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SHORT COMMUNICATION

Early pregnancy metabolite profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus

Jamie V. de Seymour · Cathryn A. Conlon · Karolina Sulek · Silas G. Villas Bôas · Lesley M. E. McCowan · Louise C. Kenny · Philip N. Baker

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Abstract Current early pregnancy screening tools to identify women at risk of developing gestational diabetes mellitus lack both specificity and sensitivity. As a result, the foetus and mother are often subjected to insult during disease progression, prior to diagnosis and treatment in later pregnancy. Metabolomics is an analytical approach, which allows for appraisal of small molecular mass compounds in a biofluid. The aim of this pilot study was to investigate the relationship between the early gestation serum metabolite profile and the subsequent development of gestational diabetes mellitus in the search for early pregnancy biomarkers and potential metabolic mechanisms. Our nested case-control study analysed maternal serum at 20 weeks' gestation, obtained from the New Zealand cohort of the Screening for Pregnancy Endpoints study. Metabolomic profiling was performed using gas chromatography coupled to mass spectrometry, and metabolites were identified using R software and an in-house mass spectral library. Statistical analysis was performed using SPSS version 21.0. Forty-eight metabolites were identified in the serum samples. Itaconic acid ($P = 0.0003$), with a false discovery rate of 0.012, was found to be significantly more abundant in women who subsequently developed gestational diabetes mellitus, when compared to controls with uncomplicated pregnancies. The current pilot study found that itaconic acid may have potential as a novel biomarker in early pregnancy to predict the subsequent development of gestational diabetes mellitus. However, the findings from this pilot study require validation with a larger, diverse population before translation into the clinical setting.

Keywords Biomarkers · Cis-aconitate · Inflammation · GC–MS · Itaconic acid · Gestational diabetes mellitus · Metabolomics

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J. V. de Seymour (✉) · K. Sulek · P. N. Baker
Liggins Institute, The University of Auckland, Building 505, 85 Park Road, Grafton, Auckland, New Zealand
e-mail: j.deseymour@auckland.ac.nz

J. V. de Seymour · C. A. Conlon
Institute of Food, Nutrition and Human Health, Massey University, Auckland, New Zealand

S. G. Villas Bôas
School of Biological Sciences, The University of Auckland, Auckland, New Zealand

L. M. E. McCowan
Department of Obstetrics and Gynaecology, The University of Auckland, Auckland, New Zealand

L. C. Kenny
The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Cork, Ireland

Introduction

Gestational diabetes mellitus (GDM) is a disorder of pregnancy with significant adverse consequences for mother and offspring. The immediate consequences include an increased likelihood of a Caesarean section, hypoglycaemia of the newborn, respiratory distress syndrome, and macrosomia [1]. The long-term implications of a pregnancy affected by GDM include a substantially increased risk of the mother developing type 2 diabetes

post-natally, as well as the offspring having an increased susceptibility to obesity and related metabolic complications in adulthood [1]. The prevalence of GDM is as high as 23 % in some populations [2] and is predicted to rise in parallel to the increasing rates of maternal obesity. To date, early pregnancy screening tools to identify women at-risk of developing either GDM in later pregnancy [3], or type 2 diabetes post-natally [4], are of limited efficacy. A recent study showed that first trimester 25-hydroxyvitamin D levels are associated with insulin resistance later in pregnancy and therefore may have some use as a potential biomarker [5]. However, the specificity of using 25-hydroxyvitamin D levels as a biomarker of GDM would need to be carefully considered.

Metabolomics is the study of low molecular weight molecules present in a biological organism (metabolites). Metabolite profiling has been found in previous studies to successfully predict the onset of later pregnancy disorders in early pregnancy. Metabolomic analysis in early pregnancy also provides a useful tool to assist in the elucidation of metabolic mechanisms underlying GDM development—crucial for the advancement of prevention and treatment strategies. In our study, we aimed to investigate the relationship between the early gestation serum metabolome and the subsequent development of gestational diabetes.

Methods

In this nested case-control study, serum samples at 20 weeks' gestation were obtained from the New Zealand Cohort of the Screening for Pregnancy Endpoints (SCOPE) study.[1] The current study used cases from SCOPE that subsequently developed GDM ($n = 22$) and matched to controls with uncomplicated pregnancies ($n = 26$) according to age (±3 years), ethnicity, and BMI (±3.5 kg/m^2).

Informed consent was obtained from the participants in the SCOPE study, and ethical approval was granted by the Auckland Ethics Committee (AKX/02/00/364).

Metabolomic analysis of the serum samples was conducted using gas chromatography coupled to mass spectrometry (GC–MS) (Thermo Scientific Trace GC Ultra coupled to an ISQ MS, Auckland, New Zealand). All chemicals used in the sample preparation were of analytical grade and supplied by Sigma-Aldrich (St. Louis, MO, USA), Finechem (Waltham, MA, USA), or Merck (Whitehouse Station, NJ, USA).

[1] The SCOPE study was a multinational prospective study that recruited women with healthy nulliparous singleton pregnancies between 2004 and 2011. The New Zealand cohort of the SCOPE study recruited 2,032 women in total, 2.1 % of whom subsequently developed GDM (for diagnostic criteria see Online Resource 1).

Twenty microliter of DL-alanine-2,3,3,3-d4 98 atom % D (10 mM) was added to each thawed sample, as an internal standard. The samples were dried using a SpeedVac Concentrator with a Refrigerated Vapor Trap (Thermo Scientific, Auckland, New Zealand). The dried serum underwent cold methanol extraction using 50 and 80 $\%_{v/v}$ methanol/water. The samples were centrifuged, and the pooled supernatants dried. Following extraction, the samples were derivatised using the modified methyl chloroformate alkylation procedure detailed by Smart et al. [6], before being analysed using a GC–MS instrument.

