Journal of Clinical & Translational Endocrinology 1 (2014) 38–43

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Research Paper

Metabolomic analysis reveals amino-acid responses to an oral glucose tolerance test in women with prior history of gestational diabetes mellitus☆

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HIGHLIGHTS

- We explored metabolite responses in women with prior gestational diabetes mellitus
- We used the oral glucose tolerance test as the provocative measure in this study
- Women with prior gestational diabetes mellitus were stratified by glucose tolerance
- We examined the relationship between metabolomic and clinical/behavioral parameters
- Greater change in metabolites was strongly associated with breastfeeding duration

ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form
27 March 2014
Accepted 28 March 2014

Keywords:
Gestational diabetes mellitus
Type 2 diabetes
Breastfeeding
Pregnancy

ABSTRACT

Objective: Although gestational diabetes mellitus (GDM) is associated with an increased risk of type 2 diabetes mellitus (T2DM) compared to normoglycemic pregnancies, the biochemical pathways underlying the progression of GDM to T2DM are not fully elucidated. The purpose of this exploratory study was to utilize metabolomics with an oral glucose tolerance test (OGTT) to examine the amino acid response in women with prior GDM to determine if a relationship between these metabolites and established risk factors for T2DM exists.

Materials/methods: Thirty-eight non-pregnant women without diabetes but with prior GDM within the previous 3 years were recruited from a community-based population. A 75 g-OGTT was administered; fasting and 2-h plasma samples were obtained. Metabolite profiles of 23 amino acids or amino acid derivatives were measured with gas chromatography-mass spectrometry. Measures of insulin resistance were derived from the OGTT and risk factors for T2DM were obtained by self-report.

Results: Twenty-two metabolite levels decreased significantly in response to the OGTT ($p < 0.05$). The clinical covariates most powerfully associated with metabolite level changes included race, body mass index (BMI), and duration of prior breastfeeding, (mean ± SD of standardized β-coefficients, $\beta = -0.38 \pm 0.05$, 0.25 ± 0.08, and 0.44 ± 0.03, respectively, all $p < 0.05$). Notably, a prior history of breastfeeding was associated with the greatest number of metabolite changes.

Conclusions: Greater change in metabolite levels after a glucose challenge was significantly associated with a longer duration of breastfeeding and higher BMI. Further exploration of these preliminary observations and closer examination of the specific pathways implicated are warranted.

Abbreviations: OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus; AA, amino acid; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; β, standardized β-coefficients; HOMA-IR, homeostatic model of assessment – insulin resistance; G/I, glucose to insulin ratio.

Conflict of interest statement: The authors have no potential conflicts of interest to disclose.

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http://dx.doi.org/10.1016/j.jcte.2014.03.003

Introduction

Gestational diabetes mellitus (GDM) affects approximately 7% of all pregnancies in the United States, and this prevalence is increasing in parallel to obesity [1] and type 2 diabetes mellitus (T2DM) [2]. Furthermore, women with GDM compared to women without a history of GDM are at increased risk for developing T2DM [3], which is influenced by risk factors such as higher body mass index (BMI), older age, GDM in past pregnancies, inadequate or deficient postpartum intervention and education, and use of insulin therapy and medical nutrition therapy [4]. Existing methods to assess T2DM risk after GDM focus on clinical, demographic, or genetic information [5]. However, the biochemical pathways underlying increased T2DM risk after GDM are still unclear.

Metabolomic profiling, an approach that examines biochemical pathways to identify biomarkers predictive of metabolic diseases, has shown promise in identifying early biomarkers of risk for several disorders including T2DM [6–11]. Therefore, we conducted a cross-sectional exploratory metabolomic analysis of samples from an oral glucose tolerance test (OGTT) in postpartum women without diabetes but with a history of GDM in order to explore their metabolomic profiles and the association of these profiles with established and putative risk factors for T2DM. These preliminary metabolomic observations offer the promise of hypothesis generation regarding the mechanism of T2DM development subsequent to GDM.

