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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

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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.

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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