JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Exploration of predictive metabolic factors for gestational diabetes mellitus in Japanese women using metabolomic analysis

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Keywords
Case–control study, Gestational diabetes mellitus, Metabolome

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J Diabetes Investig 2019; 10: 513–520

doi: 10.1111/jdi.12887

ABSTRACT

Aims/Introduction: We aimed to explore novel predictive markers for gestational diabetes mellitus using metabolomic analysis in pregnant Japanese women.

Materials and Methods: We carried out a case–control study with a cohort of participants enrolled during the first or early second trimester in the Center of Chiba Unit of the Japan Environment and Children's Study. Participants were classified as either gestational diabetes mellitus cases or matched controls based on age, body mass index and parity. Metabolite levels of their serum and urine obtained randomly before the diagnosis of gestational diabetes mellitus were analyzed using hydrophilic interaction chromatography tandem mass spectrometry. Orthogonal projections to latent structures discriminant analysis was carried out to investigate metabolome profiles for the different groups. Metabolites with a variable importance in projection value of >1.5 were identified as potential markers.

Results: In total, 242 participants were enrolled in the study, of which 121 were cases. The R2X, R2Y and Q2 parameters for the discrimination ability of the resulting models were 0.388, 0.492 and 0.45 for serum, and 0.454, 0.674 and 0.483 for urine, respectively. We finally identified three metabolites in serum and 20 in urine as potential biomarkers. Glutamine in serum and ethanolamine and 1,3-diphosphoglycerate in urine showed >0.8 area under the receiver operating characteristic curves.

Conclusions: The present study identified serum and urine metabolites that are possible predictive markers of subsequent gestational diabetes mellitus in Japanese women. Further studies are required to elucidate their efficacy.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance resulting in hyperglycemia of variable severity, with onset or first recognition during pregnancy[1]. GDM is reportedly associated with the risk of various maternal and perinatal complications, such as macrosomia, premature delivery and pre-eclampsia[2], and the onset of type 2 diabetes postpartum[3]. Additionally, GDM promotes the development of obesity and other metabolic disorders in the offspring of GDM mothers later in life[4–6]. Hence, the developmental origins of health and disease is a concept that states that environmental factors during the fetal and perinatal period affect the onset of some non-communicable diseases in adulthood[7]. Prevention of GDM development or strict control of plasma glucose in patients with GDM is considered to be important for both pregnant women and their children[8]. Given the availability of effective interventions for delaying or preventing GDM onset[9,10], earlier identification of individuals at risk is particularly crucial. Biomarkers for prediction are required for an effective prevention of GDM. Valid predictive methods have not been established thus far; however, despite the use of conventional risk factors, such as body mass index (BMI), hemoglobin A1c (HbA1c) might possibly prove to be feasible in the development of a predictive method[11]. Although some hormones and nutrients associated with glucose metabolism have also been reported as potential markers for GDM prediction[12,13], these factors alone are not sufficient to predict GDM. Recently, analytical methods to investigate all of the metabolites in an organism, also known as the metabolome, have been established. This method allows for

Received 25 January 2018; revised 21 June 2018; accepted 26 June 2018

the simultaneous analysis of various metabolites[14]. Recent studies have reported early detection or prediction of diabetes or impaired glucose metabolism using metabolomic analysis[15,16]. GDM has been one of the targets of this analytical method. Several studies using a metabolomic approach reported that various metabolites might have the potential to predict the subsequent development of GDM in European, USA or Chinese populations[17–23]. Metabolomic profiles are affected by ethnicity[24]; thus, metabolomic analysis for the Japanese population should be carried out. In the present study, we aimed to explore the novel predictive factors for GDM using the metabolomic analysis of serum and urine from pregnant Japanese women in their first or early second trimester.

METHODS

Design

This was a case–control study within a cohort of participants enrolled in the Japan Environment and Children's Study (JECS) at the Center of Chiba Unit.

Participants

JECS is a nationwide, ongoing cohort study that is planned and implemented to elucidate the influences of environmental factors in the fetus through childhood on children's development and health. Pregnant women were recruited for 3 years (2011–2014), and women who agreed to participate were enrolled before delivery. The study design of JECS has been described in detail elsewhere[25]. Various environmental factors were assessed, and biomaterials, such as maternal blood and urine, were collected[25,26]. The blood and urine samples obtained randomly at the first visit after enrollment were analyzed in the present study. Blood samples were centrifuged and divided into serum. Serum and urine samples were frozen and stored at −20°C until analysis.

