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Research report

Mitochondrial bioenergetics and oxidative status disruption in brainstem of weaned rats: Immediate response to maternal protein restriction

Diorginis José Soares Ferreira [a,b], Anderson Apolônio da Silva Pedroza [b], Glauber Ruda Feitoza Braz [b], Reginaldo Correia da Silva-Filho [b], Talitta Arruda Lima [b], Mariana Pinheiro Fernandes [b], Sonia Q. Doi [c], Claudia Jacques Lagranha [a,b,*]

[a] Neuropsychiatry and Behavioral Science Graduate Program, Federal University of Pernambuco, 50670–901, Brazil
[b] Laboratory of Biochemistry and Exercise Biochemistry, Department of Physical Education and Sports Science, Federal University of Pernambuco–CAV, Vitória de Santo Antão 55608–680, Brazil
[c] Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

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ABSTRACT

Mitochondrial bioenergetics dysfunction has been postulated as an important mechanism associated to a number of cardiovascular diseases in adulthood. One of the hypotheses is that this is caused by the metabolic challenge generated by the mismatch between prenatal predicted and postnatal reality. Perinatal low-protein diet produces several effects that are manifested in the adult animal, including altered sympathetic tone, increased arterial blood pressure and oxidative stress in the brainstem. The majority of the studies related to nutritional programming postulates that the increased risk levels for non-communicable diseases are associated with the incompatibility between prenatal and postnatal environment. However, little is known about the immediate effects of maternal protein restriction on the offspring's brainstem. The present study aimed to test the hypothesis that a maternal low-protein diet causes tissue damage immediately after exposure to the nutritional insult that can be assessed in the brainstem of weaned offspring. In this regard, a series of assays was conducted to measure the mitochondrial bioenergetics and oxidative stress biomarkers in the brainstem, which is the brain structure responsible for the autonomic cardiovascular control. Pregnant *Wistar* rats were fed *ad libitum* with normoprotein (NP; 17% casein) or low-protein (LP; 8% casein) diet throughout pregnancy and lactation periods. At weaning, the male offsprings were euthanized and the brainstem was quickly removed to assess the mitochondria function, reactive oxygen species (ROS) production, mitochondrial membrane electric potential ($\Delta\Psi$m), oxidative biomarkers, antioxidant defense and redox status. Our data demonstrated that perinatal LP diet induces an immediate mitochondrial dysfunction. Furthermore, the protein restriction induced a marked increase in ROS production, with a decrease in antioxidant defense and redox status. Altogether, our findings suggest that LP-fed animals may be at a higher risk for oxidative metabolism impairment throughout life than NP-fed rats, due to the immediate disruption of the mitochondrial bioenergetics and oxidative status caused by the LP diet.

1. Introduction

It is well known that mitochondria play several functions in eukaryotic cells. Considered as the major energy producer, by means of ATP formation, mitochondria are also the main source of reactive oxygen species (ROS) (Halliwell and Gutteridge, 2007). In physiologic conditions, mitochondrial ROS production is a tightly adjusted mechanism required for a plethora of cellular processes (e.g., cell signaling, gene expression, cellular growth and apoptosis) (Fukai and Ushio-Fukai, 2011). Since mitochondrion also has a high antioxidant capacity (Figueira et al., 2013), its dysfunction can

Abbreviations: EDTA, ethylene diamine tetra acetic acid; G6PDH, gucose-6 phosphate dehydrogenase; GSSG, oxidized glutathione; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione-S-transferase; LP, low-protein; NAD(P)H, nicotinamide adenine dinucleotide (phosphate) reduced; NP, normoprotein; RVLM, rostral ventrolateral medulla; SOD, superoxide dismutase; TRIS, tris (hydroxymethyl) aminomethane; TCA, trichloroacetic acid

* Corresponding author at: Núcleo de Educação Física e Ciências do Esporte, Rua Alto do Reservatório, s/n, Bela Vista, CEP: 55608–680 Vitória de Santo Antão, PE, Brazil.
E-mail address: lagranha@hotmail.com (C.J. Lagranha).

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trigger an imbalance between ROS generation and removal, a disorder that has been associated with several diseases, including heart failure (Munzel et al., 2015), neurogenic diseases (Abramov et al., 2010; Kwon et al., 2004; Palomo and Manfredi, 2015) and hypertension (Chan, 2006).

Previous studies have shown that into the brainstem structure, specifically in the rostral ventrolateral medulla, mitochondrial impairment (e.g., disruption in complexes I and III of the electron transport chain) as well as decrease in the antioxidant capacity contributes to neurogenic hypertension via sympathoexcitatory overstimulation (Chan et al., 2009; Hirooka et al., 2011). Although a central oxidative imbalance has been described in several hypertension models (Chan et al., 2009; Nishihara et al., 2012; Oliveira-Sales et al., 2010), the cause of the imbalance is yet unclear.

Studies involving fetal programming have demonstrated a close relationship between nutritional insults in early life and later propensity for non-communicable diseases, including hypertension (Barker et al., 1989; Barros et al., 2015; de Brito Alves et al., 2014). One of the hypotheses to explain this relationship is the predictive adaptive response, which postulates that early adversities will provide to the organism under development a predictive adaptation in response to cues from the environment, resulting in anticipated phenotype adaptation for immediate survival and improved success in an adverse environment (Gluckman et al., 2005b; Nettle et al., 2013). However, the initial adaptation will be only temporary and the disparity between the prenatal prediction and postnatal reality will result detrimental to the organism, leading to an increased risk of non-communicable diseases in adulthood (Martin-Gronert and Ozanne, 2012; Wang et al., 2012; Warner and Ozanne, 2010).

