Journal of King Saud University – Science 32 (2020) 3213–3216

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Review

Metabolomics profiles in umbilical cord blood

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ARTICLE INFO

Article history:
Received 17 February 2020
Revised 16 March 2020
Accepted 16 March 2020
Available online 26 March 2020

Keywords:
Maternal BMI
Gestational weight
Type 2 diabetes
Pregnancy

ABSTRACT

Pregnancy is a period of major endocrine and metabolic changes which modulate both maternal and child's health. Pregnancy exposures such as gestational diabetes mellitus (GDM), elevated maternal pre-pregnancy body-mass-index (BMI) and gestational weight gain (GWG) are risk factors for type 2 diabetes, overweight, and metabolic syndrome not only in the mother. Maternal diabetes and obesity to induce marked abnormalities in glucose homeostasis and insulin secretion in the fetus, and are linked to obesity, diabetes, and metabolic disease in the offspring. In recent years, the study of metabolomics has begun to receive increasing international attention, especially as it pertains to medical research. This is due in part to the potential for discovery of new biomarkers in the metabolome and to a new understanding of the "exposome", which refers to the endogenous and exogenous compounds that reflect external exposures. Consequently, metabolomics research into pregnancy-related issues has increased. Biomarkers discovered through metabolomics may shed some light on the etiology of certain pregnancy-related complications and their adverse effects on future maternal health and infant development and improve current clinical management. The discoveries and methods used in these studies will be compiled and summarized within the following paper. A further focus of this paper is the use of hair as a biological sample, which is gaining increasing attention across diverse fields due to its noninvasive sampling method and the metabolome stability. At the end we have concluded that maternal BMI and glycemia are associated with different components of the new born metabolism, consistent with their independent effects on new born size at birth. Maternal BMI is associated with a new born metabolic signature characteristic of insulin resistance and risk of type 2 diabetes in adults. Thus we can say that metabolomics is a useful tool and it helps to develop targeted therapeutic tool to investigate these complications at early stages.

Contents

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2020.03.034

1. Introduction

Maternal diabetes and obesity to induce marked abnormalities in glucose homeostasis and insulin secretion in the fetus, and are linked to obesity, diabetes, and metabolic disease in the offspring (Egeland and Meltzer, 2010). Gestational diabetes mellitus (GDM) has been shown to have profound effects on the intrauterine milieu, which may reflect and/or modulate altered function of the maternal–fetal unit (Tam et al.,2017; Lawlor et al., 2011). Individuals whose cord blood or amniotic fluid insulin levels are elevated have a 3–4-fold risks for developing glucose intolerance, obesity, and type 2 diabetes in late childhood and as adults (Kawasaki et al., 2018; Chen and Scholl, 2005). Previous studies employing metabolomics to study GDM have primarily focused on maternal blood or urine profiles, and although they have identified alterations in metabolic pathways including amino acid (AA), steroid hormone, glycerophospholipid, and fatty acid metabolism, these studies have been limited by inconsistent results. Sampling amniotic fluid provides a direct measure of the metabolites of the fetal compartment compared to maternal blood and urine (Silverman et al., 1998). This is especially meaningful in the period prior to 20 weeks of gestation, when the fetal skin is not yet keratinized, which allows for bi-directional diffusion of solute between the fetus and the amniotic fluid and the surfaces of the amnion, placenta, and umbilical cord (Underwood et al., 2005). Two previous studies profiled the amniotic fluid metabolome in women with GDM (Diaz et al., 2011). However, these studies were limited by a less sensitive analytic approach and failure to consider the effects from critical biological variables, such as fetal sex, maternal age, and gestational age (GA) at delivery. Therefore, we hypothesized that isolation, identification, and quantification of metabolites present in second trimester AF from pregnancies subsequently diagnosed with GDM using sensitive mass spectrometry and nested case-control study design will provide new insights into how GDM alters maternal-placental-fetal metabolism, which ultimately results in permanent changes in developing fetal tissues and contributes to obesity and diabetes later in life. Pregnancy is a period of major endocrine and metabolic changes which modulate both maternal and child's health (Gilmore et al., 2015). Pregnancy exposures such as gestational diabetes mellitus (GDM), elevated maternal pre-pregnancy body-mass-index (BMI) and gestational weight gain (GWG) are risk factors for type 2 diabetes, overweight, and metabolic syndrome not only in the mother, but also in the offspring, as suggested by the numerous indications for the Developmental Origins of Health and Disease (DOHaD) hypothesis (Hoffman et al., 2017).