Participants with GDM were defined as those with GDM reported in their medical records at the time of delivery[27], and without a past history or diagnosis of GDM reported by the first questionnaires carried out at the beginning of pregnancy (prior to diagnosis of GDM: p-GDM group). We then selected the same number of participants without a history or diagnosis of GDM who were matched to the cases based on age, BMI and parity for the control group at random. In Japan, GDM screening is recommended using the following stepwise methods for almost all pregnant women. Random blood glucose levels are measured at an early stage of pregnancy. Between 24 and 28 gestational weeks, pregnant women are given a 50-g glucose challenge test (cut-off value ≥140 mg/dL), or random blood glucose levels are measured (cut-off value ≥100 mg/dL). All women with positive screening test results are administered a 75-g oral glucose tolerance test for GDM diagnosis[28].

The present study was carried out according to the Declaration of Helsinki, and the study protocol was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University, Chiba, Japan. Additionally, written informed consent was obtained from the enrolled participants.

Metabolomic analysis by hydrophilic interaction chromatography tandem mass spectrometry

Metabolomic analysis was carried out as previously described[29,30]. Serum and urine levels of 42 and 263 metabolites, respectively, found to be detectable under our storage conditions (−20°C) were pre-treated[31] and analyzed using hydrophilic interaction chromatography tandem mass spectrometry methods as previously reported[31,32]. Briefly, after centrifugation at 14,000 *g* for 10 min, 40 μL of sample and 60 μL of methanol containing the internal standards (1 nmol/L lidocaine and N,N-diethyl-2-phenylacetamide for positive ion mode, and D-camphor-10-sulfonic acid for negative ion mode) to adjust for sample loss during the pretreatment procedure were pipetted onto Amicon® Ultra-0.5 3K filter columns (Merck Millipore, Tokyo, Japan). The columns were centrifuged at 14,000 *g* for 1 h, and the filtrates were analyzed using Prominence UFLC (Shimadzu, Kyoto, Japan) and QTRAP 4500 systems (AB SCIEX, Tokyo, Japan). Samples were delivered to mass spectrometry through hydrophilic interaction chromatography using a 3.5-μm XBridge BEH Amide column (Waters, Tokyo, Japan), 4.6 mm (internal diameter) × 100 mm, at a flow rate of 350 μL/min. In the present study, we analyzed the target metabolome using a gradient of mobile phase A (20 mmol/L ammonium hydroxide/20 mmol/L ammonium acetate [pH 9.0] in ultrapure water : acetonitrile [95:5]) and mobile phase B (high-performance liquid chromatography-grade acetonitrile). Peak areas from the total ion current for each metabolite selected reaction monitoring transition were integrated using MultiQuant v3.0 software (AB SCIEX). The peak integration settings used were as described by Yuan *et al.*[31]. Urine metabolites were corrected using the peak area of creatinine in each sample.

Statistical analysis

Data are presented as mean ± standard deviation or median (interquartile range). Differences in the measurement values between p-GDM and control groups were evaluated by unpaired *t*-tests, Mann–Whitney tests or Fisher's exact tests according to their distribution. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) were carried out to investigate metabolome profiles of different groups using R package ropls[33] in Microsoft R open 3.3.0 (R Core Team, 2016, R Core Team R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria). Metabolites with a variable importance in projection value >1.5 were identified as potential markers. The statistical significance and validity of the OPLS-DA model were calculated using a permutation test (number of permutations = 1,000)[34]. The sensitivity and specificity of each metabolite was assessed using the receiver operating characteristic (ROC) curve and respective area under the ROC curve

(AUC_{ROC}) carried out using R package ROCR[35]. It has been previously reported that several metabolites can be affected by gestational age[36]. Thus, we carried out comparisons and ROC analyses using the data obtained before 16 weeks-of-gestational age (serum: p-GDM $n = 32$, controls $n = 44$; urine: p-GDM $n = 11$, controls $n = 13$).

To identify pathways that changed in the p-GDM group, we used an enrichment analysis using MBRole 2 (Computational Systems Biology Group, National Center for Biotechnology [CNB-CSIC], Madrid, Spain)[37]. In this study, we used a false discovery rate value[38] of <0.05 as the threshold to judge the significantly enriched metabolic pathways of submitted metabolites using the Kyoto Encyclopedia of Genes and Genomes ID of potential biomarkers[39].