Recently, studies on adult rats born from mothers fed a low-protein diet (LP) during gestation and lactation, showed that these animals have an increase in arterial blood pressure and in the cardiovascular sympathetic tone (Barros et al., 2015). Based on the fact that central oxidative stress might lead to those cardiovascular responses, we have previously studied the oxidative stress in the brainstem of animals using an experimental model published by Barros et al. We found a significant increase in oxidative damage to lipids in conjunction with a marked decrease in antioxidant defenses (Ferreira et al., 2015). Further investigations have shown that in animals at 30 days of age, born to an LP-fed mother, the arterial blood pressure was not affected, despite the higher sympathoexcitatory responses to peripheral chemoreceptor stimulation (de Brito Alves et al., 2014).

Due to the suggested relationship between oxidative imbalance and several pathologies, various studies have been conducted to clarify how the adult oxidative balance can be affected by early nutritional insult (Ferreira et al., 2015; Simmons, 2006; Tarry-Adkins et al., 2013). However, studies addressing the immediate adaptive response to nutritional insult are still scarce. Our present work was designed to test the hypothesis that a maternal LP diet during gestation and lactation periods affects negatively the mitochondrial bioenergetics and oxidative status in the brainstem of offsprings that this begins immediately after exposure to the maternal protein restriction.

2. Results

2.1. Mitochondrial function

Total mitochondrial protein content was not significantly different between the groups (NP 20.2 ± 6.4 μg/μl x LP 18.3 ± 3.4 μg/μl). However, the brainstem mitochondrial function was affected by the maternal LP diet. In the basal state of mitochondria respiration, LP-weaned rats showed a 66% higher O_2 consumption (10.75 ± 1.6 nmolO_2/mL) when compared to the NP-weaned rats (6.4 ± 1.07 nmolO_2/mL) (Fig. 1). In addition, when mitochondria were stimulated with ADP, the LP-weaned rats were unable to increase respiration to a rate equivalent to the NP-weaned rats, showing a 2.8-(30.6 ± 10.5 nmolO_2/mL) and 11.9-fold increase (77.12 ± 14.33 nmolO_2/mL), respectively (Fig. 1). Thus, LP-weaned animals showed a lower respiratory control ratio (5.83 ± 0.73) when compared to NP-weaned rats (8.30 ± 0.53) (Fig. 1 insert).

Assessing additional mitochondrial functions, we found that the LP-weaned rats produced 228% more ROS than NP-weaned rats (a.u. of fluorescence: LP, from 40.4 ± 1.8 to 95.2 ± 4.3; NP, from 27.4 ± 1.6 to 127 ± 7.5) (Fig. 2(A)), while the $\Delta\Psi_m$ decreased 33% in LP-weaned rats (a.u. of fluorescence: LP, from 2280.6 ± 380.6 to 1528.0 ± 255.0; NP, from 4317 ± 260 to 1683.6 ± 101.5) (Fig. 2(B)).

2.2. Oxidative stress biomarkers

Two oxidative stress biomarkers, malondialdehyde and carbonyl levels were measured. The results have shown that these biomarkers were affected differently by the perinatal LP diet in the weaned rats. While carbonyl levels were augmented by 123% (LP: 26.14 ± 3.4 x NP: 11.70 ± 1.27 μM/mg prot) in LP-weaned rats (Fig. 3(B)), no significant difference was observed in MDA levels between the LP and NP groups (Fig. 3(A)).

2.3. Enzymatic antioxidant system

Oxidative stress can be induced either by an increase in pro-oxidative agents or by a decrease in antioxidant defense. We measured several antioxidant enzymes and the majority showed no significant difference between LP and NP groups (SOD: NP 5.10 ± 0.77 *vs* LP 5.74 ± 0.18 U/mg protein, n=5–8; CAT: NP 1.17 ± 0.03 *vs* LP 1.11 ± 0.03 U/mg protein, n=5–8; GPx: NP 1.26 ± 0.03 *vs* LP 1.21 ± 0.09 U/mg protein, n=5; G6PDH: NP 0.44 ± 0.07 *vs* LP 0.33 ± 0.01 U/mg protein, n=5). Except for the GST activity, which decreased 47% in LP compared to NP (NP 13.9 ± 0.84 *vs* LP 7.3 ± 0.8 U/mg protein, n=5) (Fig. 4(E)).

2.4. Non-enzymatic antioxidant system

The antioxidant defense is constituted by enzymatic and non-enzymatic molecules. Therefore, we also measured the reduced glutathione (GSH), a major antioxidant non-enzymatic, molecule, which is responsible for counterbalancing excess pro-oxidative agents. Our data showed that LP-weaned rats had a 21% decrease

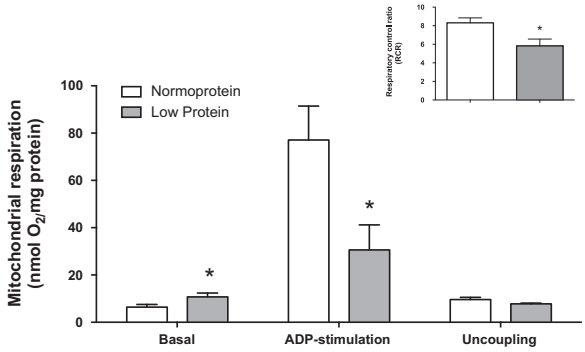

Fig. 1. Mitochondrial respiration capacity. Mitochondrial O_2 consumption in basal state, stimulated with ADP, uncoupled with carbonyl cyanide m-chlorophenyl hydrazone and the respiratory control ratio in brainstem of 22 day old male rats born to dams fed through perinatal period with either normo- (17% casein) or low-protein (8% casein) diet; n=4–8. Values are expressed as means ± SEM. ($^*p \leq 0.05$ unpaired Student's *t* test).