Gestational diabetes mellitus (GDM) refers to the condition of having normal glucose metabolism before pregnancy but impaired glucose tolerance and elevated fasting glucose concentrations during pregnancy. The prevalence of GDM varies from country to country and even from region to region. The latest epidemiological studies show that the prevalence of GDM in the USA is 9%, while the incidence of GDM in Asian countries is 3.0–21.2% (Yuen and Wong, 2015). With the increase in the rate of obesity and the increase in maternal age, the incidence of gestational diabetes is increasing (Ramachandran et al., 2016).

A group of biological fields comprising genomics, transcriptomics, proteomics, and metabolomics allow for high-throughput, simultaneous analysis of vast numbers of molecules within a biological sample. The last of these, metabolomics, is defined as the study of metabolites, a recognized field of study arising in the 1960s aimed at developing a better understanding of cellular biology (Kumar and Kocour, 2017). However, its nomenclature is relatively new; the term "metabolomics" was coined in the early 2000s. The study of metabolomics facilitates an increased understanding of all the endogenous and exogenous low-molecular weight molecules (<1500 Da) which are downstream products from interactions on a genomic or proteomics level (Bouatra et al., 2013). Metabolomics has become a fast growing area of interest for research into the prognosis, screening, diagnosis, and treatment of many diseases (Zhao et al.,2015). Medical metabolomics research focused on the identification and investigation of relevant metabolites formed during certain pathological metabolic reactions. The process begins with uncarpeted screening of the metabolome (the entire set of small molecules contained in a biological sample). Overall associations between metabolites with significantly altered levels from control are identified and analyzed to generate a hypothesis. A more targeted approach is then utilized, focusing on the metabolites with significantly altered values to further investigate the consistency of results and prove or disprove the proposed hypothesis. A variety of biological specimens, namely, serum, urine, tissue, cerebrospinal fluid, hair, saliva, stool, and exhaled breath, have been used in analysis to study human pathology (Wishart et al., 2008).

GDM is one of the most common complications of pregnancy and its prevalence is constantly rising. If uncontrolled, GDM results in overt hyperglycemia which may significantly increase perinatal morbidity and mortality. Women with GDM have a higher risk of preeclampsia and cesarean section. Whereas complications for their newborns include a higher risk for macrosomia and fetal hypoglycemia (Lukács et al., 2018). Potential long-term consequences for the health of motherand child may be an impaired glucose tolerance, obesity, and metabolic disorders. Even though GDM usually resolves after birth and blood glucose returns to normal levels, mothers that developed GDM during pregnancy have an increased risk for type 2 diabetes mellitus (T2DM) (Crowther et al., 2005). Therefore, screening and treatment for GDM are common in most developed countries. Randomized controlled trials have shown improved maternal and neonatal outcomes for these strategies (Padayachee and Coombes, 2015). But even with strict glycaemic control GDM still represents a risk for adverse pregnancy outcomes. It is known that ethnicity, higher maternal age, obesity, greater weight gains during pregnancy, and hypertension display risk factors for GDM. The pathogenesis of GDM is multifactorial and exact mechanisms underlying the development of the disease are still poorly understood. A traditional pathophysiologic concept proposes that pancreatic β-cells are not able to account for the physiologic pregnancy-related decline in tissue sensitivity to insulin. Glucose intolerance occurs as a result of an inadequate increase in insulin secretion (Desoye and Hauguel-de Mouzon, 2007). The placenta secretes cytokines and other factors which add to pregnancy-induced insulin resistance. Other potentially contributing factors discussed in the literature include chronic low-grade inflammation different genetic, epigenetic and non-genetic environmental factors including nutrition. Moreover, fetal sex (Yang et al., 2017; Jaskolka et al., 2015) and fetal genes have been shown to correlate with maternal glucose concentrations during pregnancy and thus may modulate the risk for maternal GDM.

