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Asymmetric dimethylarginine and arginine metabolites in women with and without a history of gestational diabetes

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ABSTRACT

Aims: Dysregulation of arginine metabolism, as evidenced by increased circulating levels of asymmetric dimethylarginine (ADMA), has been proposed as an early event in the natural history of cardiovascular disease. Since the diagnosis of gestational diabetes mellitus (GDM) identifies a patient population at increased future risk of cardiovascular disease later in life, we sought to characterize arginine metabolism in women with and without a history of recent GDM.

Methods: In this prospective observational cohort study, 225 women (72 who had GDM; 153 who did not) underwent cardiometabolic characterization, including oral glucose tolerance test, at 1- and 3-years postpartum. Circulating ADMA and its stereoisomer symmetric dimethylarginine (SDMA) were measured by liquid chromatography–mass spectrometry at both visits.

Results: Serum ADMA and SDMA were not significantly different between the GDM and non-GDM groups at either 1-year or 3-years postpartum. On multiple linear regression analyses, high-density-lipoprotein cholesterol ($t = -2.62$, $p = 0.009$) and creatinine ($t = -2.62$, $p = 0.01$) were independently associated with ADMA at 3-years, while creatinine ($t = 7.09$, $p < 0.0001$) and BMI ($t = -2.24$, $p = 0.026$) predicted SDMA.

Conclusion: Women with recent GDM do not exhibit altered serum concentrations of ADMA or SDMA at 1- and 3-years postpartum, suggesting that ADMA dysregulation is not a feature of their cardiometabolic profile in the early years after delivery.

1. Introduction

Dysregulation of arginine metabolism has been proposed as an early marker for cardiovascular disease (CVD). In particular, asymmetric dimethylarginine (ADMA), a metabolic product of arginine metabolism, has been identified as a potential mediator of endothelial dysfunction,[1] the development of which is an early event in the natural history of CVD. ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), such that increased circulating ADMA may alter the bioavailability of the vasodilator nitric oxide and thereby compromise endothelial function. Consistent with this model, elevated circulating levels of ADMA have been reported in the setting of several cardiometabolic conditions, including hypercholesterolemia,[2] hypertension,[3] type 2 diabetes mellitus,[4] chronic heart failure,[5] and atherosclerosis.[6] Moreover, ADMA has consistently emerged as an independent predictor of incident cardiovascular events,[1,7–9] supporting its growing recognition as an early biomarker of future CVD.

The diagnosis of gestational diabetes mellitus (GDM), or glucose intolerance with first onset and recognition in pregnancy, identifies a population of young women who have an elevated future risk of developing type 2 diabetes (T2DM)[10,11] and CVD[12–15] over their lifetime as compared to their peers. Importantly, although T2DM clearly increases the likelihood of cardiovascular outcomes, their future risk of CVD is elevated even if they do not develop T2DM.[15] Thus, women with a history of GDM can provide a model for studying early events in the natural history of CVD. Compared to their peers, women with recent GDM exhibit an enhanced cardiovascular risk factor profile by

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as early as 3-months postpartum, including higher prevalence rates of dysglycemia, dyslipidemia, and metabolic syndrome.[16–18] Current understanding of the natural history of vascular disease would suggest that chronic exposure to this enhanced risk factor profile may lead to endothelial dysfunction over time and ultimately to CVD. Accordingly, there is growing interest in the assessment of endothelial health in women with a history of GDM.

In this context, ADMA is a biomarker of particular interest. Indeed, a recent systematic review of 17 metabolomic profiling studies found that ADMA and non-esterified free fatty acids were the circulating metabolites during pregnancy that were most consistently associated with GDM.[19] However, there have been limited studies of ADMA in women with a history of GDM in the non-gravid state,[20–23] the setting in which it is more likely to portend cardiovascular implications. Furthermore, the few such studies conducted to date[20–23] have been limited by small sample sizes ($n \leq 84$), cross-sectional designs, lack of appropriate controls for comparison, and incomplete covariate adjustment. Thus, our objective in this study was to systematically evaluate arginine metabolism and the determinants thereof at both 1- and 3-years postpartum in a well-characterized cohort of 225 women with and without a history of recent GDM.

2. Methods

2.1. Study participants

This analysis was performed in the setting of a prospective observational cohort study wherein women are recruited at the time of GDM screening in pregnancy and then undergo cardiometabolic characterization in the years after delivery. The full study protocol has been described previously.[24] Briefly, at our institution, all pregnant women are screened for GDM in late 2nd trimester. For this study, women were recruited at that time and underwent a 3-h 100 g oral glucose tolerance test (OGTT) to ascertain gestational glucose tolerance status, thereby enabling their classification as either GDM (defined by National Diabetes Data Group criteria[25]) or non-GDM. Participants returned at 3- and 12-months postpartum to undergo reassessment of glucose tolerance by 2-h 75 g OGTT. At their 1-year postpartum visit, the participants were recruited into the long-term follow-up study in which they undergo assessment biannually. The current analysis was performed in the first 225 participants to complete their 3-year postpartum visit. In these 225 women, arginine metabolism at 1- and 3-years postpartum was characterized by high-performance liquid chromatography mass spectrometry. The protocol for this study was approved by the Mount Sinai Hospital Research Ethics Board. All women provided written informed consent for their participation.