Methods

Thirty-nine non-lactating women with a GDM pregnancy within the past 3 years were enrolled in a randomized-controlled lifestyle intervention; details of this trial are described elsewhere [12,13]. At baseline, participants provided clinical and self-reported behavioral data. After a 10-h, overnight fast, participants underwent a 75 g-OGTT where fasting and 2-h plasma samples were collected. For our cross-sectional analysis, women were classified as normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or T2DM based on criteria from the American Diabetes Association for fasting and 2-h glucose plasma levels [14]. Women classified as IFG, IGT, or IFG + IGT were considered to have prediabetes. Body mass index (BMI) was measured as weight (in kg) divided by height (in m) squared. The study was approved by the University of Michigan Institutional Review Board and the Partners Human Research Committee. All participants provided informed, written consent prior to study enrollment.

The University of Michigan's Diabetes and Research Training Center performed all biochemical assays. Methods for glucose [12], insulin [12], and sex hormone binding globulin [15] assays are described elsewhere. The Human Adiponectin Radioimmunoassay kit (Linco Research, St. Charles, MO) was used to assay adiponectin (standard curve concentrations 0.78–200 ng/mL; assay sensitivity limit 1 ng/mL; and inter-assay CV 15.5% at 20 ng/mL and 10.2% at 72 ng/mL). Metabolomic analyses were performed by the University of Michigan's Nutrition and Obesity Research Center. Amino acids (AA) for analyses were chosen and analyzed based on the methodology described by Wang et al. [11] in conjunction with currently available platform metabolites. Plasma purification and derivatization was performed with the "EZ:faast" free AA analysis kit via gas chromatography-mass spectrometry. AA separation and detection was done with a 6890 gas chromatography with a 5973 mass selective detector from Phenomenex (Torrance, CA) [16].

Student paired *t*-tests were used to compare metabolite levels at fasting vs. 2-hour time points and analyze differences between glucose tolerance groups. Pearson correlation coefficients were used to examine the correlation of AA levels before and after the OGTT. The change in AA levels was defined as the 2-h AA level minus the fasting AA level. Two additional measures were calculated in order to place our data in context with current literature: the fold change from fasting to 2-h post-glucose load was calculated as the change in AA divided by the fasting AA level; and the percent change was calculated as the fold change multiplied by 100. Forward stepwise regressions (inclusion criteria $p < 0.15$) were used to examine the ability of clinical covariates to predict the change in AA. Independent clinical variables examined were age; race (white, black, Asian, or other); ethnicity (Hispanic: yes, no); BMI; parity (continuous variable for the number of previous deliveries, 1–5); family history of type 2 diabetes (yes, no); fasting glucose levels; 2-h glucose levels; glucose to insulin (G/I) ratio, homeostatic model of assessment – insulin resistance (HOMA-IR), insulin levels, duration of breastfeeding following their GDM pregnancy (no breastfeeding, breastfed (0–3 months, 3 months–1 year, or ≥1 year)); and adiponectin levels. The variable inclusion level was set to $p < 0.15$ to allow metabolites with limited but not significant effects to be included as these potentially influenced the inclusion and coefficients of other variables. However, only those variables with a $p < 0.05$ were subsequently included in results and conclusions. Using these regressions, standardized β-coefficients (β) based on standard deviations with $p < 0.05$ were calculated for each metabolite response to compare across clinical or behavioral parameters and metabolites. HOMA-IR and G/I ratios were calculated [17,18], which have been shown to be adequate measures of insulin resistance [19,20]. Women with NGT were compared with women with prediabetes with an independent samples *t*-test and χ^2-test. AA levels were reported as unitless liquid chromatography-tandem mass spectrometry peak areas. Data were presented as mean ± SD and $p < 0.05$ was considered statistically significant. SAS software v9.2 was used for all analyses (SAS Institute Inc., Cary, NC).