However, it is not clear if the fetus can impact on the maternal organism in such a regulating manner. Pathophysiologic pathways of development and progression of GDM still need to be investigated more thoroughly in order to better understand potential involvement of a fatal influence. Metabolomics is an investigative approach that analyses products of biochemical pathways in a detailed way (Moid et al., 2020). It is a robust, rapid, and efficient method to analyze a large number of small molecules in tissues, urine, blood and other biological fluids. This approach is well suited to find biomarkers for the prediction, diagnosis, and monitoring of several diseases including metabolic disorders like GDM (Fanos et al., 2013). It can also help to better understand physio-

logic and pathophysiologic processes on a molecular level and, as such, in a more detailed manner.

2. Metabolomics

Metabolites be directly linked to endogenous enzymatic activities encoded by the human genome, but also those derived from food, medications, the microbiota that inhabit the body, and the environment. Our dependence on diet as a source for nine of the 20 amino acids for which there are codons in the human genome but no endogenous biosynthetic route is an example that highlights why it is important to account for "exogenous" metabolites in our study of the metabolome. Although broadening the scope of analyses to measure more metabolites increases the difficulty level, it is clear that comprehensive metabolomics heralds exciting new opportunities for discovery. Metabolomics is an objective lens to view the complex nature of how physiology is linked to external events and conditions, as well as measure its response to perturbations such as those associated with disease.

Metabolites have been described as proximal reporters of disease because their abundance in biological specimens is often directly related to pathogenic mechanisms (Gerszten and Wang, 2008) and this concept is routinely demonstrated in clinical chemistry laboratory results. Historically, often a decade or more could pass between the initial discovery of a disease marker, its validation in human trials, and its routine implementation as a clinical test. To realize the potential of precision medicine, we need to accelerate the discovery of specific markers of disease and drug pharmacodynamics, as well as metabolite profiles associated with external environment and their associations with disease risk. Current metabolomics technologies can enable more rapid discovery and validation of metabolic indicators of disease. Techniques used in metabolomics, such as liquid chromatography–mass spectrometry (LC-MS), can routinely measure tens to hundreds of metabolites with excellent precision and are suitable for discovery studies in human cohorts (Moid et al., 2020). Confidence comes from experience with recent applications to find early metabolic indicators of disease in longitudinal cohorts years before symptoms are clinically apparent—for example, in pancreatic cancer type 2 diabetes, memory impairment, and many other conditions (Mayers et al., 2014). Metabolomics studies have also inspired work revealing novel insights into relationships between diet and disease, such as observations linking elevated branched chain amino acids and obesity to insulin resistance. Bringing the microbiome into the mix, metabolite profiling studies by (Koeth et al., 2013) recently showed that elevated plasma levels of trimethylamine-N-oxide are at the nexus of a relationship among diets abundant in red meat, the composition of the gut microbiome, and risk for cardiac events.

3. Conventional metabolites and targeted metabolomics assays

Briefly, conventional metabolite levels [lactate, triglycerides, 3-hydroxybutyrate, glycerol, nonessential fatty acids (NEFAs)] were measured on a Beckman Coulter Unicel DxC 600 clinical analyzer (Beckman Coulter, Brea, CA). Targeted metabolomics assays for acylcarnitines and amino acids were performed by tandem mass spectrometry with the addition of known quantities of stable isotope-labeled internal standards on an Acquity triplequadruple system (Waters Corporation, Milford, MA).

