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Commentary

Unravelling the impact of intrauterine growth restriction on heart development: insights into mitochondria and sexual dimorphism from a non-hominoid primate

George W. Booz[1], Gaelle P. Massoud[2], Raffaele Altara[3] and Fouad A. Zouein[2]

[1]Department of Pharmacology and Toxicology, School of Medicine, University of Mississippi Medical Center, Jackson, MS, U.S.A.; [2]Department of Pharmacology and Toxicology, American University of Beirut Medical Center, Faculty of Medicine, Riad El-Solh, Beirut, Lebanon; [3]Department of Pathology, School of Medicine, University of Mississippi Medical Center, Jackson, MS, U.S.A.

Correspondence: George W. Booz (gbooz@umc.edu) or Fouad A. Zouein (fz15@aub.edu.lb)

Fetal exposure to an unfavorable intrauterine environment programs an individual to have a greater susceptibility later in life to non-communicable diseases, such as coronary heart disease, but the molecular processes are poorly understood. An article in *Clinical Science* recently reported novel details on the effects of maternal nutrient reduction (MNR) on fetal heart development using a primate model that is about 94% genetically similar to humans and is also mostly monotocous. MNR adversely impacted fetal left ventricular (LV) mitochondria in a sex-dependent fashion with a greater effect on male fetuses, although mitochondrial transcripts increased more so in females. Increased expression for several respiratory chain and adenosine triphosphate (ATP) synthase proteins were observed. However, fetal LV mitochondrial complex I and complex II/III activities were significantly decreased, likely contributing to a 73% decreased LV ATP content and increased LV lipid peroxidation. Moreover, MNR fetal LV mitochondria showed sparse and disarranged cristae. This study indicates that mitochondria are targets of the remodeling and imprinting processes in a sex-dependent manner. Mitochondrial ROS production and inadequate energy production add another layer of complexity. Altogether these observations raise the possibility that dysfunctional mitochondria in the fetus may contribute in turn to epigenetic memory of *in utero* stress in the adult. The role of mitoepigenetics and involvement of mitochondrial and genomic non-coding RNAs in mitochondrial functions and nuclei–mitochondria crosstalk with *in utero* stress awaits further investigation.

Introduction

Genetics and lifestyle are two commonly acknowledged determinants of non-communicable chronic diseases in adults; however, early-life adaptations that result in long-term dysfunction are now recognized to be important as well, a concept articulated as the Developmental Origins of Health and Disease (DOHaD) hypothesis. One aspect of this hypothesis is that fetal exposure to an unfavorable intrauterine environment programs an individual to have a greater susceptibility later in life to non-communicable diseases, such as coronary heart disease [1]. The unfavorable environment may result from among others things maternal nutrient reduction (MNR), a common case of intrauterine growth restriction (IUGR) in the developing world [1] or placental dysfunction and/or preeclampsia [2]. Evidence from numerous epidemiological studies support the connection between fetal stress and adult disease. For instance, in a cohort in the United Kingdom, Barker and colleagues linked ischemic heart disease in adults to low birth weight [3].

