



Sexual dimorphism in the fetal cardiac response to maternal nutrient restriction



Sribalashubashini Muralimanoharan^{a,e}, Cun Li^{a,c}, Ernesto S. Nakayasu^d, Cameron P. Casey^d, Thomas O. Metz^d, Peter W. Nathanielsz^{a,c}, Alina Maloyan^{a,b,*}

^a Center for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, The University of Texas Health Science Center, San Antonio, TX 78229, USA

^b Knight Cardiovascular Institute, Oregon Health and Science University, Portland, Oregon 97239, USA

^c College of Agriculture and Natural Resources, University of Wyoming, Laramie, Wyoming 82071, USA

^d Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, USA

^e Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75390-9038, USA

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ABSTRACT

Poor maternal nutrition causes intrauterine growth restriction (IUGR); however, its effects on fetal cardiac development are unclear. We have developed a baboon model of moderate maternal undernutrition, leading to IUGR. We hypothesized that the IUGR affects fetal cardiac structure and metabolism. Six control pregnant baboons ate ad-libitum (CTRL) or 70% CTRL from 0.16 of gestation (G). Fetuses were euthanized at C-section at 0.9G under general anesthesia. Male but not female IUGR fetuses showed left ventricular fibrosis inversely correlated with birth weight. Expression of extracellular matrix protein TSP-1 was increased ($p < 0.05$) in male IUGR. Expression of cardiac fibrotic markers TGF β , SMAD3 and ALK-1 were downregulated in male IUGRs with no difference in females. Autophagy was present in male IUGR evidenced by upregulation of ATG7 expression and lipidation LC3B. Global miRNA expression profiling revealed 56 annotated and novel cardiac miRNAs exclusively dysregulated in female IUGR, and 38 cardiac miRNAs were exclusively dysregulated in males ($p < 0.05$). Fifteen (CTRL) and 23 (IUGR) miRNAs, were differentially expressed between males and females ($p < 0.05$) suggesting sexual dimorphism, which can be at least partially explained by differential expression of upstream transcription factors (e.g. HNF4 α , and NF κ B p50). Lipidomics analysis of fetal cardiac tissue exhibited a net increase in diacylglycerol and plasmalogens and a decrease in triglycerides and phosphatidylcholines. In summary, IUGR resulting from decreased maternal nutrition is associated with sex-dependent dysregulations in cardiac structure, miRNA expression, and lipid metabolism. If these changes persist postnatally, they may program offspring for higher later life cardiac risk.

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1. Introduction

The “fetal origins of disease” hypothesis proposes that maternal undernutrition causes permanent structural and metabolic changes in developing organs that increase the subsequent risk of cardiovascular and metabolic disease [1]. Poor maternal nutrition during pregnancy is

thought to account for 14% of intrauterine growth restriction (IUGR) in developing countries (4th Report on The World Nutrition Situation - ACC/SCN 2000) while other important etiologic factors such as malaria, acute and chronic infections and cigarette smoking could contribute as well. In developed countries, smoking is the most important determinant of IUGR, followed by factors such as maternal nutrition, pre-eclampsia, genetic factors and alcohol or drug use [2].

The cellular processes, by which prenatal maternal nutritional challenges predispose to later life offspring disease are termed fetal or developmental programming. Programming can be defined as the responses of the developing organism to a challenge during a critical window of development that alter offspring phenotype with potential persistent effects on life course health. The first human epidemiological studies on programming reported life course cardiovascular disease associated with poor fetal growth in both males and females [3,4]. Similarly, numerous animal studies addressed the short- and long-term consequences of poor maternal nutrition for the offspring health [5].

