall, the median scores for GDM women were lower in amino acids and glycerophospholipids while they are relatively higher for lysophosphatidylcholines, as compared with non-GDM women. In the second approach, each metabolite's relation with GDM status was analyzed using univariate logistic regression models and scores

Fig. 1. Heat-map of partial Pearson correlation coefficients adjusted for gestational age for the 127 significantly changed metabolites ordered by pathway groups.

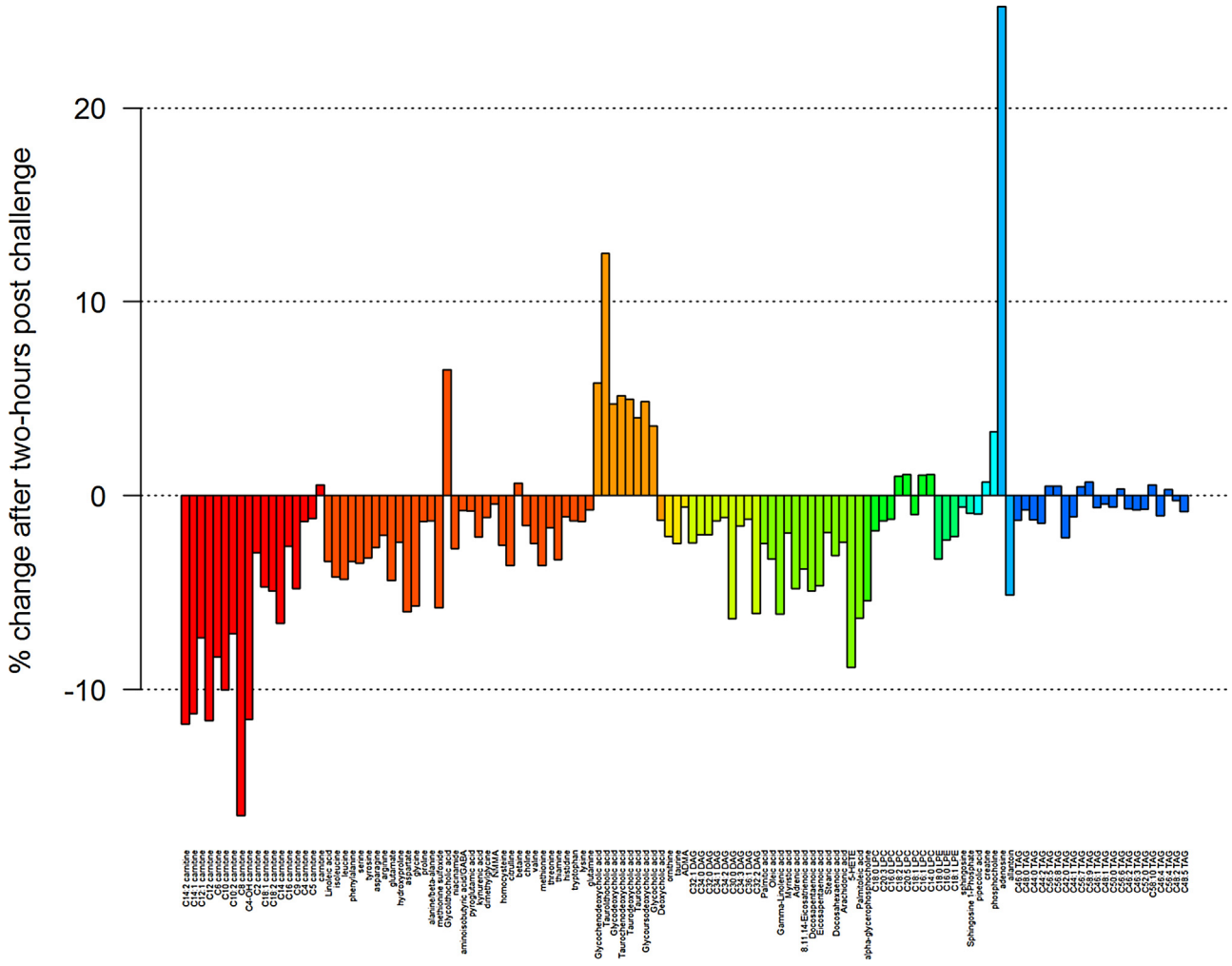

Fig. 2. Changes in metabolite levels in response to two-hour post challenge are shown. Metabolites were ordered by pathway groups.

