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Influence of moderate maternal nutrition restriction on the fetal baboon metabolome at 0.5 and 0.9 gestation

C. Hellmuth [a], O. Uhl [a], F.F. Kirchberg [a], U. Harder [a], W. Peissner [a], B. Koletzko [a,*], P.W. Nathanielsz [b,c]

[a] *Ludwig-Maximilians-Universität, Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Universtiy of Munich Medical Center, 80337, Muenchen, Germany*
[b] *Department of Animal Science, University of Wyoming, Laramie, 82701, WY, USA*
[c] *Texas Pregnancy & Life-course Health Research Center, San Antonio, 7620 NW Loop 410, 78227, TX, USA*

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Abstract *Background and aims:* Moderately reduced maternal nutrient availability during pregnancy has adverse effects on the fetuses' growth and metabolism during and after pregnancy. The aim of this study was to explore effects of maternal nutrition restriction (MNR) on key metabolites of the fetal energy metabolism, particularly amino acids (AA), nonesterified fatty acids (NEFA), acylcarnitines and phospholipids. These effects may reflect mechanisms relating MNR to later adverse outcomes.

Methods and results: Plasma and liver samples of fetal baboons, whose mothers were fed ad libitum (CTR) or MNR (70% of CTR), were collected at 0.5 and 0.9 gestation (G – term 184 days). Metabolites were measured with liquid chromatography coupled to mass spectrometry. In both, CTR and MNR, fetal metabolic profiles changed markedly between 0.5G and 0.9G. Fetal liver glucose concentrations were strongly increased. Hepatic levels of NEFA, sphingomyelins, and alkyl-linked phospholipids increased while plasma NEFA and acyl-linked phospholipids levels decreased with progression of gestation.

At 0.5G, MNR fetal plasma levels of short- and medium-chain acylcarnitines were elevated, but did no longer differ between groups at 0.9G. At 0.9G, plasma levels of methionine and threonine as well as hepatic threonine levels were lower in the MNR group.

Conclusion: Small differences in the concentrations of plasma and liver metabolites between MNR and CTR fetuses reflect good adaptation to MNR. Fetal liver metabolic profiles changed markedly between the two gestation stages, reflecting enhanced liver glucose and lipid levels with advancing gestation. Decreased concentrations of AA suggest an up-regulation of gluconeogenesis in MNR.

Acronyms: AA, Amino acid; APCI, Atmospheric pressure chemical ionization; BCAA, Branched-chain amino acids; Carn, Acylcarnitines; CTR, Control group; Cys, Cysteine; ESI, Electrospray ionization; FA, Fatty acid; G, Gestation; HPLC, High-performance liquid chromatography; Ile, Isoleucine; IUGR, Intrauterine growth restriction; LC-MS, Liquid chromatography coupled to MS detection; Leu, Leucine; lysoPCa, Lysophosphatidylcholines; Met, Methionine; MNR, Moderate maternal nutrition restriction; MRM, Multiple reaction monitoring; MS, Mass spectrometer; NEFA, Nonesterified fatty acids; PCaa, Diacyl-phosphatidylcholines; PCae, Acyl-alkyl-phosphatidylcholines; PCA, Principal component analysis; PEPCK, Phosphoenolpyruvate carboxykinase; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARG, Peroxisome proliferator activated receptor γ; QC, Quality control; Ser, Serine; SM, Sphingomyelins; Thr, Threonine; Val, Valine.

* Corresponding author. Ludwig-Maximilians-University of Munich, Div. Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center, Lindwurmstr. 4, D-80337, Muenchen, Germany. Tel.: +49 89 4400 52826; fax: +49 89 4400 57742.
E-mail address: office.koletzko@med.lmu.de (B. Koletzko).

