Early Human Development 89 (2013) 315–320

Contents lists available at SciVerse ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Metabolomics analysis of umbilical cord blood clarifies changes in saccharides associated with delivery method

Fusako Hashimoto [a], Shin Nishiumi [b], Osamu Miyake [c], Hitomi Takeichi [d], Mari Chitose [e], Hiromi Ohtsubo [a], Shingo Ishimori [a], Takeshi Ninchoji [a], Yuya Hashimura [a], Hiroshi Kaito [a], Naoya Morisada [a], Ichiro Morioka [a], Hideoki Fukuoka [f], Masaru Yoshida [b,g,h,1], Kazumoto Iijima [a,*,1]

[a] Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan
[b] Division of Gastroenterology, Kobe University Graduate School of Medicine, Kobe, Japan
[c] Division of Pediatrics, Palmore Hospital, Kobe, Japan
[d] Department of Nutritional Science, Kobe Gakuin University, Kobe, Japan
[e] Department of Human Science, Kobe Shoin Women's University, Kobe, Japan
[f] Institute of Epigenetic Regulation of Fetal Development, Waseda University, Tokyo, Japan
[g] Integrated Center for Mass Spectrometry, Kobe University Graduate School of Medicine, Kobe, Japan
[h] Division of Metabolomics Research, Kobe University Graduate School of Medicine, Kobe, Japan

ARTICLE INFO

Article history:
Received 1 September 2012
Received in revised form 27 October 2012
Accepted 31 October 2012

Keywords:
Allose
Glucose
Mannose
Fructose

ABSTRACT

Background: A metabolomic approach using umbilical cord blood from infants at birth has not been studied widely yet.
Aim: We examined changes in metabolite levels in umbilical cord blood at birth via gas chromatography/mass spectrometry (GC/MS)-based metabolomics, with the aim of achieving a detailed understanding of fetal stress during labor.
Study design: All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine. This was a cohort study of pregnant women based in Palmore Hospital, which is located in an urban area of Japan, and was carried out between December 2010 and May 2011.
Subject: Umbilical cord arterial blood samples were obtained from 41 infants immediately after delivery.
Outcome measures: Metabolites in the blood samples were measured using GC/MS to investigate whether the delivery method (spontaneous onset of labor, induction of labor or elective cesarean section) affected the metabolite profile in umbilical cord blood.
Results: Elective cesarean section without labor led to lower levels of isoleucine, fructose, mannose, glucose, allose, glucuronic acid, inositol and cysteine in comparison with vaginal delivery following spontaneous labor and without medication.
Conclusion: It is proposed that the stress associated with labor be involved in alterations in the levels of metabolites, particularly saccharides such as glucose, in umbilical cord blood.

1. Introduction

The mode of delivery during birth is strongly associated with fetal and maternal stress. In previous studies, it has been reported that fetal stress was decreased in newborns delivered by cesarean section compared with vaginal delivery, and cesarean section was associated with significantly lower maternal hormonal responses compared with vaginal delivery [1]. In addition, cortisol concentrations of second babies were significantly lower in comparison to first babies [1], and neonatal glucose concentration was lower in cesarean section infants than that in vaginal delivery infants [2]. Umbilical cord blood is the peripheral blood of the fetus, and various molecules derived from the maternal body are generally moved to the cord blood. To date, the low molecular weight metabolites in umbilical cord blood have not been comprehensively analyzed. Therefore, clarifying the metabolites in umbilical cord blood may be useful for the assessment of fetal nutrition or delivery stress for infants.

Metabolomics (metabolome analysis) provides comprehensive data about the metabolic processes of a cell or organism. Metabolomics has recently developed rapidly, and has been applied to various research fields including medicine. We have studied pancreatic [3], lung [4], and gastrointestinal [5] cancers, and gastroenterological diseases [6], and have revealed alterations in the serum metabolite profile associated with these diseases. In perinatology, analyses of neural tube defects [7]

* Corresponding author at: Department of Pediatrics, Kobe University Graduate School of Medicine, 5-1 Kusunoki-cho 7 chome, Chuo-ku, Kobe, 650-0017, Japan. Tel.: +81 78 382 6090; fax: +81 78 382 6099.
E-mail address: iijima@med.kobe-u.ac.jp (K. Iijima).
[1] These authors share senior authorship.

0378-3782/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.earlhumdev.2012.10.010

and intrauterine growth restriction [8,9] have been performed. Metabolomics analysis of human umbilical vein endothelial cells has demonstrated that activation of AMP-activated protein kinase induces metabolic effects in cell metabolism [10]. Here, we examined changes in metabolite levels in umbilical cord blood at birth via gas chromatography/mass spectrometry (GC/MS)-based metabolomics with the aim of achieving a detailed understanding of fetal stress during labor.