Abbreviations: ALK1, activin receptor-like kinase-1; ATG7, autophagy-related protein-7; CL, cardiolipin; CTGF, connective tissue growth factor; CDX2, caudal type homeo box transcription factor 2; DG, diglycerides; HNF4 α , hepatocyte nuclear factor 4 alpha; IUGR, intrauterine growth restriction; LC3B, microtubule-associated protein 1B-light chain 3; MNR, maternal nutrient restriction; NF κ B1, nuclear factor kappa B subunit 1; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PPAR γ , peroxisome proliferator-activated receptor gamma; SM, sphingomyelins; TSP-1, Thrombospondin-1; TGF β , transforming growth factor β ; TG, triglycerides.

* Corresponding author at: Knight Cardiovascular Institute, Oregon Health and Science University, Portland, Oregon, 97239, USA.

E-mail address: maloyan@ohsu.edu (A. Maloyan).

Rat and mouse models are clearly the preferred nonhuman models and both have shown that long-term effects of adverse maternal nutrition include changes in postnatal growth [6] and altered metabolism in offspring [7–11]. Maternal low protein diet results in high blood pressure in offspring [10], altered endocrine function [9], immune function [7], and renal development [11]. Same long-term consequences of maternal malnutrition were observed in long-gestation, precocial species including sheep [12,13] and nonhuman primates [14–16]. The pig is currently considered a reliable large animal model for translational research in IUGR as result of deficiencies in maternal nutritional supply or placental development [17]. In pigs, IUGR offspring have compromised health, reduced growth potential and high predisposition for adiposity [18].

Few published studies focused on effect of maternal nutrition on myocardial development clearly show that the morphology and the metabolism of the heart are sensitive to the intrauterine insults. For example, Dong et al. have reported that maternal nutrient restriction in sheep results in enlarged ventricular size associated with an upregulation of insulin-like growth factor (IGF) receptor [19]. Using the same model of maternal nutrient restriction, Han et al. have reported significant changes in cardiac gene expression measured by hybridization assay [12]. Gilbert et al. have demonstrated a dysregulation of the renin-angiotensin system in midgestation left ventricle of fetal sheep exposed to 50% reduction in maternal nutrition [13]. Beauchamp et al. [20] showed impaired cardiac muscle energetics as result of maternal undernutrition and low birth weight. Maternal nutrient restriction in rats results in decreased cardiomyocytes number [21], whereas the rat model of maternal protein restriction showed an increase in myocardial expression and deposition of collagens and alters the expression of pro-fibrotic genes in adult offspring [22].

We have developed a baboon model of moderate maternal nutrient reduction during pregnancy that produces IUGR offspring [14,23–30]. In this model, throughout pregnancy, undernourished mothers ate 70% of the diet consumed by controls – a 30% reduction in intake. We have previously shown that the offspring are growth restricted at birth, with male and female offspring of restricted mothers weighing approximately 11% less than offspring of controls [25]. Metabolic, functional, and epigenetic abnormalities were reported in the fetal and/or neonatal brain [15], kidney [28] and liver [14,23] of IUGR baboons. In adult life, the IUGR baboons showed an increase in insulin resistance [31] and engage in more aggressive and affiliative behaviors compared to control animals [32]. In this study, we sought to determine how maternal undernutrition affects fetal cardiac development.

2. Materials and methods

2.1. Animal care and maintenance

All procedures were approved by the Texas Biomedical Research Institute (Texas Biomed) Institutional Animal Care and Use Committee and conducted in AAALAC-approved facilities. Thirty-eight female baboons (*Papio hamadryas anubis*) from the Southwest National Primate Research Center at San Antonio, Texas, USA were recruited for this study and maintained in group housing. The caging system allows control and monitoring of food intake while still permitting normal social and physical activity and has been described in detail [14,29,30]. Briefly, groups of 16 females carefully selected to produce a homogeneous group were assembled and socialized in the presence of a vasectomized male while eating Purina Monkey Diet 5038 (Purina, St. Louis, Missouri, USA) ad libitum. After acclimation, the vasectomized male was replaced by a proven breeder male. Females were observed for turgescence (sex skin swelling) and signs of vaginal bleeding to enable timing of pregnancy [33]. Pregnancy was confirmed by ultrasound at 0.16G after which they were randomly assigned to either a control group of females that continued to receive ad lib feed ($n = 24$) or a group that underwent maternal nutrient restriction and received 70% of the feed eaten by controls on a weight-adjusted basis ($n = 14$). The system of individual

feeding of adult baboons maintained in an outdoor group social environment has been previously described in detail [14].