RESULTS

From 2011 to 2014, approximately 6,000 participants were recruited at the Chiba Unit Center, of which 167 developed GDM based on medical record reviews used to match the p-GDM group. Serum samples were available from 121 participants in the p-GDM group for metabolomic analysis. We then selected 121 participants for the control group. The characteristics of the p-GDM and control groups are shown in Table 1. Age, pre-pregnancy weight, height, pre-pregnancy BMI and ratio of primipara did not significantly differ between the groups. HbA1c values and gestational age in weeks at the time of sample collection in the p-GDM group were significantly higher than in the control group. The urine samples of 67 (p-GDM: 36, control: 31) of 242 participants were also analyzed.

To identify metabolic differences between the p-GDM and control groups, we quantified serum and urine metabolites during early pregnancy. Using hydrophilic interaction chromatography tandem mass spectrometry, 32 of the 187 and 42 of the 263 targeted metabolites in serum and urine were detected in >50% of analyzed samples, respectively.

We carried out OPLS-DA to generate discrimination models (Figure S1). In serum, the predictive ability of R2X, R2Y and Q2 was 0.388, 0.492 and 0.45, respectively, whereas in urine, the predictive ability of R2X, R2Y and Q2 was 0.454, 0.674 and 0.483, respectively. The original model did not belong to the population of 1,000 randomly permuted models (for serum and urine, R2Y and Q2: $P < 0.001$), showing the significance of the fitness and prediction ability attached with the original OPLS-DA model. Variable importance in projection scores was calculated for each metabolite based on its contribution to statistical discrimination. In total, 3 and 20 metabolites were found to meet the selection criteria (variable importance in projection >1.5) in serum (Table 2) and urine (Table 3), respectively, and were therefore subjected to further evaluation.

In serum, glutamine ($P < 0.001$), pyrophosphate ($P < 0.001$) and octulose-1,8-bisphosphate ($P < 0.001$) significantly differed between the p-GDM and control groups. Shikimate-3-phosphate ($P < 0.001$), ethanolamine ($P < 0.001$), 1,3-diphosphoglycerate ($P < 0.001$), N-acetyl-L-alanine ($P < 0.001$) and methionine ($P < 0.001$) in urine were also significantly different between the groups (Table 4). In the p-GDM group, one serum sample had been obtained during the third trimester, whereas all others had been obtained during the first or second trimester. We carried out the same analyses excluding the serum sample obtained during the third trimester. The results were comparable with those described above, and therefore we carried out the subsequent analyses without this serum sample.

Among these metabolites, glutamine in serum and ethanolamine and 1,3-diphosphoglycerate in urine showed >0.80 AUC_{ROC} values in the prediction model for discrimination between the p-GDM and control groups (Table 4). These results suggest that glutamine in serum, and ethanolamine and 1,3-diphosphoglycerate in urine are candidate predictive markers for GDM (Figure S2). In contrast, the AUC_{ROC} value for HbA1c was 0.58.

Using the data from before 16 weeks-of-gestational age, we compared the aforementioned three metabolites between the groups. The levels of these three metabolites were significantly different between the groups (data not shown). The AUC_{ROC} values of glutamine in serum, and ethanolamine and 1,3-diphosphoglycerate in urine were 0.81, 0.92 and 0.78, respectively.

Enrichment analysis showed that the metabolites contributing to discrimination in urine were related to seven pathways

Table 1 | Participant characteristics

Characteristics	p-GDM	Control	*P*-value
Age (years)	32.0 (5.0)	31.9 (4.8)	0.90
Pre-pregnant weight (kg)	57.5 (12.8)	57.8 (13.2)	0.82
Height (cm)	158.4 (5.5)	158.7 (5.3)	0.58
Pre-pregnancy BMI (kg/m^2)	22.4 (5.4)	22.9 (4.8)	0.49
Gestational weight gain (kg)	8.6 (5.1)	9.3 (4.7)	0.24
Parity	0.81 (1.06)	0.86 (0.91)	0.68
Gestational weeks at sample collection (weeks)	18.6 (3.8)	17.3 (3.5)	<0.01
HbA1c	5.15 (0.41)	5.03 (0.30)	0.01

Values are shown as mean (standard deviation). BMI, body mass index; HbA1c, hemoglobin A1c; p-GDM, prior to diagnosis of gestational diabetes mellitus.

Table 2 | Metabolite markers identified in serum

	VIP	Coefficient
Glutamine	2.39	–0.1866
Pyrophosphate	2.34	–0.1900
Octulose-1,8-bisphosphate (OBP)	2.24	–0.1366

Metabolites with variable importance in projection (VIP) of >1.5 are shown.

