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Early pregnancy urinary biomarkers of fatty acid and carbohydrate metabolism in pregnancies complicated by gestational diabetes

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ABSTRACT

Aims: Alterations in organic acid biomarkers from fatty acid and carbohydrate metabolism have been documented in type 2 diabetes patients. However, their association with gestational diabetes mellitus (GDM) is largely unknown.

Methods: Participants were 25 GDM cases and 25 non-GDM controls. Biomarkers of fatty acid (adipate, suberate and ethylmalonate) and carbohydrate (pyruvate, L-lactate and β-hydroxybutyrate) metabolism were measured in maternal urine samples collected in early pregnancy (17 weeks) using liquid chromatography–mass spectrometry methods. Logistic regression were used to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results: GDM cases and controls differed in median urinary concentrations of ethylmalonate (3.0 vs. 2.3 μg/mg creatinine), pyruvate (7.4 vs. 2.1 μg/mg creatinine), and adipate (4.6 vs. 7.3 μg/mg creatinine) (all p-values <0.05). Women in the highest tertile for ethylmalonate or pyruvate concentrations had 11.4-fold (95%CI 1.10–117.48) and 3.27-fold (95%CI 0.72–14.79) increased risk of GDM compared with women in the lowest tertile for ethylmalonate and pyruvate concentrations, respectively. Women in the highest tertile for adipate concentrations, compared with women in the lowest tertile, had an 86% reduction in GDM risk (95%CI 0.02–0.97).

Conclusions: These preliminary findings underscore the importance of altered fatty acid and carbohydrate metabolism in the pathogenesis of GDM.

1. Introduction

Gestational diabetes (GDM), a common complication of pregnancy occurring in 5–10% of all pregnancies [1,2], is characterized by glucose intolerance that first appears during pregnancy. GDM is associated with adverse maternal (e.g., C-sections and increased risk of type 2 diabetes [T2DM]) and offspring (e.g., fetal hyperinsulinism, macrosomia, and birth injuries) outcomes [3–5]. Underlying pathophysiological disturbances commonly

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identified in GDM include reduced insulin secretion and abnormal insulin resistance [1] which have been linked to abnormal fatty acid and carbohydrate metabolism.

Higher maternal dietary intake of saturated fat, cholesterol, red and processed meats prior to pregnancy or during early pregnancy were significantly associated with increased GDM risk [6–8]. During pregnancy, women with GDM do have higher serum triglyceride concentrations as compared with women with pregnancies not complicated by GDM [9,10]. Endogenous hepatic glucose production has been shown to be less sensitive to increased insulin concentration among women with GDM affected pregnancies [11], accompanied by an increased contribution of carbohydrates to oxidative metabolism [11]. Although limited, available evidence also suggests that diets with low-dietary glycemic index are associated with reduced risk of GDM [12].

Organic acids, organic compounds with acidic properties measured in human blood and urine, are degradation products of amino acids, neurotransmitters, and intestinal bacterial action on food components that provide information on energy production, neurotransmitter metabolism, intestinal dysbiosis, dietary fat, carbohydrate and protein metabolism [13]. Measurement of organic acids such as ethylmalonate, suberate, and adipate reflect metabolic processes involved in long-chain fatty acid metabolism (such as carnitine-dependent pathways) and related mitochondrial function [13]. Additionally, altered concentrations of organic acids such as pyruvate, L-lactate and β-hydroxybutyrate are biological markers of disturbed carbohydrate metabolism [13].

Alterations in organic acid biomarkers, including those that are intermediate products of fatty acid or carbohydrate metabolism have been documented in experimental models of insulin resistance [14–17]. Furthermore, some [18–23], but not all, studies [14] of organic acid profiles among humans have documented alterations of circulating or urinary organic profiles associated with insulin resistance, hyperglycemia or type 2 diabetes (T2DM). Some investigators have suggested that quantitative profiling of organic acids in urine may be a simple, sensitive test that can reveal evidence of functional inadequacy of specific nutrients for laboratory evaluations in insulin resistance and T2DM [21].