Theory

Metabolomics, the systematic study of small molecule products of biochemical pathways, has shown promise in the identification of key metabolites for the prediction, diagnosis and monitoring of several metabolic disorders, including GDM [21]. An exploratory study of biomarkers from 2nd trimester maternal urine and blood plasma observed that women who developed GDM showed early changes in biotin status, altered amino acid levels, and/or gut metabolism [22]. Another investigation found associations between 1st trimester biomarkers, hs-CRP and SHBG, and an increased risk of GDM [23]. Because of the importance of early risk stratification for GDM, as well as the variable predictive power of current models for GDM diagnosis, improved 1st trimester biomarker determination is necessary.

Several longitudinal studies have shown associations between metabolites and future development of insulin resistance, prediabetes, or T2DM in humans [6–11]. Recent investigations have also shown that metabolomic analyses of samples from participants before and after an OGTT can be used to detect early shifts in metabolism during the progression from early insulin resistance to T2DM [7,24]. For example, in a community-based population of 377 men and women without diabetes, Ho et al. evaluated biochemical changes after an OGTT for individuals at risk for T2DM [7]. AA changes identified in regards to an OGTT were found to be physiologically consistent with biochemical pathways of insulin action. Of note, four metabolites (pyridoxic acid, β-hydroxybutyric acid, lactic acid and isoleucine) showed blunted responses in insulin-resistant participants.

These data are relevant because prior studies have suggested that the progressive decline of glucose tolerance first detected during

pregnancy likely parallels the pathogenesis of T2DM [25]. Although women with a history of GDM are at a higher risk for T2DM than those without GDM [3], there is limited information on the mechanisms underlying the progression from GDM to subsequent T2DM. Therefore, metabolomic analyses of samples from an OGTT on women with a history of GDM may be utilized to strengthen extant models of T2DM pathophysiology and potentially expose the mechanistic linkage between these two metabolic diseases.

Results

Thirty-eight women were included in analyses. Although 39 women were enrolled in the original study [12], one woman had a measured 2-h glucose level >200 mg/mL, which is consistent with diabetes; consequently, she was excluded from further analyses. Of the remaining 38 women, 61% were NGT, 3% had IFG, 21% had IGT, and 15% had both IFG and IGT. The mean age was 35 ± 4 years; 71% self-reported white race; 61% were NGT (Table 1). No participant was breastfeeding at the time of the study. After delivery, 9% of women with NGT and 7% of women with prediabetes never breastfed; 13% of women with NGT and 20% of women with prediabetes breastfed from 0 to 3 months; 48% of women with NGT and 40% of women with prediabetes breastfed 3 months–1 year; and 30% of women with NGT and 33% of women with prediabetes breastfed for more than a year (Table 1). Twenty of the 23 AA and AA derivatives had statistically significant reductions in response to the OGTT ($p < 0.0001$). Alanine (mean ± SD; −39.17 ± 77.36, $p = 0.0035$), sarcosine (−0.52 ± 0.90, $p = 0.0014$), and glutamine (−75.74 ± 241.33, $p = 0.0607$) had less statistically significant reductions while cystine increased (2.18 ± 3.02, $p < 0.0001$) (Table 2). As expected, all of the metabolites changes were significantly correlated with each other (Fig. 1).

Clinical and biochemical parameters, such as postpartum behaviors, race, parity, G/I ratio and history of breastfeeding, were associated with changes in metabolite levels (Table 3). White race was negatively associated with changes in alanine, tryptophan, serine, asparagine, glutamine, ornithine, glutamate, and lysine (β = −0.16, −0.31, −0.35, −0.44, −0.28, −0.54, −0.36, −0.58, respectively, all $p < 0.05$). Parity and adiponectin were positively associated with a change in cystine levels ($\beta = 0.44$, $p < 0.001$ and 0.24, $p = 0.031$). Increasing fasting glucose levels were negatively associated with changes in levels of alanine, valine, and glutamate (β = −0.24, −0.32, −0.52, respectively, all $p < 0.007$). Breastfeeding was positively associated with several metabolites in response to the OGTT: phenylalanine, tryptophan, 4-hydroxyproline, serine, threonine, asparagine, ornithine, aspartic acid, and lysine (β = 0.49, 0.40, 0.50, 0.38, 0.44, 0.45, 0.35, 0.32, 0.60, respectively, all $p < 0.05$). When comparing the women with NGT to the women with prediabetes, the women with prediabetes demonstrated a blunted percent decrease in AA compared to the women with NGT (Fig. 2); however, this trend in attenuated AA decrease between the two groups did not achieve statistical significance.