Table 3 | Metabolite markers identified in urine

	VIP	Coefficient
Shikimate-3-phosphate	2.91	0.0510
Ethanolamine	2.59	–0.0620
1,3-Diphopshateglycerate	2.36	0.0625
Leucine-isoleucine	2.04	–0.0413
p-Hydroxybenzoate	1.96	0.0309
Hydroxyphenylpyruvate	1.87	–0.0326
N-acetyl-L-alanine	1.83	0.0420
Hydroxyproline	1.83	–0.0353
Octulose-monophosphate	1.76	–0.0261
N-acetyl-glucosamine	1.74	–0.0327
Shikimate	1.73	–0.0273
Cellobiose	1.71	0.0337
Threonine	1.70	–0.0354
Methylcysteine	1.70	–0.0331
Methionine sulfoxide	1.69	–0.0350
Urea	1.66	–0.0311
Deoxyribose-phosphate	1.65	–0.0305
Methionine	1.61	–0.0379
Acadesine	1.54	–0.0324
D-sedoheptulose-7-phosphate	1.52	–0.0299

Metabolites with variable importance in projection (VIP) of >1.5 are shown.

(Table 5). These pathways are associated with amino acid metabolism and the pentose phosphate pathway.

DISCUSSION

In the present study, we found that glutamine in serum, and ethanolamine and 1,3-diphosphoglycerate in urine might be new predictive metabolites for GDM in Japanese women during early pregnancy using metabolomic analysis.

Gestational diabetes mellitus is reported to be associated with both maternal and fetal adverse outcomes; thus, blood glucose levels must be controlled within an appropriate range to prevent the onset of adverse outcomes. A previous study showed that an intensive therapeutic approach might be useful for this purpose[8]. Prediction of GDM before its onset is also required, as it allows for the provision of early interventions for susceptible women to prevent GDM.

Several factors have been reported to predict GDM, such as age, pre-pregnancy BMI, family history of diabetes and history of large-for-gestational age infant delivery. As we selected the control group based on age, pre-pregnancy BMI and parity of p-GDM group, which are risk factors for GDM, their predictive values could not be evaluated in the present study. However, we examined the predictive value of HbA1c. Although maternal HbA1c level in early pregnancy significantly differed between the groups, the predictive ability of HbA1c was not sufficient in our cohort, as suggested by its low AUC_{ROC} value. It was reported that HbA1c within the normal range alone might not be sufficient to predict GDM[12]. Here, most of the participants showed HbA1c <5.7%, which might be the reason why HbA1c did not show sufficient predictive ability. However, several studies have reported that HbA1c was useful in predicting subsequent GDM or to exclude women who are at low risk for GDM[40–42]. The inconsistency in the results might have been due to the difference in study population or other risk factors. Larger cohort studies are required to elucidate the ability of HbA1c to predict GDM. Our cohort had no family history of

Table 4 | Area under the receiver operating characteristic curve for metabolite markers, and differences in the serum and urine metabolite markers stratified by pre-gestational diabetes vs control groups

	p-GDM	Control	*P*-value	FDR	AUC
Serum					
Glutamine	0.15 (0.098–0.21)	0.29 (0.25–0.35)	3.74E-16	1.57E-14	0.804
Pyrophosphate	0.0071 (0.00–0.043)	0.092 (0.0054–0.18)	9.03E-10	3.70E-08	0.725
Octulose-1,8-bisphosphate	0.15 (0.035–0.23)	0.24 (0.15–0.36)	4.25E-07	1.70E-05	0.689
Urine					
Shikimate-3-phosphate	0.047 (0.032–0.062)	0.032 (0.023–0.040)	1.13E-04	2.94E-02	0.768
Ethanolamine	0.039 (0.028–0.053)	0.070 (0.055–0.096)	2.47E-06	6.47E-04	0.821
1,3-Diphosphoglycerate	0.037 (0.023–0.053)	0.015 (0.011–0.022)	2.38E-07	6.27E-05	0.848
N-acetyl-L-alanine	0.071 (0.054–0.11)	0.046 (0.027–0.073)	8.00E-04	2.08E-01	0.735
Methionine	0.0064 (0.0041–0.0098)	0.013 (0.0072–0.017)	2.29E-03	5.93E-01	0.718

Data are presented as median (interquartile range). AUC, area under the receiver operating characteristic curve; p-GDM, prior to diagnosis of gestational diabetes.