4. Nontargeted analyses

Nontargeted gas chromatography (GC)/mass spectrometry (MS) assays were performed to analyze the full range of metabolites in plasma. Methanol, the extraction solvent, was spiked with a retention-time-locking internal standard of perdeuterated myristic acid. Extracts were prepared for GC/MS by methoximation and trimethyllsilylation. Plasma from Northern European and Thai ancestries were run on a 6890N GC-5975B inert mass spectrometer (Agilent Technologies, Santa Clara, CA). Afro-Caribbean and Mexican American samples were run on a 7890B GC-5977B inert mass spectrometer (Agilent Technologies).

Samples for GC/MS were run in batches of equal size during 50 days for each ancestry group and balanced by the field center, maternal phenotypes, and newborn outcomes. Quality control pools, constructed using small volumes from all cord blood samples and prepared the same as above were injected at the first, middle, and last samples of each day GC/MS batch. Data from these quality control samples were used to control technical variability attributable to batch and run order, as applied using the metabomxtr R package. Peaks were decontrolled with AMDIS freeware and annotated against a retention-time-locking spectral library built upon that of Fiehn and colleagues with additions from our laboratory. Manual curation included grouping metabolite peaks across samples according to similar GC retention time and mass spectra. Detected peak areas were log2 transformed for analysis. In total, 73 GC/MS metabolites that had not been assayed using targeted approaches were used for subsequent data analysis.

5. Conclusion

In summary, cord blood metabolites are associated with newborn size and hyperinsulinemia, even when accounting for maternal BMI and glucose during pregnancy. 1,5-Anhydroglucitol, a marker of glycemia, may be an emerging marker of newborn adiposity. The known associations of medium-chain acylcarnitines with obesity in adolescents and adults appear to be present at birth although the relationship of branched-chain amino acids to lean vs fat mass in the newborn period is less clear. Further study is needed to elucidate the mechanisms underlying these associations to better understand how the complex relationship between metabolite signatures of adiposity and hyperinsulinemia in newborns relates to obesity and type 2 diabetes phenotypes in adolescents and adults.

6. Future prospective

To explore the association between targeted and nontargeted cord blood metabolites with measures of newborn adiposity and hyperinsulinemia to determine whether a unique cord blood metabolic signature might emerge as a proxy for the metabolic environment of the fetus and provide mechanistic insight into fetal fat deposition, insulin sensitivity, and future obesity and metabolic disease risk.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ramachandran, Ambady, Snehalatha, Chamukuttan, Nanditha, Arun, 2016. Classification and diagnosis of diabetes. Diabetes Care 39 (Suppl1), S13–S22.
Lukács, Andrea, Lukácsa, Andrea, Barkai, László, et al., 2018. Screening risk factors for type 2 diabetes in overweight and obese adolescents in school settings of Hungary: a population-based study. J. King Saud Univ. – Sci. 4, 176–179.
Moid, Ahmad, AlAmmari, et al., 2020. Trace identification of endocrine-disrupting bisphenol A in drinking water by solid-phase extraction and ultra-performance