Four AA (leucine, isoleucine, proline, and alpha-aminoisobutyric acid) did not show any significant association with clinical parameters in the forward stepwise regression. Age, BMI, family history of T2DM, fasting and 2-h glucose, HOMA-IR, fasting insulin, and adiponectin had positive associations with a few AA changes in response to the OGTT, while SHBG and HbA1c did not show any significant associations with any AA responses (Table 3). Additionally, when considering insulin resistance, 2-h glucose, HOMA-IR, and G/I ratio, all demonstrated associations with OGTT-induced AA changes; however, no metabolite common to all of these parameters emerged.

In order to compare and contrast our data with what was found by Ho et al. [7], we calculated the fold change in metabolites following the OGTT in the women with NGT versus prediabetes (Table 4). Although we are unable to strictly compare these two different populations of participants, the magnitudes of the three fold-changes are not widely discrepant. In addition, when considering physiologic clustering of AA, we did not observe any patterns

Table 1
Baseline characteristics of participants

	Normal glucose tolerance ($n = 23$)	Prediabetes ($n = 15$)	p-value
Age, years	33.9 ± 3.9	37.9 ± 3.2	<0.01
Months postpartum	16.0 ± 13.1	19.4 ± 10.5	0.40
Months postpartum after GDM	16.3 ± 12.9	21.5 ± 10.4	0.19
HbA1c, % (mmol/mol)	5.4 ± 0.5 (36 ± 18)	5.6 ± 0.5 (38 ± 18)	0.49
SHBG (nmol/L)	69.3 ± 42.4	56.4 ± 24.4	0.25
Fasting glucose (mg/dL)	85.0 ± 7.8	96.0 ± 9.3	<0.01
2-h glucose (mg/dL)	98.1 ± 17.2	161.9 ± 18.7	<0.01
Fasting insulin (IU/L)	30.5 ± 73.6	21.1 ± 8.6	0.55
2-h insulin (IU/L)	19.8 ± 17.4	19.4 ± 9.0	0.93
Adiponectin (ng/mL)	6581.8 ± 2357.5	6065.7 ± 3169.8	0.61
BMI (kg/m^2)	27.3 ± 5.4	29.3 ± 5.1	0.26
G/I ratio	5.7 ± 1.8	5.4 ± 2.5	0.74
HOMA-IR	6.7 ± 16.8	5.0 ± 2.2	0.65
Parity (n)	2.7 ± 1.5	2.7 ± 1.2	0.87
Current smokers, %	17	20	0.84
Family history of type 2 diabetes, %	35	60	0.23
Race, White, %	70	70	0.80
Ethnicity, Hispanic, %	4.35	0	0.41
Breastfeeding			
No breastfeeding, %	9	7	0.82
Breastfed 0–3 months, %	13	20	0.90
Breastfed 3 months–1 year, %	48	40	0.89
Breastfed ≥ 1 year, %	30	33	0.85

GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin; SHBG, sex hormone-binding globulin; BMI, body mass index; G/I, glucose to insulin ratio; HOMA-IR, homeostatic model of assessment – insulin resistance.
Data are means ± standard deviation unless otherwise indicated.