Table 5 | Altered metabolites identified as potential markers in urine using enrichment analysis

Annotation	*P*-value	FDR correction	Matching identification
Valine, leucine and isoleucine biosynthesis	2.23E-04	5.13E-03	C00123 C04236 C00188
ABC transporters	5.36E-04	6.16E-03	C00123 C00185 C00188 C00086
Betaine–homocysteine S-methyltransferase	4.23E-04	1.14E-02	C00155 C00073
Methionine synthase	1.46E-03	1.97E-02	C00155 C00073
Aminoacyl-tRNA biosynthesis	4.05E-03	2.17E-02	C00123 C00188 C00073
Phenylalanine, tyrosine and tryptophan biosynthesis	6.14E-03	2.35E-02	C03175 C01179
Pentose phosphate pathway	8.56E-03	2.81E-02	C00673 C05382

FDR, false discovery rate; tRNA, transfer ribonucleic acid.

diabetes, which is an important predictor of GDM[11]. Several studies have shown that the alterations of the metabolites are useful in predicting GDM, especially when added to clinical findings, such as family history[23,43]. The present results should be validated using other cohorts in combination with these clinical findings.

The present data suggest that glutamine in serum, and ethanolamine and 1,3-diphosphoglycerate in urine are potential predictive markers, as shown by their AUC_{ROC} values. Glutamine is reported to increase insulin secretion and suppress β-cell apoptosis in diabetic rats[44]. Conversely, glutamine stimulated α-cell proliferation through mammalian target of rapamycin signal[45]. Meta-analysis of metabolomics data showed that blood glutamine was inversely associated with the risk of type 2 diabetes development[46]. Glutamine is considered to play an important role in glucose metabolism in p-GDM pregnant women. Ethanolamine is a metabolite of glycerophospholipid metabolism. Glycerophospholipid metabolism is reportedly associated with GDM or pre-diabetes[17,47]. However, the role of ethanolamine itself in glucose metabolism still remains unclear and warrants further studies. 1,3-Diphosphoglycerate is a metabolite of glycolysis and gluconeogenesis. Alteration of urinary 1,3-diphosphoglycerate might reflect the changes in glycolytic or gluconeogenic pathways of patients in the p-GDM state. The precise mechanism of alteration of these metabolites has not yet been clarified; hence, further studies are required.

Several amino acids are reportedly associated with the p-GDM state, such as glutamate and serine[48]. The present results showed this trend for glutamate, although its contribution to prediction was unremarkable. Urinary choline was reported to be one of the predictor candidates for GDM[48–50]. In our study, choline did not show an ability to predict GDM. Metabolites that were not detected to have a positive predictive ability for GDM in the present study have also been reported to predict GDM in the previous literature[17]. Because there are several differences between the results from previous studies and the present results, such as the timing of sample collection, analytical methods or ethnicity, we could not detect other metabolites in this study. Bentley-Lewis *et al.*[22] reported that metabolomic profiles were different among USA, Chinese and Japanese people. They explained that these differences might be caused by dietary habits or gut microbiome[22]. It is useful to analyze the metabolomic profiles of individuals from each ethnic group. In addition, GDM is thought to have a common pathogenic pathway. Therefore, we need to study GDM prediction using metabolomic analysis from the point of view of the ethnic-specific characteristics and common pathogenic mechanism.

The present data showed that maternal urine had more metabolites that were associated with subsequent GDM than serum. Given these findings and that urine collection is less invasive than blood collection, urine might be a suitable biological material obtained from pregnant women in their first to early second trimester to examine the risk of subsequent GDM.

Enrichment analysis showed that amino acid metabolism and the pentose phosphate pathway were related to p-GDM status. In several studies in which metabolomic analysis has been applied for the prediction of GDM, metabolites in amino acid metabolism were possible biomarkers[17–23]. The alteration of amino acid metabolism is reportedly associated with type 2 diabetes and the development of diabetes in the future[15,16]. Amino acids activate the mammalian target of rapamycin signal pathway, which is involved in insulin receptor signaling and glucose metabolism[51]. In the present study, several amino acid metabolic pathways were associated with subsequent GDM. These data suggest that the alterations in amino acid metabolism were associated with p-GDM and can resemble type 2 diabetes or a pre-diabetes status.