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Introduction

Sub-optimal nutrition in pregnancy is associated with adverse metabolic offspring outcomes in later life such as reduced glucose tolerance, promotion of an atherogenic lipid profile, higher body mass index (BMI), as well as development of cardiovascular diseases [1]. These outcomes are generally attributed to developmental programming which can be defined as the response to a specific challenge to the mammalian organism during a critical developmental time window [2]. This may alter the trajectory of development, modifying the organism's phenotype with effects on health that can persist throughout the life-course [3]. Several different animal models have been used to examine the effect of reduced maternal nutrition on metabolic disorders. Most studies have been conducted in rodents [4,5] which cannot simply be extrapolated to humans that have a very different developmental profile from monotocous, precocial species [6]. Thus, studies with nonhuman primates are required in order to understand early development of metabolic diseases in humans. In baboons, moderate maternal nutrition restriction (MNR) has only minor effects on overall fetal growth at 0.5 gestation (G – term 184 days) [7,8], but at 0.9G fetuses show intrauterine growth restriction [9]. Decreased maternal nutrition in pregnant baboons was shown to alter the placental nutrient transfer [10], fetal metabolism [7] and was associated with an increased risk for post-natal adverse outcomes such as insulin resistance at 3.5 yrs [11]. Metabolic adaptations during development in response to decreased fetal nutrition may persist after birth, even when nutrition has returned to the normal range, as shown in animal models and in humans [12]. For instance, fetal baboons experiencing MNR have increased hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity, indicating elevated gluconeogenesis [13] and they also have higher fasting glucose levels 3.5 years after birth [11]. Thus, the aim of the present study was to explore the effects of MNR in pregnancy on the metabolic signature, with focus on but not limited to gluconeogenetic amino acids (AA), of fetal baboons. Most AA are precursors for endogenous gluconeogenesis but they enter species-specific pathways [14]. They are actively transported across the placenta to enter the fetal circulation to support fetal growth [15]. Lipids or fatty acids (FA) are released from placenta into the fetal circulation in the form of nonesterified fatty acids (NEFA) and are esterified in phospholipids by the fetal liver [16]. For this study, we used a subset of samples from a previously published fetal baboon study and analyzed AA, NEFA, and polar lipids (acylcarnitines and phospholipids).

Methods

Ethics statement

All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Texas Health Science Center and Southwest National Primate Research Center Institutional Animal Care and Use Committees. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Animal care

Twenty-seven baboons (Papio hamadryas anubis) from the Southwest National Primate Research Center, San Antonio Texas were studied (Table 1). Animals were housed in outdoor gang cages providing full social and physical activity [17]. Animals were fed between 7 am and 9 am or 11 am and 1 pm as described [17]. Water was continuously available in the feeding cages via individual waterers (Lixit, Napa, CA). Animals received Monkey Diet 15% 5LE0 (Supplemental Table 1, ≥15% crude protein, ≥4% crude fat, ≥10% crude fiber; Purina, St. Louis, MO). At the start of the feeding period, ad libitum-fed baboons were given 60 biscuits in their individual cage. Biscuits remaining were counted after baboons returned to their group cage.

Study

Healthy non-pregnant female baboons of similar body weights (10–15 kg) were randomly assigned to one of two group cages in social groups of 10–16 animals, and a vasectomized male. After acclimation to the feeding cages (30 d), a fertile male was substituted. Pregnancy was dated initially by timing of ovulation and changes in sex skin color, and confirmed by ultrasound at 30 d of gestation. From this day on, 12 of the 27 pregnant baboons were fed 70% of food eaten by contemporaneous controls on a per-kilogram basis (MNR), while the remaining 15 pregnant baboons were still ad libitum-fed (control, CTR). Before pregnancy, the two groups did not differ in morphometric characteristics [18] (Table 1).

Standard cesarean sections were performed at 0.5G and 0.9G (term 184 days) after an overnight fast. The umbilical cord was identified and elevated to the incision to enable fetal exsanguination, as approved by the American Veterinary Medical Association. The fetus was removed from the uterus and immediately submitted for morphometric measurements, complete pathologic evaluation, and tissue sampling. The complete fetal liver was removed and half of the sample was taken for this study and immediately flash frozen in liquid nitrogen [19].

Preparation of liver tissue

Briefly, an aliquot of about 50 mg of the liver tissue was diluted with 600 μl methanol and bead–based homogenized in cryo-vials with a MINILYS homogenizer (Precellys, Bertin Technologies, Montigny-le-Bretonneux, France). Subsequently, samples were centrifuged for 10 min at 23306*g* (room temperature). 20 μl (presenting 1.67 mg liver) of the supernatant were transferred and processed as described for plasma and standards for each application.