Table 3a
Differences in metabolite levels in response to OGTT between GDM and non GDM cases.

Metabolite	Adjusted for age			Adjusted for age and BMI		
	Estimate	SE	*P*-value	Estimate	SE	*P*-value
C58:10 TAG	−0.085	0.037	0.030	−0.082	0.037	0.031
C58:9 TAG	−0.069	0.033	0.041	−0.069	0.033	0.042
Adenosine	0.705	0.321	0.031	0.705	0.322	0.031
Methionine sulfoxide	0.356	0.131	0.008	0.357	0.131	0.008

Table 3b
Effect of scores on GDM status evaluated by logistic regression.

	Estimate	SE	*P*-value
Score based on pca	−29.3788	8.6236	< 0.001
Score based on univariate analysis Normalized	−0.1714	0.05857	0.003

were computed by multiplying the delta value of each metabolite concentrations at baseline and post-OGTT with its corresponding regression coefficient and taking the sum over all metabolites. This score is also found to be associated with GDM status (β coefficient = −0.113, SE = 0.046, $P = 0.0143$).

Discussion

Using a targeted metabolomics approach, we found significant metabolic differences in various biological pathways in response to a glucose challenge during pregnancy. Specifically, we identified relative decreases in acylcarnitine, fatty acid, and diacylglycerol pathways while relative increases were noted in bile acid pathways. In addition, we found that C58:10 triacylglycerol, C58:9 triacylglycerol, adenosine, and methionine sulfoxide were significantly associated with GDM status even after adjusting for age and BMI.

Our findings showing decreases in acylcarnitines and diacylglycerols following glucose ingestion are not surprising [12]. Acylcarnitines, involved in β-oxidation of fatty acid or amino acid metabolism, are markers of mitochondrial dysfunction [28] and have been implicated in insulin resistance and energy homeostasis [29]. Moreover, acylcarnitines are synthesized by the enzyme carnitine palmitoyltransferase 1 (CPT 1) that is known to be responsible for the transport of long-chain fatty acids into the mitochondrial matrix [30]. Fatty acids can be metabolized via a mitochondrial FA oxidation (FAO) pathway which yields energy. The FAO competes with glucose oxidation in a process known as glucose-FA or Randle cycle [29]. Available evidence suggests that acylcarnitines play a significant role in energy metabolism and GDM pathogenesis [31]. Investigators have also postulated an alternative mechanism in which FAO rate outpaces the tricarboxylic acid cycle (TCA) thereby leading to the accumulation of

intermediary metabolites such as acylcarnitines that may interfere with insulin sensitivity [29].

Lipotoxicity, excess lipid supply and accumulation, has been one of the theories proposed for the induction of insulin resistance in glucose and lipid metabolism [29,32]. Diacylglycerols (DAG) are lipid intermediates that have been implicated in insulin resistance and are signalling molecules and building blocks of cellular membranes, which harbour the insulin receptor. Animal studies have demonstrated that long-term ingestion of DAG prevents high fat-induced body weight gain and fat accumulation [33,34]. Our study results showing decreases in DAG following glucose ingestion confirm prior observation in animal [35] and human studies [36] suggesting a key role for lipid intermediates such as DAG in the development of metabolic disorders such as GDM.