Recently, the pentose phosphate pathway has been reportedly associated with adiposity, a higher glycemic phenotype[52] and diabetes complications[48]. In studies involving a cohort of university students, it was shown using glucose tolerance tests that alterations in the pentose phosphate pathway were associated with glucose metabolism[30,53]. The pentose phosphate pathway is associated with purine metabolism, which is reportedly associated with GDM[18,22]. The key enzyme of pentose phosphate pathway, glucose-6-phosphate dehydrogenase, has been reported to be associated with insulin resistance[54]. Increased expression of glucose-6-phosphate dehydrogenase promotes β-cell dysfunction and apoptosis[55]. Conversely, high glucose levels can activate the pentose phosphate pathway[56]. These data

and the present findings show that the pentose phosphate pathway or purine metabolism play an important role in impaired glucose metabolism including p-GDM status. Additionally, alteration of the pentose phosphate pathway might be caused by altered glucose metabolism reflecting a p-GDM state.

The present study was subject to several limitations. The JECS is a multi-region, multi-facility cohort study; thus, we could not review the accuracy of the diagnostic process or oral glucose tolerance test results for all enrolled participants. Hence, the diagnostic criteria for GDM might not have been consistently used[27]. We did not obtain the history of the study participants regarding whether delivery of a large-for-gestational age neonate occurred. We sampled blood and urine randomly. Therefore, we could not determine the accurate relationship between the metabolites and meals. Metabolic status is considered to be affected by diet[57]; however, we did not make adjustments for diet in any of our analyses. Serum and urine were stored at −20°C; therefore, it is possible that some metabolite profiles were altered, although the stability of urine samples at −20°C has been previously reported[58]. Consequently, the present data might not reflect the precise metabolic status of the participants.

In conclusion, the present study identified metabolites in randomly obtained serum and urine samples that distinguish the p-GDM group from the control group during early pregnancy. Glutamine in serum, as well as ethanolamine and 1,3-diphosphoglycerate in urine, are possible predictive markers of subsequent GDM in Japanese women. Amino acid metabolism and the pentose phosphate pathway could be key metabolic pathways associated with p-GDM status. These findings might provide new insights into the prediction of GDM. Further studies are required to elucidate the efficacy of these metabolites as predictive markers at an earlier gestational age in other cohorts.

ACKNOWLEDGMENTS

We express our sincere appreciation for the cooperation and support of all study participants and members of the JECS. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the above-mentioned governmental organization. The JECS was funded by the Ministry of the Environment in Japan. This work was partly supported by JSPS KAKENHI grant numbers 16H01781 and 16K16198, and a research support grant from Eli Lilly Japan K.K.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes–2009. *Diabetes Care* 2009; 32(Suppl 1): S13–S61.
2. HSCR Group, Metzger BE, Lowe LP, *et al.* Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; 358: 1991–2002
3. Bellamy L, Casas JP, Hingorani AD, *et al.* Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009; 373: 1773–1779.
4. Ouyang F, Parker MG, Luo ZC, *et al.* Maternal BMI, gestational diabetes, and weight gain in relation to childhood obesity: the mediation effect of placental weight. *Obesity (Silver Spring)* 2016; 24: 938–946.
5. Kim SY, England JL, Sharma JA, *et al.* Gestational diabetes mellitus and risk of childhood overweight and obesity in offspring: a systematic review. *Exp Diabetes Res* 2011; 2011: 541308.
6. Boney CM, Verma A, Tucker R, *et al.* Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005; 115: e290–e296.
7. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science* 2004; 305: 1733–1736.
8. Crowther CA, Hiller JE, Moss JR, *et al.* Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005; 352: 2477–2486.
9. Lamminpaa R, Vehvilainen-Julkunen K, Schwab U. A systematic review of dietary interventions for gestational weight gain and gestational diabetes in overweight and obese pregnant women. *Eur J Nutr* 2017; 57: 1721–1736.
10. Rogozinska E, Chamillard M, Hitman GA, *et al.* Nutritional manipulation for the primary prevention of gestational diabetes mellitus: a meta-analysis of randomised studies. *PLoS One* 2015; 10: e0115526.
11. Farrar D, Simmonds M, Bryant M, *et al.* Risk factor screening to identify women requiring oral glucose tolerance testing to diagnose gestational diabetes: a systematic review and meta-analysis and analysis of two pregnancy cohorts. *PLoS One* 2017; 12: e0175288.
12. Powe CE. Early pregnancy biochemical predictors of gestational diabetes mellitus. *Curr Diab Rep* 2017; 17: 12.
13. de Seymour JV, Conlon CA, Sulek K, *et al.* Early pregnancy metabolite profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus. *Acta Diabetol* 2014; 51: 887–890.
14. Zhang A, Sun H, Wang X. Serum metabolomics as a novel diagnostic approach for disease: a systematic review. *Anal Bioanal Chem* 2012; 404: 1239–1245.
15. Menni C, Fauman E, Erte I, *et al.* Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. *Diabetes* 2013; 62: 4270–4276.
16. Wang TJ, Larson MG, Vasan RS, *et al.* Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011; 17: 448–453.
17. Chen Q, Francis E, Hu G, *et al.* Metabolomic profiling of women with gestational diabetes mellitus and their offspring: review of metabolomics studies. *J Diabetes Complications* 2018; 32: 512–523.