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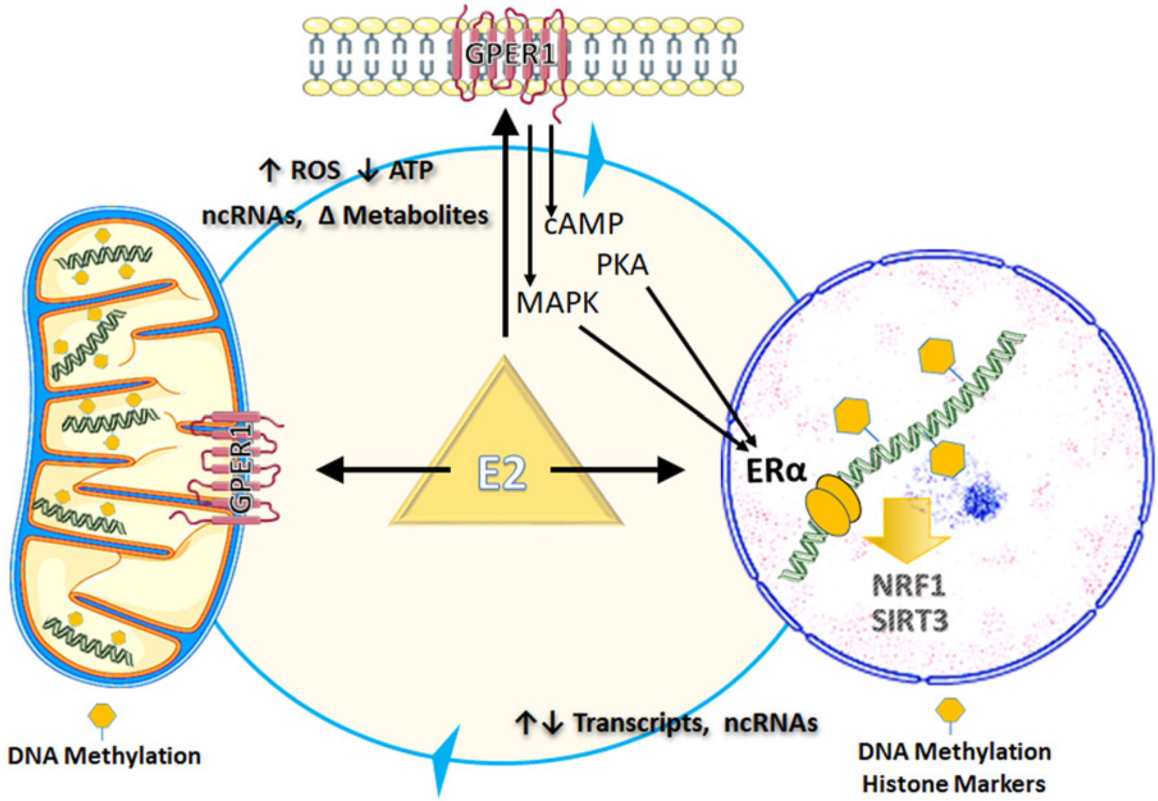

Figure 1. Mitochondrion-nucleus crosstalk in fetal imprinting

Mitochondria determine gene expression by affecting genomic DNA methylation and histone markers, with alterations in ROS, ATP, and metabolites playing a role. Aberrant expression of mitochondrial genes can further exacerbate mitochondrial dysfunction, synergistically contributing to stress-related epigenetic markings. Mitochondrial and genomic ncRNAs likely contribute to fetal programing also. The protective role of estrogen/estradiol (E2) in mitochondrial function and dynamics, operating via the estrogen receptor (e.g., ERα) and GPER1, at the nuclear and perhaps mitochondrial levels, adds another layer of complexity. Some of the content is adapted from Servier Medical Art (https://smart.servier.com/). See text for additional details.

Other studies have demonstrated a link between low birth weight and other chronic diseases, such as hypertension, diabetes, obesity, and atherosclerosis [4–9]. Numerous animal studies have affirmed this relationship as well, demonstrating in many cases an impact in particular of fetal stress on heart development [10–12]. In a remarkable study appearing recently in *Clinical Science*, Pereira et al. reported findings on the impact of MNR on the fetal heart using baboons [13]. Their findings are obviously of great interest given the fact that baboons are close evolutionary relatives to humans, but also for providing mechanistic insights into the programing process that underlies the DOHaD hypothesis.