Table 2
Difference in metabolites' response to an OGTT (2-h minus fasting)

Metabolite	Mean	Standard deviation	p-value
Alanine	−39.17	77.37	0.0035
Sarcosine	−0.52	0.90	0.0014
Glycine	−52.86	61.06	<0.0001
Alpha ABA	−3.77	3.35	<0.0001
Valine	−65.93	45.73	<0.0001
Leucine	−53.87	26.20	<0.0001
Isoleucine	−26.42	13.92	<0.0001
Threonine	−28.90	22.03	<0.0001
Serine	−43.21	40.85	<0.0001
Proline	−43.58	30.43	<0.0001
Asparagine	−11.15	9.67	<0.0001
Aspartic acid	−5.46	7.03	<0.0001
Methionine	−5.47	3.95	<0.0001
4-hydroxyproline	−2.34	2.52	<0.0001
Glutamate	−21.63	22.62	<0.0001
Phenylalanine	−15.56	11.17	<0.0001
Glutamine	−75.74	241.33	0.0607
Ornithine	−11.94	15.22	<0.0001
Lysine	−32.01	31.44	<0.0001
Histidine	−11.52	10.25	<0.0001
Tyrosine	−12.55	9.19	<0.0001
Tryptophan	−5.70	5.64	<0.0001
Cystine	2.18	3.02	<0.0001

Amino acid levels were calculated as unitless liquid chromatography-tandem mass spectrometry peak areas.
Means defined as the 2-h amino acid level minus the fasting amino acid level after oral glucose tolerance test was administered.

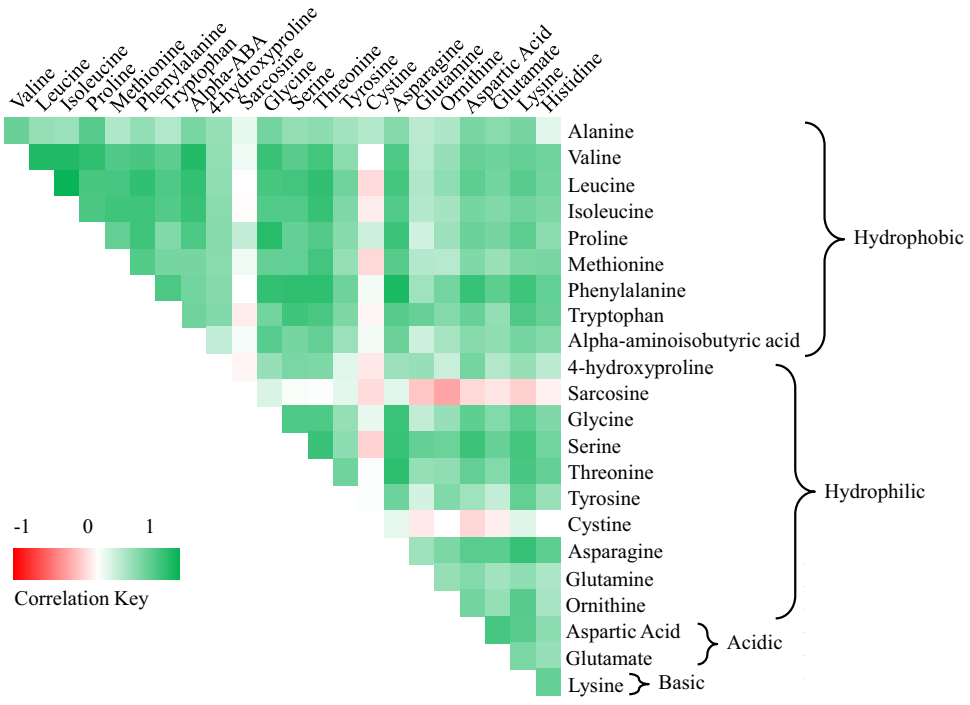

Figure 1. Correlations among amino acid changes during an oral glucose tolerance test. Correlations among amino acid changes from fasting to 2-h sample during an oral glucose tolerance test (OGTT). Pearson correlation coefficients are shown. General correlations across amino acids were observed; greater correlations based on chemical groupings were not observed. Cystine demonstrated primarily inverse correlations because it increased from fasting to 2-h samples. Sarcosine demonstrated weaker correlations due to a less significant decrease during the OGTT ($p = 0.002$).

of responsiveness with respect to branched chain AA or chemical grouping (acidic or basic).