In addition to inconsistencies in previous reports, most prior investigations were case-control studies that did not help to clarify the temporal relationship between altered organic acids concentrations and risk of T2DM. Importantly, we are aware of no prior published studies that have evaluated urinary organic acid profiles in GDM, which is biochemically and epidemiologically similar to T2DM. We therefore, used urine samples (which were collected 16 weeks gestation, on average) from a prospective cohort study and examined the extent to which selected organic acids known to be biomarkers of fatty acid (adipate, suberate and ethylmalonate) and carbohydrate (pyruvate, L-lactate and β-hydroxybutyrate) metabolism are associated with incident GDM risk.

2. Materials and methods

Study subjects were selected from participants of the Omega study, a prospective cohort study designed to investigate risk factors of pregnancy complications such as preeclampsia and GDM. Study population and data collection procedures, described before [6], are briefly summarized here. Participants were women who attended prenatal care clinics affiliated with the Swedish Medical Center, Seattle, WA, USA. Eligible women were those who initiated prenatal care before 20 weeks gestation, spoke and read English, were ≥18 years of age, and planned to carry the pregnancy to term and deliver at the study hospital. Participants completed a questionnaire administered in English by a trained interviewer at or near enrollment. These questionnaires were used to gather information on socio-demographic, anthropometric, and behavioral characteristics and reproductive and medical histories. After delivery, maternal and infant medical records were abstracted for information on the course and outcomes of pregnancy. The Institutional Review Board of the Swedish Medical Center approved study protocols. All participants provided written informed consent. Between 1996 and 2006, 5063 eligible women were approached and 4000 (approximately 79%) agreed to participate. A total of 3886 pregnant women provided biological samples (i.e., blood and urine samples) and completed interviews.

Participants provided a clean-catch spot urine sample around 16–17 weeks of gestation. Immediately after collection, samples were separated into 2 ml aliquots and stored at −80 °C until analysis. Pregnancy outcome information was abstracted from hospital and clinic medical records. We used the food frequency questionnaire (FFQ) from the Women's Health Initiative Clinical Trial [24] to assess maternal dietary intake during the three-month period (before conception and during the first trimester). Participants completed FFQs at an average of 15.9 weeks gestation (standard deviation: 4.6 weeks). Dietary intake values of nutrients were estimated using food composition tables from the University of Minnesota Nutrition Coding Center nutrient database (Nutrition Coordinating Center, Minneapolis, MN).

In our study settings, according to the recommendations from the American Diabetes Association (ADA) [1], pregnant women were screened at 24–28 weeks gestation using a 50 g 1-h oral glucose challenge test. Those patients who failed this screening test (glucose ≥7.8 mmol/l or 140 mg/dl) were then followed-up within 1–2 weeks with a 100 g 3-h oral glucose tolerance test (OGTT). We also abstracted laboratory results from participants' 50 g 1-h glucose challenge test and from the diagnostic 100 g 3-h OGTT. Women were diagnosed with GDM if two or more of the 100 g OGTT glucose concentrations exceeded the ADA criteria: fasting ≥5.3 mmol/l (≥95 mg/dl); 1-h ≥10.0 mmol/l (≥180 mg/dl); 2-h ≥8.6 mmol/l (≥155 mg/dl); 3-h ≥7.8 mmol/l (≥140 mg/dl) [1]. From the sample of women who developed GDM and delivered a live born infant, we sampled 25 women to serve as cases. For controls, we sampled 25 women who did not develop GDM and who delivered a singleton live born infant. The 25 controls were frequency-matched to GDM cases for gestational age a urine collection.

Urinary biomarkers were measured using liquid chromatography with tandem mass spectrometric detection (LC/MS–MS) method in Metametrix Clinical Laboratory (now named Genova Diagnostics) (Duluth, Georgia). Intra-assay coefficients of variation for this method are 11.8%. Urinary biomarkers were