18. Law KP, Zhang H. The pathogenesis and pathophysiology of gestational diabetes mellitus: deductions from a three-part longitudinal metabolomics study in China. *Clin Chim Acta* 2017; 468: 60–70.
19. McCabe CF, Perng W. Metabolomics of diabetes in pregnancy. *Curr Diab Rep* 2017; 17: 57.
20. Graca G, Duarte IF, Barros AS, *et al.* Impact of prenatal disorders on the metabolic profile of second trimester amniotic fluid: a nuclear magnetic resonance metabonomic study. *J Proteome Res* 2010; 9: 6016–6024.
21. Diaz SO, Pinto J, Graca G, *et al.* Metabolic biomarkers of prenatal disorders: an exploratory NMR metabonomics study of second trimester maternal urine and blood plasma. *J Proteome Res* 2011; 10: 3732–3742.
22. Bentley-Lewis R, Huynh J, Xiong G, *et al.* Metabolomic profiling in the prediction of gestational diabetes mellitus. *Diabetologia* 2015; 58: 1329–1332.
23. Enquobahrie DA, Denis M, Tadesse MG, *et al.* Maternal early pregnancy serum metabolites and risk of gestational diabetes mellitus. *J Clin Endocrinol Metab* 2015; 100: 4348–4356.
24. Dumas ME, Maibaum EC, Teague C, *et al.* Assessment of analytical reproducibility of 1H NMR spectroscopy based metabonomics for large-scale epidemiological research: the INTERMAP Study. *Anal Chem* 2006; 78: 2199–2208.
25. Kawamoto T, Nitta H, Murata K, *et al.* Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014; 14: 25.
26. Michikawa T, Nitta H, Nakayama SF, *et al.* The Japan Environment and Children's Study (JECS): a preliminary report on selected characteristics of approximately 10 000 pregnant women recruited during the first year of the study. *J Epidemiol* 2015; 25: 452–458.
27. Mizuno S, Nishigori H, Sugiyama T, *et al.* Association between social capital and the prevalence of gestational diabetes mellitus: an interim report of the Japan Environment and Children's Study. *Diabetes Res Clin Pract* 2016; 120: 132–141.
28. Minakami H, Hiramatsu Y, Koresawa M, , *et al.* Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2011 edition. *J Obstet Gynaecol Res* 2011; 37: 1174–1197
29. Eguchi A, Sakurai K, Watanabe M, *et al.* Exploration of potential biomarkers and related biological pathways for PCB exposure in maternal and cord serum: a pilot birth cohort study in Chiba. *Japan. Environ Int* 2017; 102: 157–164.
30. Miki T, Lee EY, Eguchi A, *et al.* Accelerated oligosaccharide absorption and altered serum metabolites during oral glucose tolerance test in young Japanese with impaired glucose tolerance. *J Diabetes Investig* 2017; 9: 512–521.
31. Yuan M, Breitkopf SB, Yang X, *et al.* A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. *Nat Protoc* 2012; 7: 872–881.
32. Soga T, Igarashi K, Ito C, *et al.* Metabolomic profiling of anionic metabolites by capillary electrophoresis mass spectrometry. *Anal Chem* 2009; 81: 6165–6174.
33. Thevenot EA, Roux A, Xu Y, *et al.* Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *J Proteome Res* 2015; 14: 3322–3335.
34. Pitman EJG. Significance tests which may be applied to samples from any populations III. The analysis of variance test. *Biometrika* 1938; 29: 322–335.
35. Sing T, Sander O, Beerenwinkel N, *et al.* ROCR: visualizing classifier performance in R. *Bioinformatics* 2005; 21: 3940–3941.
36. Diaz SO, Barros AS, Goodfellow BJ, *et al.* Following healthy pregnancy by nuclear magnetic resonance (NMR) metabolic profiling of human urine. *J Proteome Res* 2013; 12: 969–979.
37. Lopez-Ibanez J, Pazos F, Chagoyen M. MBROLE 2.0-functional enrichment of chemical compounds. *Nucleic Acids Res* 2016; 44: W201–W204.
38. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J Roy Stat Soc B Met* 1995; 57: 289–300.
39. Kanehisa M, Araki M, Goto S, *et al.* KEGG for linking genomes to life and the environment. *Nucleic Acids Res* 2008; 36: D480–D484.
40. Balaji V, Madhuri BS, Ashalatha S, *et al.* A1C in gestational diabetes mellitus in Asian Indian women. *Diabetes Care* 2007; 30: 1865–1867.
41. Wu K, Cheng Y, Li T, *et al.* The utility of HbA1c combined with haematocrit for early screening of gestational diabetes mellitus. *Diabetol Metab Syndr* 2018; 10: 14.
42. Benaiges D, Flores-Le Roux JA, Marcelo I, *et al.* Is first-trimester HbA1c useful in the diagnosis of gestational diabetes? *Diabetes Res Clin Pract* 2017; 133: 85–91.
43. D'Anna R, Scilipoti A, Giordano D, *et al.* myo-Inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. *Diabetes Care* 2013; 36: 854–857.
44. Medras ZJH, El-Sayed NM, Zaitone SA, *et al.* Glutamine up-regulates pancreatic sodium-dependent neutral aminoacid transporter-2 and mitigates islets apoptosis in diabetic rats. *Pharmacol Rep* 2018; 70: 233–242.
45. Dean ED, Li M, Prasad N, *et al.* Interrupted glucagon signaling reveals hepatic alpha cell axis and role for L-glutamine in alpha cell proliferation. *Cell Metab* 2017; 25: e1365.