Discussion

We conducted an exploratory metabolomic analysis in 38 non-pregnant, non-lactating women without diabetes but with insulin-resistance as reflected by G/I ratio and HOMA-IR within 3 years of a GDM pregnancy. We found that, with the exception of cystine which increased significantly, plasma AA levels decreased significantly from fasting to 2-h post-OGTT; however, we did not observe a statistically significant difference between the women with prediabetes and the women with NGT (Fig. 2). The trend for an attenuated response to an OGTT in women with prediabetes and a history of GDM is similar to the pattern seen in previous studies of participants with diabetes or prediabetes compared to those without diabetes. In addition, breastfeeding, race, and BMI were associated with the most metabolite profile changes following the OGTT and parity had the most profound effect on metabolite levels.

Longer breastfeeding duration was associated with the greatest number of AA changes (phenylalanine, tryptophan, 4-hydroxyproline, serine, threonine, asparagine, ornithine, aspartic acid, and lysine) with standardized β-values ranging between 0.35 and 0.60. Moreover, the high levels of standardized βs relative to those observed in other clinical-metabolite associations indicate a strong effect of a history of breastfeeding at the metabolite level. Also, because the measures of insulin resistance are negatively associated in relation to the positive associations found with breastfeeding for various amino acids (phenylalanine, serine, aspartic acid), this suggests that breastfeeding has a positive influence on insulin resistance. This is consistent with data that suggests a protective effect of breastfeeding against metabolic syndromes [26,27].

We observed a strong positive association between changes in cystine levels and increasing parity which has not been previously reported in the literature. We found metabolite decreases of similar magnitude to those found by Ho et al. [7]. In addition, we observed an association between non-white race and a greater decrease in several metabolite plasma levels. However, the role of race is one to be examined in a larger, more racially/ethnically diverse population of women. Although we were unable to perform cluster analyses or conduct a comparison of normoglycemic controls to women with GDM, investigations of these questions may be of interest. Furthermore, we did not find abnormalities of branched chain AA with increasing BMI as reported by Batch et al. [28] which might be because of our small population size.

One notable limitation of this study is the sample size. We were unable to adjust for multiple comparisons as might be done in a large study population. Moreover, the limited sample size and the high degree of correlation among delta amino acids magnified the risk of overfitting the data due to collinearity, thereby limiting our ability to perform cluster analyses and draw extensive conclusions from these data. Consequently, we were similarly restricted in our ability to draw conclusions regarding relationships between metabolite changes and subgroups of race and parity.

Recent examinations have examined metabolite profiles in populations of currently pregnant women with uncomplicated [22] and complicated [22,29,30] pregnancies and found that certain AA were elevated in women with insulin-resistance compared to women with NGT. These metabolites, however, have not been consistent across studies. Future studies with larger cohorts could utilize cluster analysis based on measures of insulin resistance such as the G/I ratio or HOMA-IR in order to begin to clarify metabolic pathways of clinical interest. For example, Wang et al. [11] demonstrated a substantial increase in the predictive value of AA in participants with T2DM versus those with NGT when three AA were considered together rather than separately. This study, however, extends the current literature by examining the relationship between metabolite responses and the biochemical and behavioral parameters associated with insulin resistance and prediabetes status.