2.2. Study design

Briefly, once a day prior to feeding, all baboons were placed in individual feeding cages. Baboons passed along a chute, over a scale, and into an individual feeding cage. The weight of each baboon was obtained as it crossed an electronic scale system (GSE 665; GSE Scale Systems, Livonia, MI, USA). The weight recorded was the mean of 50 individual measurements over 3 s. If the SD of the weight measurement was $> 1\%$ of the mean weight, the weight was automatically discarded, and the weighing procedure was repeated. Once housed in an individual cage, each animal was fed between either 07:00 and 09:00 or 11:00 and 13:00. Water was available continuously in the individual feeding cage and the group cages. Animals were fed Purina Monkey Diet 5038 (Purina, St. Louis, MO, USA), described by the vendor as “a complete life-cycle diet for all Old World Primates.” The biscuit contains stabilized vitamin C as well as all other required vitamins. Its basic composition is crude protein $\geq 15\%$, crude fat $\geq 5\%$, crude fiber $\leq 6\%$, ash $\leq 5\%$, and added minerals $\leq 3\%$. At the start of the feeding period, each baboon was given 60 biscuits in the feeding tray of the individual cage. At the end of the 2 h feeding period, the baboons were returned to the group cage. Biscuits remaining in the tray, on the floor of the cage, and in the pan beneath the cage were counted. Food consumption of animals, weights, and health status were recorded each day.

2.3. Cesarean sections

Surgical procedures were performed by a fully certified MD or DVM, and postsurgical care was prescribed and monitored by a primate center veterinarian. Cesarean sections were performed at 165d G (0.9G) using standard techniques that have been previously described in detail [22]. All baboons were premedicated with ketamine hydrochloride (10 mg/kg, i.m.). After intubation, isoflurane (2%) was used to maintain a surgical plane of anesthesia throughout surgery. Following hysterotomy, the umbilical cord was identified and used for fetal exsanguination while under general anesthesia as approved by the AVMA Panel on Euthanasia. The placenta and fetus were removed from the uterus and immediately submitted for morphometric analyses and tissue sampling. Fetal morphometrics was obtained at necropsy. Post-operative maternal analgesia was by buprenorphine hydrochloride (Buprenorphine HCl Injection, Hospira, Inc., Lake Forest, IL) 0.015 mg/kg/d split as two doses for 3 days. After recovery in individual cages, mothers were returned to their group housing.

2.4. Materials

Antibody against HNF4 α was purchased from Origene. Antibody against ATG7 and LC3B were part of the Autophagy Antibody Sampler Kit from Cell Signaling Technologies (Danvers, MA, USA). TGF β , SMAD3, and NF κ B p50 were also purchased from Cell Signaling. The anti-TSP1-1 antibody was from R&D Systems and anti-CTGF – from GeneTex Inc. The anti-ALK1 and anti-PPAR γ antibodies were purchased from Santa Cruz Biotechnology, and antibody against β -Actin – from Sigma (St. Louis, MO, USA).

2.5. Histology

The excised hearts were washed in PBS, fixed overnight in 4% paraformaldehyde, and embedded in paraffin. After serial sectioning of hearts, (apex to base) 7- μ m sections were stained either with hematoxylin-eosin to visualize tissue architecture or with Masson trichrome to visualize the fibrotic tissue. Areas of fibrosis within sections were measured by visualizing blue-stained areas, exclusive of staining that colocalized with perivascular or intramural vascular structures. Using

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