46. Guasch-Ferre M, Hruby A, Toledo E, *et al.* Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. *Diabetes Care* 2016; 39: 833–846.
47. Zeng H, Tong R, Tong W, *et al.* Metabolic biomarkers for prognostic prediction of pre-diabetes: results from a longitudinal cohort study. *Sci Rep* 2017; 7: 6575.
48. Chen L, Cheng CY, Choi H, *et al.* Plasma metabonomic profiling of diabetic retinopathy. *Diabetes* 2016; 65: 1099–1108.
49. Graca G, Goodfellow BJ, Barros AS, *et al.* UPLC-MS metabolic profiling of second trimester amniotic fluid and maternal urine and comparison with NMR spectral profiling for the identification of pregnancy disorder biomarkers. *Mol BioSyst* 2012; 8: 1243–1254.
50. Diaz SO, Barros AS, Goodfellow BJ, *et al.* Second trimester maternal urine for the diagnosis of trisomy 21 and prediction of poor pregnancy outcomes. *J Proteome Res* 2013; 12: 2946–2957.
51. Langenberg C, Savage DB. An amino acid profile to predict diabetes? *Nat Med* 2011; 17: 418–420.
52. Hootman KC, Trezzi JP, Kraemer L, *et al.* Erythritol is a pentose-phosphate pathway metabolite and associated with adiposity gain in young adults. *Proc Natl Acad Sci USA* 2017; 114: E4233–E4240.
53. Williams JF, Blackmore PF, Arora KK. The significance of sedoheptulose 1,7-bisphosphate in the metabolism and regulation of the pentose pathway in liver. *Biochem Int* 1985; 11: 599–610.
54. Ham M, Choe SS, Shin KC, *et al.* Glucose-6-Phosphate dehydrogenase deficiency improves insulin resistance with reduced adipose tissue inflammation in obesity. *Diabetes* 2016; 65: 2624–2638.
55. Lee JW, Choi AH, Ham M, *et al.* G6PD up-regulation promotes pancreatic beta-cell dysfunction. *Endocrinology* 2011; 152: 793–803.
56. Peiro C, Romacho T, Azcutia V, *et al.* Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. *Cardiovasc Diabetol* 2016; 15: 82.
57. Shrestha A, Mullner E, Poutanen K, *et al.* Metabolic changes in serum metabolome in response to a meal. *Eur J Nutr* 2017; 56: 671–681.
58. Gika HG, Theodoridis GA, Wilson ID. Liquid chromatography and ultra-performance liquid chromatography-mass spectrometry fingerprinting of human urine: sample stability under different handling and storage conditions for metabonomics studies. *J Chromatogr A* 2008; 1189: 314–322.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Score plots of orthogonal projections to latent structures discriminant analysis model from serum and urine.
Figure S2 | Receiver operating characteristic curves of metabolites in serum and urine.