Table 3
Associations between clinical parameters and 2-h minus fasting metabolite response to an OGTT

Metabolite	Age beta (*p*-value)	Race beta (*p*-value)	BMI beta (*p*-value)	Parity beta (*p*-value)	Family history beta (*p*-value)	Fasting glucose beta (*p*-value)
Alanine		−0.16 (0.015)	−0.08 (0.042)	0.18 (0.005)		−0.24 (<0.001)
Valine			0.19 (0.01)			−0.32 (0.007)
Leucine						
Tryptophan		−0.31 (0.017)	0.54 (<0.001)		0.27 (0.018)	
4-hydroxyproline					0.29 (0.005)	
Sarcosine	−0.39 (0.010)		0.10 (0.043)	−0.37 (0.045)		
Serine		−0.35 (0.027)	0.26 (0.005)			
Cystine				0.44 (<0.001)		
Asparagine		−0.44 (0.008)	0.26 (0.028)			
Glutamine		−0.28 (0.048)				
Ornithine		−0.54 (<0.001)			−0.36 (0.005)	
Aspartic acid					0.10 (0.038)	
Glutamate		−0.36 (0.003)				−0.52 (<0.001)
Lysine		−0.58 (<0.001)	0.47 (0.002)			

	2-h glucose beta (*p*-value)	G/I ratio beta (*p*-value)	HOMA-IR beta (*p*-value)	Insulin beta (*p*-value)	Breastfeeding beta (*p*-value)	Adiponectin beta (*p*-value)
Phenylalanine		−0.31 (0.016)			0.49 (0.007)	
Tryptophan					0.40 (0.009)	−0.38 (0.004)
4-hydroxyproline					0.50 (<0.001)	
Sarcosine	0.50 (0.036)					
Glycine		−0.35 (0.015)				
Serine		−0.27 (0.003)	−0.28 (0.006)		0.38 (0.022)	
Threonine					0.44 (0.008)	
Tyrosine				0.12 (0.022)		
Cystine						0.24 (0.031)
Asparagine					0.45 (0.01)	
Glutamine	−0.31 (0.036)					
Ornithine					0.35 (0.007)	
Aspartic acid	−0.29 (0.009)	−0.36 (<0.001)			0.32 (0.026)	
Lysine					0.60 (<0.001)	
Histidine				0.16 (0.008)		

BMI, body mass index; G/I, glucose to insulin ratio; HOMA-IR, homeostatic model of assessment − insulin resistance.
Data for amino acid-clinical parameter associations which did not achieve statistical significance are not included.
Data are presented as standardized β-coefficients (*p*-values). Standardized β-coefficients were computed from standard deviations with *p*-values <0.05.
Categorical variables examined were race (White, Black, Asian or other); family history of type 2 diabetes (yes, no); and duration of breastfeeding following their GDM pregnancy (no breastfeeding, breastfed 0–3 months, breastfed 3 months–1 year, or breastfed ≥1 year).