liquid chromatography-tandem mass spectrometry. J. King Saud Univ. – Sci. 3, 1634–1640.
Crowther, C.A., Hiller, J.E., Moss, J.R., McPhee, A.J., Jeffries, W.S., Robinson, J.S., 2005. Australian carbohydrate intolerance study in pregnant women (ACHOIS) trial group: effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N. Engl. J. Med. 352, 2477–2486.
Chen, X., Scholl, T.O., 2005. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. Curr. Diabetes Rep. 5, 282–288.
Diaz, S.O., Pinto, J., Graca, G., Duarte, I.F., Barros, A.S., Galhano, E., Pita, C., Almeida Mdo, C., Goodfellow, B.J., Carreira, I.M., et al., 2011. Metabolic biomarkers of prenatal disorders: an exploratory nmr metabonomics study of second trimester maternal urine and blood plasma. J. Proteome Res. 10, 3732–3742.
Wishart, D.S., Lewis, M.J., Morrissey, J.A., et al., 2008. The human cerebrospinal fluid metabolome. J. Chromatogr. A 164–173.
Desoye, G., Hauguel-de Mouzon, S., 2007. The human placenta in gestational diabetes mellitus. The insulin and cytokine network. Diabetes Care 30, S120–S126.
Egeland, G.M., Meltzer, S.J., 2010. Following in mother's footsteps? Mother-daughter risksfor insulin resistance and cardiovascular disease 15 years after gestational diabetes. Diabet. Med. 27, 257–265.
Fanos, V., Atzori, L., Makarenko, K., Melis, G.B., Ferrazzi, E., 2013. Metabolomics application in maternal-fetal medicine. BioMed Res. Int. 702514
Gerszten, R.E., Wang, T.J., 2008. The search for new cardiovascular biomarkers. Nature 451, 949–952.
Kumar, G., Kocour, M., 2017. Applications of next-generation sequencing in fisheries research: a review. Fish. Res. 11–22.
Gilmore, L.A., Klempel-Donchenko, M., Redman, L.M., 2015. Pregnancy as a window to future health: excessive gestational weight gain and obesity. Semin. Perinatol. 39, 296–303.
Hoffman, D.J., Reynolds, R.M., Hardy, D.B., 2017. Developmental origins of health and disease: current knowledge and potential mechanisms. Nutr. Rev. 75, 951–970.
Jaskolka, D., Retnakaran, R., Zinman, B., Kramer, C.K., 2015. Sex of the baby and risk of gestational diabetes mellitus in the mother: a systematic review and meta-analysis. Diabetologia 58, 2469–2475.
Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T., Britt, E.B., Fu, X., Wu, Y., Li, L., et al., 2013. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. 19, 576–585.
Kawasaki, M., Arata, N., Ogawa, Y., 2018. Obesity and abnormal glucose tolerance in the offspring of mothers with diabetes. Curr. Opin. Obstet Gynecol.
Lawlor, D.A., Lichtenstein, P., Langstrom, N., 2011. Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. Circulation 123, 258–265.
Mayers, J.R., Wu, C., Clish, C.B., Kraft, P., Torrence, M.E., Fiske, B.P., Yuan, C., Bao, Y., Townsend, M.K., Tworoger, S.S., et al., 2014. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. Nat. Med., 1193–1198
Padayachee, C., Coombes, J.S., 2015. Exercise guidelines for gestational diabetes mellitus. World J. Diabetes 6, 1033–1044.
Silverman, B.L., Rizzo, T.A., Cho, N.H., Metzger, B.E., 1998. Long-term effects of the intrauterine environment. The northwestern university diabetes in pregnancy center. Diabetes Care 21 (Suppl. 2), B142–B149.
Bouatra, S., Aziat, F., Mandal, R., et al., 2013. The human urine metabolome. PLoS One.
Tam, W.H., Ma, R.C.W., Ozaki, R., Tutino, G.E., et al., 2017. In utero exposure to maternal hyperglycemia increases childhood cardiometabolic risk in offspring. Diabetes Care 40, 679–686.
Underwood, M.A., Gilbert, W.M., Sherman, M.P., 2005. Amniotic fluid: not just fetal urine anymore. J. Perinatol. 25, 341–348.
Yuen, L., Wong, V.W., 2015. Gestational diabetes mellitus: challenges for different ethnic groups. World J. Diabetes 6, 1024–1032.
Zhao, Y.Y., Cheng, X.L., Vaziri, N.D., Liu, S., Lin, R.-C., 2015. UPLC-based metabonomic applications for discovering biomarkers of disease sin clinical chemistry. Clin. Biochem. 16–26.
Yang, X., Darko, K.O., Huang, Y., He, C., Yang, H., He, S., Li, J., Li, J., Hocher, B., Yin, Y., 2017. Resistant starch regulates gut microbiota: structure, biochemistry and cell signalling. Cell Physiol. Biochem. 42, 306–318.