IUGR and fetal heart remodeling: polytocous versus monotocous

The impact of MNR on the fetal heart has been studied experimentally using various animal models, such as rodents (e.g., mice, rats, and guinea pigs) [14–17], rabbits [18–20], and sheep [21,22]. These studies have revealed that with MNR the fetal heart undergoes structural remodeling, including cardiac hypertrophy with increased myocardial interstitial fibrosis, a reduced number of cardiomyocytes, and a reduction in microvascularization [10,23,24]. Ultrastructural changes observed involve shorter sarcomeres, mitochondrial rearrangements, and increased cytoplasmic volume of cardiomyocytes [19,23,25–27]. Altered gene expression related to oxygen and energy homeostasis have been reported [19,23], as well as cardiac mitochondrial complex II dysfunction and an increase in Sirt3 expression as perhaps a compensatory response [10,18]. Notably, the right ventricles of adult IUGR-born male rats were reported to exhibit reduced contractility as well as mitochondria that were more susceptible to a brief period of hypoxia [28]. Cardiac tissue of adult female mice that underwent *in utero* undernutrition had mitochondria that showed reduced respiration, although no effect on the total number of mitochondria or the levels of complexes I–IV were noted [29]. Other preclinical studies have provided evidence for reactive oxygen species (ROS) in the remodeling process, but the source of this ROS is not well established [2,11].

While these studies have provided an understanding of the impact of IUGR on heart development, a drawback is that the pregnancies are polytocous and thus the pregnant dams have a greater nutritional burden than the human, which is monotocous. For this reason, and genetically, the baboon model of MNR on fetal development is anticipated to provide findings that are more relevant to humans and are more translational. A series of studies reported the effect on the adult offspring of feeding baboon mothers 70% of the control ad libitum diet during pregnancy. They showed that IUGR caused premature cardiac aging with reduced systolic and diastolic function [30]. Both right and left ventricles were affected with sex differences noted in the rates and directions of right ventricular (RV) volume changes and decreased end-systolic RV sphericity [31]. The extent of adult RV dysfunction was indicative of the combined hemodynamic effects of impaired LV function and pulmonary hypertension [32]. These data show that alterations in cardiac function in IUGR baboons make them more susceptible to early cardiac aging and cardiovascular diseases. Examination of exhaled breath volatile organic compounds of juvenile IUGR baboon offspring with documented cardiometabolic dysfunction provided evidence for sex-dependent altered metabolism [33]. In addition, Muralimanoharan et al. reported sex-dependent dysregulations in cardiac structure, miRNA expression, and lipid metabolism in near-term IUGR baboon fetuses [34]. Only IUGR male fetuses exhibited LV myocardial fibrosis, the extent of which was inversely correlated to birthweight. Sex-related differences in miRNAs were partially explained by differential expression of upstream transcription factors [34]. Lipidomics analysis of fetal cardiac tissue showed that there was a net increase in plasmalogens and diacylglycerol, along with a decrease in phosphatidylcholines and triglycerides.

Mitochondrial dysfunction: sex differences

The recent study of Pereira et al. in *Clinical Science* is noteworthy for its detailed analysis of the impact of MNR on LV mitochondria and the finding of sexual dimorphism in the process [13]. Analyses were performed on near-term fetuses (0.9 gestation) of mothers fed a control diet or who received 70% of control nutrition for most of the pregnancy (from 0.16 gestation). MNR caused a 2-fold increase in fetal LV mitochondrial DNA (mtDNA) copy number in female fetuses but not males. Moreover, there were 21 mitochondrial transcripts that were differentially expressed between control and MNR fetuses, with the majority (85%) being up-regulated. Most of these encoded subunits of the mitochondrial oxidative phosphorylation system and adenosine triphosphate (ATP) synthase proteins. The changes were more notable in female MNR fetuses. A number of increases were noted in LV mitochondrial protein levels from NMR fetuses, but surprisingly nearly all were restricted to males. These included complex I subunit NDUFB8, complex III subunit UQCRC1, and cytochrome *C* (CYT C), which can contribute to apoptosis. Also increased in male MNR fetuses were outer membrane channel VDAC1 and cyclophilin D, a modulator of the mitochondrial permeability transition pore (mPTP) and related cell death. In both sexes, MNR was associated with an increase in mitochondrial fission 1 protein (Fis1), whereas the mitochondrial fusion protein MFN2 was increased by MNR in females only.