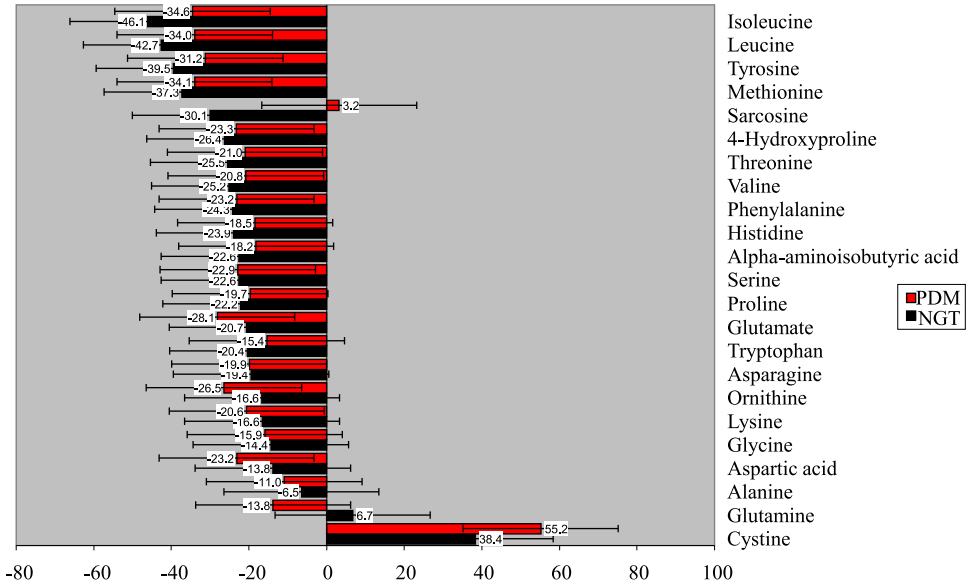

Figure 2. Percent change in metabolites during an oral glucose tolerance test. Percent change from fasting to 2-h plasma samples during an oral glucose tolerance test (OGTT) in women with prior gestational diabetes mellitus (GDM) and either normal glucose tolerance (NGT) or prediabetes (PDM). Prediabetes status was determined as having impaired glucose tolerance, impaired fasting glucose, or both, according to American Diabetes Association guidelines (see Methods section). Blunted decreases in women with prediabetes compared to NGT were observed in metabolites with the greater percent changes, although these differences did not achieve statistical significance.

Table 4
Comparisons between fold-change in metabolites following an OGTT between the current study and Ho et al. *Diabetes*, 2013

Metabolite	Ho et al. (2013)	Normal glucose tolerance $n = 23$	Prediabetes $n = 15$
Isoleucine	−0.47	−0.46	−0.35
Leucine	−0.45	−0.43	−0.34
Methionine	−0.36	−0.37	−0.34
Tyrosine	−0.34	−0.39	−0.31
Threonine	−0.27	−0.25	−0.21
Aspartate	−0.27	−0.14	−0.23
Phenylalanine	−0.26	−0.24	−0.23
Ornithine	−0.25	−0.17	−0.26
Serine	−0.24	−0.23	−0.23
Valine	−0.23	−0.25	−0.21
Lysine	−0.17	−0.17	−0.21
Histidine	−0.17	−0.24	−0.18
Tryptophan	−0.16	−0.20	−0.15
Glycine	−0.14	−0.14	−0.16
Glutamate	−0.14	−0.21	−0.28
Proline	−0.14	−0.22	−0.20
Glutamine	−0.10	0.07	−0.14
Alanine	−0.07	−0.06	−0.11

Data are the fold-change in metabolites following an oral glucose tolerance test in women with normal glucose tolerance versus prediabetes.

Conclusion

To our knowledge, this is the first study to focus on the metabolomic responsiveness to an OGTT in women with a history of GDM. The identification of the role of postpartum behaviors, like breastfeeding, on metabolite responsiveness to an OGTT is novel and may facilitate our understanding of how best to risk-stratify and intervene in those women at high risk for metabolic diseases. The importance of this exploration in postpartum women is the impetus for closer examination of these relationships and the specific pathways implicated in the pathogenesis of T2DM after GDM.

Acknowledgments

Thanks to Drs. David M. Nathan and Robert Gerszten for their critical review and to Rebecca Walmer, B.A. of the Massachusetts General Hospital Diabetes Research Center for her assistance with manuscript preparation. These data were presented at an oral presentation at the Endocrine Society's 95th Annual Meeting in San Francisco, CA.

Funding: Robert Wood Johnson Foundation Harold Amos Medical Faculty Development Program Award and NIH 1R03DK096152 awarded to R.B-L.; NIH grant DK089503 to the University of Michigan to support the Chemistry Core of the Michigan Diabetes Research and Training Center which performed all biochemical assays; and P30 DK089503 to the University of Michigan Nutrition Obesity Research Center which performed the metabolomic analyses.

Author contributions: R.B-L. participated in study design, data analysis, and manuscript development; G.X. participated in data analysis and manuscript development; A.Y. and J.H. participated in data management and manuscript review; H.L. participated in data analysis and manuscript review; C.K. participated in study design, data acquisition, and manuscript review.

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