The GC–MS raw data were deconvoluted using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) (online software distributed by the National Institute of Standards and Technology, USA—http://www.amdis.net/) combined with an in-house R-based software for metabolite identification and peak integration (relative quantification). Analyses were carried out in 'R' platform version 2.15.0 (http://www.r-project.org/). Independent samples T tests were conducted to analyse differences in metabolite levels between cases and controls using SPSS version 21.0.

Results

Demographic characteristics of the 48 participants are summarised in Online Resource 1. Forty-eight metabolites

Table 1 List of identified metabolites

Metabolites		
2-Hydroxybutyric acid	Creatinine	Nicotinamide
4-Methyl-2-oxopentanoic acid	Cysteine	Oleic acid
9-Heptadecenoic acid	Docosahexaenoic acid	Ornithine
11,14-Eicosadienoic acid	Eicosapentaenoic acid	Palmitic acid
Adrenic acid	Gamma linolenic acid	Palmitoleic acid
Alanine	Glutamic acid	Phenylalanine
Arachidonic acid	Glycine	Proline
Asparagine	Isoleucine	Pyruvic acid
Aspartic acid	Itaconic acid	Quinic acid
Azelaic acid	Lactic acid	Serine
Benzoic acid	Leucine	Stearic acid
Bishomogamma linolenic acid	Linoleic acid	Succinic acid
Cis-aconitic acid	Lysine	Threonine
Cis-vaccenic acid	Margaric acid	Tryptophan
Citraconic acid	Methionine	Tyrosine
Citric acid	Myristic acid	Valine

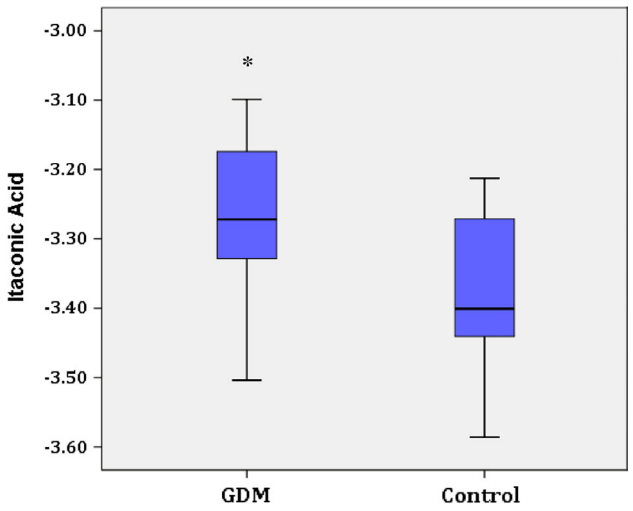

Fig. 1 *Box plot* of the distribution of itaconic acid levels among participants. *Note* Itaconic acid levels are reported as the logarithm of abundance relative to internal standard. *Statistically significant difference $P \leq 0.001$

were identified using an in-house mass spectral library of known metabolites (Table 1). Among the metabolites identified were 18 amino acids, 16 fatty acids, and 12 organic acids.

Using adjusted significance levels (Benjamini–Hochberg procedure) to account for multiple comparisons, itaconic acid levels ($P = 0.0003$) were found to be significantly higher in cases when compared to controls (Fig. 1), with a false discovery rate of 0.012. Cis-aconitate levels were also higher in GDM cases in our study when compared to controls, verging on statistical significance ($P = 0.013$), with an FDR of 0.159.

Discussion

Of the 48 metabolites that were identified in the early pregnancy serum of participants in our study, itaconic acid level was significantly higher in women who subsequently developed GDM when compared to controls. Our study is the first to identify itaconic acid as differing significantly between GDM cases and controls in early pregnancy, which, if confirmed in further studies, has potential as a novel biomarker in early pregnancy.

Understanding of the role of itaconic acid in human systems is limited. However, a recent study [7] investigated the gene, immunoresponsive gene 1, coding an enzyme essential for the production of itaconic acid in humans, through the decarboxylation of cis-aconitate. Interestingly, cis-aconitate levels were found to be higher in GDM cases in our study when compared to controls, verging on statistical significance. The expression of the immunoresponsive gene 1 is upregulated in macrophages in response to inflammation [7]. Itaconic acid's association with inflammation may demonstrate a potential role of inflammation in early pregnancy, in the development of GDM. It is recognised that inflammation often accompanies GDM; however, there have been few studies of the role of inflammation in its development, prior to diagnosis. Cases and controls were matched for BMI; thus, differences are unlikely to have resulted from obesity-related inflammation.

A major strength of our study was the use of samples from the SCOPE study biobank which were collected and stored under strictly standardised conditions in an exceptionally well-maintained biobank—of particular importance for metabolomic investigations where sample degradation can interfere with results. A limitation of our study was that the New Zealand cohort of the SCOPE study had a low prevalence of GDM, limiting the sample numbers available from this study population. Our study was focused on relative quantification of metabolites; absolute quantification would be recommended as the next step after validation.

The results from our pilot study have the potential to assist the direction of future research and early-stage interventions to address inflammation in early pregnancy and prevent the onset of GDM. Results from our pilot study require validation with a larger, diverse sample before translation into the clinical setting, as a potential GDM biomarker in early pregnancy.

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Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights All procedures followed were in accordance with the ethical standards of the Auckland Ethics Committee (AKX/02/00/364) and with the Declaration of Helsinki of 1975, as revised in 2008 (5).

Informed consent Informed consent was obtained from all patients included in the study.

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