2.2. Participant evaluation at 1-year and 3-years postpartum

At the 1-year and 3-year postpartum visits, participants completed interviewer-administered questionnaires providing information on medical, obstetrical and family history. Height, weight, body mass index (BMI), and waist circumference were measured as described previously.[24] Participants underwent a 2-h 75 g OGTT, enabling ascertainment of current glucose tolerance status. As per the Canadian Diabetes Association guidelines,[26] glucose tolerance on the OGTT can be classified into the following 5 groups: normal glucose tolerance; impaired fasting glucose (IFG); impaired glucose tolerance (IGT); combined IFG and IGT; and diabetes. Pre-diabetes is comprised of IFG, IGT, or combined IFG and IGT. Area-under-the-glucose curve ($AUC_{glucose}$) on the OGTT was calculated by trapezoidal rule and provided a continuous measure of glycemia to complement the categorical glucose tolerance.

2.3. Clinical biochemistry

All OGTTs were performed in the morning after an overnight fast. Venous blood samples were drawn at fasting and at 30-, 60- and 120-min post-ingestion of the 75 g glucose load. Specific insulin was measured from these samples using the Roche Elecsys 1010 immunoassay analyzer and electrochemiluminescence immunoassay kit (Roche Diagnostics, Laval, QC). As previously described,[24] insulin and glucose measurements from the OGTT enabled measurement of indices of insulin sensitivity/resistance and pancreatic beta-cell function. Specifically, insulin sensitivity/resistance was assessed by the Matsuda index[27] and the Homeostasis Model of Assessment (HOMA-IR).[28] Beta-cell function was measured with the Insulin Secretion-Sensitivity Index-2 (ISSI-2)[29,30] and insulinogenic index/HOMA-IR (IGI/HOMA-IR).[31] Lipid profile was assessed from fasting serum at 3-years postpartum. Total cholesterol, high-density-lipoprotein (HDL) cholesterol, and triglycerides were measured using the Roche Cobas 6000 c 501 analyzer (Roche Diagnostics, Laval, QC). LDL cholesterol was determined by Friedewald formula.

2.4. Liquid chromatography–mass spectrometry (LC–MS/MS)

Arginine metabolism was assessed by LC–MS/MS analysis, with measurement of serum ADMA, its stereoisomer symmetric dimethylarginine (SDMA), and arginine from frozen serum samples obtained at both 1- and 3-years postpartum. Sample analysis was carried out on a SCIEX QTrap5500 mass spectrometer (Framingham, MA) with an Agilent 1290 HPLC system (Agilent Technologies, Santa Clara, CA). Chromatography ran at a flow rate of 400 μL/min on an Acclaim OA column 2.1 × 150 mm, 3.0 μm (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a gradient starting at 100% A (water + 0.1% formic acid) and ramping up to 15% B (Acetonitrile + 0.1%formic acid) with a total analysis time of 8 min. Data were acquired in positive ESI scheduled MRM mode using a source temperature of 550 °C and ion spray voltage of 5200 V. Peak integration and data analysis were performed using Analyst software spectrometer (Sciex, Framingham, MA). Sample concentrations were calculated by plotting peak area ratios (Analyte/Internal Standard) against calibration curves.

2.5. Statistical analyses

All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC). Continuous variables were tested for normality of distribution, and natural log transformations of skewed variables were used, where necessary, in subsequent analyses. Continuous variables with normal distributions are presented as mean ± standard deviation, and those with skewed distributions are presented as median and interquartile range (25th–75th percentile). The study characteristics of women with GDM were compared to those of women who did not have GDM (non-GDM), using two-sample t-test for continuous variables (if normally distributed) or Wilcoxon two-sample test (if skewed), and chi-square test for categorical variables (Table 1). The respective univariate associations of cardiometabolic factors with ADMA, SDMA, and arginine at 3-years postpartum were assessed by Spearman correlation analysis (Table 2). A series of multiple linear regression models were constructed to determine independent predictors of the three outcomes: (A) ADMA at 3-years, (B) SDMA at 3-years, and (C) arginine at 3-years, respectively (Table 3). Model construction was performed in a sequential manner, as follows. Model 1 consisted of clinical risk factors for diabetes (age, ethnicity, family history of diabetes, BMI), GDM, and $AUC_{glucose}$ at 3-years (i.e. a continuous measure of current glycemia). Model II consisted of the Model I covariates with further adjustment for creatinine at 3-years (because of the known renal clearance of SDMA). Models III, IV and V consisted of the Model II covariates with further adjustment for insulin

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