Our findings showing post-glucose increases in conjugated bile acid pathways are consistent with prior studies [12]. It has long been known that bile acids are amphipathic molecules that function as powerful detergents to facilitate absorption of lipids and nutrients and excretion of cholesterol and toxic metabolites [37]. In response to glucose ingestion, cholecystokinin stimulates the gallbladder to contract and release bile into the enterohepatic circulation [38]. Several lines of evidence have demonstrated that bile acids play important roles in glucose regulation and energy homeostasis [39,40]. For example, studies from animal models have shown that the primary bile acids such as cholic acid synthesized from hepatic cholesterol increases energy expenditure and prevents the development of high-fat-induced obesity and insulin resistance [41]. These metabolic effects of bile acid are mediated by the G-protein-coupled receptor TGR5, leading to the induction of type-2 iodothyronine deiodinase. Recently, Vincent et al. have found that the postprandial bile acid response in obese patients with type-2 diabetes mellitus is greater than that in normoglycaemic individuals [42]. In the past decade, there has been an increased recognition that bile acids are natural ligands for the farnesoid X receptor (FXR-α) and were found to activate specific nuclear receptors such as pregnane X receptor, vitamin D receptor, G protein-coupled receptors and cell signalling pathways [43]. A recent study by Fall et al. found that increased concentrations of three 12α-hydroxylated bile acids (deoxycholic acid, glycocholic acid, and glycodeoxycholic acid) were associated with incident diabetes [44]. In addition, a genetic variant within the CYP7A1 locus, encoding the rate-limiting enzyme in bile acid synthesis, was found to be associated with lower type-2 diabetes risk [44]. Collectively these observations, coupled with our findings, provide additional insights into the key role bile acid pathways play in the pathogenesis of GDM and other metabolic disorders.

We found a sub-group of maternal metabolites (i.e., C58:10 triacylglycerol, C58:9 triacylglycerol, adenosine, and methionine sulfoxide) to be statistically significantly associated with GDM. These findings indicate the roles endothelial dysfunction and oxidative stress pathways play in the pathophysiology of GDM [45]. For instance, adenosine acts as a vasodilator in several vascular beds and acts as a stimulator of endothelial cell proliferation [46]. Adenosine has been implicated in physiological responses of different tissues [47,48]. It contributes to endothelial dysfunction in endothelial cells from the umbilical veins of patients with GDM [49]. Of note, the transport and metabolism of adenosine is markedly impaired in foetal endothelial cells isolated from pregnancies complicated by GDM [47,48]. In addition, increased concentration of adenosine upregulate expression of endothelial nitric oxide synthase via A2A adenosine receptors, which leads to increased nitric oxide synthesis, a potential cause of vascular dysfunction in GDM [50].

Investigators have reported that methionine sulfoxide can be considered a marker of oxidative stress and is associated with increased high sensitivity c-reactive protein [51,52]. These findings, if replicated, should facilitate the identification of additional circulating biomarkers suitable for predicting the development or progression of GDM.

Some strengths and limitations should be considered in interpreting our study findings. Using samples before and after glucose ingestion allowed subjects to serve as their own biological controls. This eliminated confounding by subject characteristics. However, there are several caveats that merit consideration. First, the cross-sectional design of our study does not allow for determination of the temporal relationship between perturbations of metabolites and GDM risk although the changes in metabolites were in agreement with known physiological actions of glucose. Longitudinal studies are needed following women earlier in their pregnancies. Second, the changes observed in metabolites were in relative concentrations. We were not able to provide absolute concentrations of metabolites. Absolute quantification of metabolites is a future area of investigation. Lastly, our study had a limited sample size and was conducted in women attending prenatal clinics in Peru and hence may not be generalizable to other populations.

In summary, we identified changes in several metabolites representing distinct biological pathways during an OGTT offering novel perspectives on the GDM pathogenesis. We found relative decreases in acylcarnitine, fatty acid, and diacylglycerol pathways while relative increases were noted in bile acid pathways. In addition, we found that C58:10 triacylglycerol, C58:9 triacylglycerol, adenosine, and methionine sulfoxide were significantly associated with GDM status even after adjusting for age and BMI.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary materials (Supplemental Fig. 1a and 1b, and 2a and 2b) associated with this article can be found at http://www.sciencedirect.com at https://doi.org/10.1016/j.diabet.2018.01.004.

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