It is difficult to interpret what the particular pattern of altered mitochondrial proteins represents, particularly in the context of what has been reported for differential mitochondrial protein expression in other studies, for instance related to aging [35–37]. However, Pereira et al. did observe that LV mitochondrial complex I and complex II/III activities were significantly decreased by MNR in males and in male and female fetuses, respectively [13]. Consistent with that, LV ATP content was decreased by 73% in MNR fetuses, mostly in males. The alterations in mitochondrial protein expression profile may simply reflect dysfunctional mitochondria or have caused disturbances in mitochondrial respiratory complex or supercomplex assembly. Notably, the latter has been associated with ROS generation [37]. Pereira et al. did report indirect evidence for increased oxidative stress and lipid peroxidation [13]. LV malondialdehyde (MDA) levels were increased by MNR, most notably in male fetuses. In contrast reduced glutathione (GSH) levels were 2.4-fold greater with MNR in females than males, indicating greater protection against oxidative stress or lower levels of such stress. In addition, while sparse and disarranged cristae were seen with MNR in mitochondria of both sexes, these changes were more prominent in males. However, as seems to be the case with aging [38], alterations in mitochondrial function and increased ROS generation may not be a direct driving force for phenotypic changes associated with MNR in either the fetus or adult. Additional studies in this regard are warranted. In any case, mitochondrial alterations induced by MNR were more severe in male fetuses, suggesting that females are protected

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somehow. The major female sex hormone estradiol is known to regulate mitochondrial metabolism and morphology via both nuclear and mitochondrial-mediated events, including activation of nuclear respiratory factor-1 (NRF-1) [39]. Recently, evidence was reported that activation of G protein-coupled estrogen receptor 1 (GPER1) by estradiol protects H9c2 cardiomyoblasts from cell death due to oxidative stress by preserving mitochondrial structure and function, and delaying the opening of mPTP [40]. There is also the intriguing possibility that GPER1 may be located in mitochondria or endoplasmic reticulum [41].

Mitochondria in fetal imprinting

Population and preclinical studies support the conclusion that epigenetics forms the link between *in utero* stress, such as MNR, hypoxia, maternal obesity, and hypertension, and the adult phenotype [1,2,23,42]. Epigenetics includes traditional factors, histone modifications (acetylations and methylations) and DNA methylations, as well as non-coding RNAs (ncRNAs). The driving force for these changes has been ascribed to increased glucocorticoids and/or activity of the hypothalamic-pituitary-adrenal axes, in the case of MNR, as well as oxidative stress [1,2,42]. The study by Pereira et al. raises the possibility that mitochondria are master regulators of both the remodeling and imprinting processes. Mitochondria are anticipated to affect genomic DNA methylation and histone acetylation, methylation, and phosphorylation via either coupling of the folate and methionine cycles or alterations in tricarboxylic acid (TCA) cycle metabolites, including α-ketoglutarate, succinate, and fumarate [42] (Figure 1). Increased ROS and decreased ATP are likely contributors as well. Mitochondrial dysfunction in turn induces changes in the expression of mitochondrial genes that can further exacerbate both the dysfunction and aberrant epigenetic imprinting. Mitoepigenetics, including mitochondrial DNA methylation, and the involvement of both mitochondrial and genomic ncRNAs in mitochondrial functions and nucleus–mitochondria crosstalk, are rapidly developing areas of investigation that likely contribute to fetal programing also [43,44]. The protective role of estrogen/estradiol (E2) in mitochondrial function and dynamics, operating at the nuclear and perhaps mitochondrial levels, adds another layer of complexity [39].

Conclusions and future directions

The study by Pereira et al. [13] is the first report that MNR impacts fetal cardiac LV mitochondria, in a close evolutionary relative to humans, and in a sex-dependent fashion. The basis for the protection in females needs to be defined. Network or pathway analyses are needed to define the underlying molecular events that account for these changes, including the role of ncRNAs. Further studies are needed to unravel the processes by which fetal cardiac mitochondria have epigenetic impacts that determine the adult susceptibility to cardiovascular disease.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

E2, estrogen/estradiol; GSH, glutathione; GPER1, G protein-coupled estrogen receptor 1; LV, left ventricular; MDA, malondialdehyde; MNR, maternal nutrient reduction; mPTP, mitochondrial permeability transition pore; ncRNA, non-coding RNA; NRF-1, nuclear respiratory factor-1; TCA, tricarboxylic acid.

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