2. Methods

2.1. Study design, participants, and location

All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine. This was a cohort study of pregnant women based in Palmore Hospital, which is located in an urban area of Japan, and was carried out between December 2010 and May 2011. The study consisted of 60 pregnant women who volunteered to participate, or to have the study explained, after they had read our advertisement (Fig. 1). Informed consent was obtained from 54 of the 60 potential participants; the remaining six women declined. Thus, a total of 54 singleton pregnant women and their single infants participated. All of these pregnancies were full term. Forty-seven infants were delivered vaginally; the remaining seven infants were delivered by cesarean section prior to spontaneous labor owing to placenta previa or recurrent cesarean. The mother of one of these seven infants exhibited maternal hyperthyroidism, so this infant was excluded from the study. Among the 47 infants delivered vaginally, 19 were delivered without medication, and 16 were delivered vaginally with prostaglandin E2 and/or oxytocin independently. A total of 12 infants were excluded from the analysis because the mothers had been administered antibiotics, or because the infants had ventricular septal defects, low birth weight (birth weight <2500 g), or exhibited intrauterine growth retardation, or there was poor information available regarding the infants. All of the pregnant women were non-smokers and had no obesity, diabetes mellitus, or gestational diabetes mellitus. A total of 41 infants from 41 pregnant women were therefore analyzed. Our study also included 13 healthy adult volunteers, who had given a blood sample during a health examination at the hospital. Information about the participants is summarized in Supplemental Table 1.

2.2. Serum collection and preparation

Immediately after delivery of the newborn, a segment of the umbilical cord was doubly clamped, and an arterial blood sample was collected from this into a tube. The blood was centrifuged at 3000×*g* for 10 min at 4 °C, and the serum was transferred to a clean tube and stored at −80 °C until use. Previously described methods were used to extract low-molecular-weight metabolites from the sera [11], and to complete oximation and subsequent derivatization for GC/MS measurements [11].

2.3. GC/MS analysis and data processing

As previously described [12], GC/MS analysis was performed using GCMS-QP2010 Ultra (Shimadzu Co., Kyoto, Japan) with a fused silica capillary column (CP-SIL 8 CB low bleed/MS; 30 m×0.25 mm (inner diameter), film thickness: 0.25 μm; Agilent Co., Palo Alto, CA). Data processing was performed using MetAlign software (Wageningen UR, The Netherlands) and in-house analytical software (AIoutput). The metabolite identification was performed according to previous reports [12,13]. In this analysis, the peak detection and alignment was performed by MetAlign software. In AIoutput, the retention time for the obtained data was also corrected using an internal standard. The metabolite database used in our study includes the information about retention time and EI spectrum for each metabolite, and the retention time of each metabolite in this database was also corrected on the basis of the results from n-alkane mix analysis. For semi-quantitative analysis, the peak height intensity of each ion was calculated and normalized using the peak height of 2-isopropylmalic acid as an internal standard. In GC/MS analysis, multiple peaks are sometimes detected for a particular metabolite owing to TMS-derivatization or isomeric form. In such cases, the peak that most closely reflected the level of the metabolite was adopted for semi-quantitative evaluation. The results obtained were also checked manually, and the low-trust data were excluded. Thus, the data analysis including database was strictly controlled.

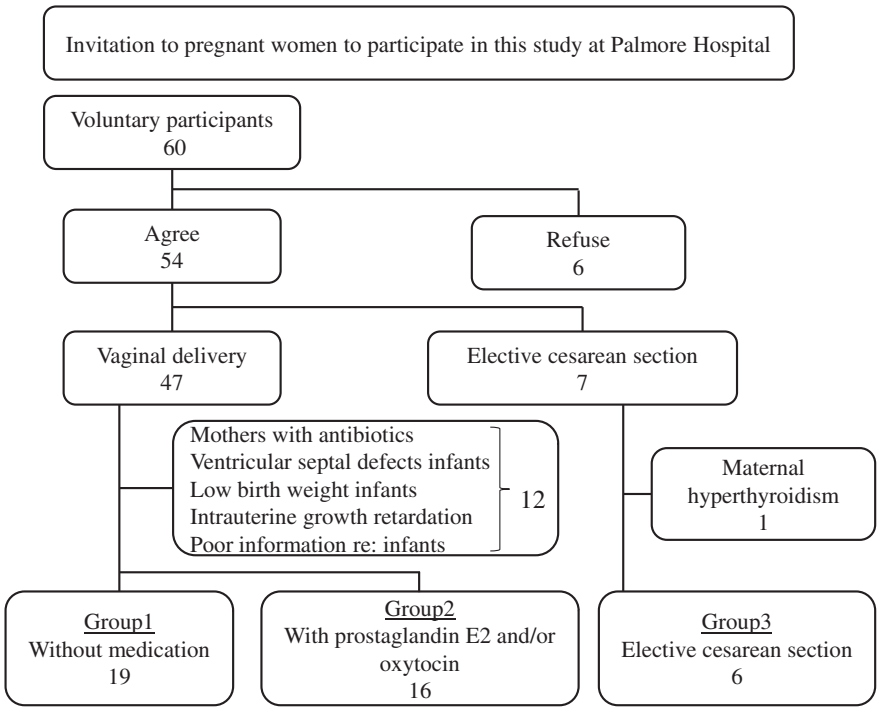

Fig. 1. Derivation of the cohort.