Maternal protein restriction compromises myocardial contractility in the young adult rat by changing proteins involved in calcium handling

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Maternal protein restriction (MPR) during pregnancy is associated with increased cardiovascular risk in the offspring in adulthood. Therefore, we hypothesized that maternal protein restriction might increase the afterload, resulting in cardiac hypertrophy and dysfunction (27). In addition to the impairment of heart function caused by the elevated afterload, maternal protein restriction might be associated with cardiac dysfunction in the offspring after birth due to fetal reprogramming (3). Indeed, one study showed that maternal protein restriction induced considerable depression of the heart contractile function in the offspring, most likely due to loss of cardiomyocytes caused by increased apoptosis (8). According to other authors, maternal protein restriction might induce alterations in the functional and metabolic components of the neonatal heart, including changes in the gene and protein expression involved in the homeostasis of the myoplasmic calcium and glucose metabolism, which might contribute to the depression of the heart contractile function (28, 29).

According to Barker’s (4) theory, maternal protein restriction might predispose the offspring to developing cardiovascular diseases in adulthood. Therefore, we hypothesized that the depression of the heart contractile function that occurs in neonatal hearts of rats subjected to protein restriction, as mentioned above, could be maintained in young rats and might be associated with changes of contractile machinery and higher cardiovascular diseases in adulthood. Thus the aim of this study was to assess cardiac contractility and the proteins involved in cardiac calcium homeostasis in the young offspring of rats subjected to protein restriction. For that purpose, the diet suggested by Teodósio et al. (30) was used, which is based on a nutrition survey that reproduces in rats a situation similar to the protein malnutrition that still exists in some areas of Northeastern Brazil.

MATERIALS AND METHODS

Diets

Two types of diets were used for this study. The diet used to induce malnutrition was the regional basic diet (RBD), as described previously (9, 24, 30). The RBD consists of grains (Phaseolus vulgaris), sweet potatoes (Ipomea batatas), jerked beef, and manioc flour (Manihot esculenta). These components were macerated, molded into “pellets”, and heated to 50°C. The RBD pellets provide a total of (g/g%) proteins 9, carbohydrates 78, lipids 1.1, fiber 7, minerals 4, sodium chloride 0.17, and kilocalories 356. No vitamin supplement was added. Moreover, diet was supplemented with 0.2% (g/g) sodium chloride to equal the amount with standard diet. The control diet was a commercial standard diet (Labina). The standard diet contains the...
following content (g/g%): protein 23, carbohydrates 41, lipids 2.5, fibers 9, minerals 8, sodium chloride 0.37, and kilocalories 278.

**Animals and Experimental Groups**

This study used Wistar rats bred at the animal facility of the Department of Physiology and Pharmacology, Center of Biological Sciences, Federal University of Pernambuco. The animals were kept in cages under controlled temperature and a 12:12-h light-dark cycle, with free access to water and food.

The care and use of animals were in accordance with the NIH guidelines, and all experiments were conducted in compliance with the ethical standards described in the 1964 Declaration of Helsinki and the Brazilian Guidelines for the Care and Use of Animals for Scientific and Educational Purposes. All protocols were approved by the Animal Experimentation Ethics Commission, Federal University of Pernambuco (no. 23076.008507/2010-16).

The animals were allocated to two experimental groups, control or maternal protein restriction (MPR), as follows.

**Control group.** The mothers were fed the standard diet (Labina) during the premating and mating periods, pregnancy, and lactation. After weaning (day 21), the male offspring was separated, fed the same standard diet, and subjected to the experimental protocol at age 3 mo old.

**MPR group.** The mothers were fed the standard diet in the premating period, and RBD during the mating period, pregnancy, and lactation. After weaning, the male offspring was fed the standard diet until age 3 mo old, when it was subjected to the experimental protocol.

**Biometry Assessment**

The animals were weighed at birth, immediately after weaning, and at 3 mo old (onset of the experimental protocol). In addition, following dissection of the left papillary muscle, the right and left ventricles and liver were separated and weighed.

**Hemodynamic Measurements**

Arterial pressure was measured indirectly in conscious 3-mo-old rats by using noninvasive tail-cuff plethysmography method (ITC Life Science noninvasive blood pressure, version 1.35). One week before, all rats were kept in the restrainers 15 min/day, in a warm and quiet room and conditioned to cuff inflation-deflation cycles to habituate to this protocol. At the day of procedure, rats were kept restrained for 5-10 min and conditioned to cuff inflation-deflation cycles prior to the recordings. Next, systolic pressure was measured, and the average values of three measurements were recorded and analyzed, as previously reported (2).

Thereafter, rats were anesthetized with urethane (1.2 g/kg ip), the left carotid artery was dissected, and a polyethylene catheter (PE-50, Clay-Adams) filled with heparinized saline (50 IU/ml) was inserted. Then the catheter was introduced into the left ventricle to measure the intraventricular pressure and calculate the positive and negative derivatives of intraventricular pressure (+dP/dt max and −dP/dt max, respectively). Next, the catheter was placed in the carotid artery again, and the pressure pulse was evaluated to establish whether aortic valve damage had occurred. The rats that exhibited increased pressure pulse with reduced diastolic pressure and absence of dicrotic notches were discarded. The records were made over 30 min using a pressure transducer (model TSD 104A) connected to a preamplifier and a data-acquisition system (model MP30, BIOPAC System, Santa Barbara, CA).

**Isolated Papillary Muscles**

After the hemodynamic evaluation, the hearts of the animals in both groups were quickly removed and perfused through the aortic stump for dissection of the papillary muscles on the anterior and posterior walls of the left ventricle. To measure the isometric tension, the papillary muscles were mounted and kept in a 20-ml organ bath containing Krebs-Henseleit solution (KHS) (in mM: 118 NaCl, 1.25 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 23 NaHCO₃, and 11 glucose) at 26 ± 0.5°C to prevent hypoxia (29) and at pH 7.4, being continuously aerated with 95% O₂ and 5% CO₂. The specimens were connected to an isometric transducer (TSD125-Biopac Systems) and stimulated by means of rectangular pulses (10–15 V, 12 ms long) applied through a pair of platinum electrodes placed along the full muscle length. The standard stimulation rate was 0.5 Hz. The force developed during contractions was measured as grams per milligram (developed force divided by the muscle weight). Correction for the papillary weight was performed to normalize the data from different sized samples. Resting tension was adjusted to produce maximal contractions (Lmax), and following a 45- to 60-min period of stabilization, the experimental protocols were begun as follows.

**Steady-state contractility.** Developed force, time to peak force (time elapsed to reach peak of developed force) and relaxation time (time to relax 50% of developed force), and time derivatives of force change (+dF/dt max and −dF/dt max) were assessed.

**Inotropic response to β-adrenergic receptor (β-AR) stimulation.** Force and time derivative of force were also assessed under increasing isoproterenol concentrations (10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³, and 10⁻² M) to evaluate the response of myocardial machinery to β-adrenergic receptor activation.

**Contractions dependent on calcium influx.** The muscles were kept in calcium-free solution with caffeine (5 mM) during 10 min to deplete calcium in the sarcoplasmatic reticulum (SR), followed by two washings and 10 min without any electrical stimulus. To induce new contractions, the calcium-free solution was replaced by modified Krebs solution (with calcium 1.25 mM) and immediately followed by the onset of electrical stimulation. This post-rest contraction, analyzed in relation to steady state, was induced before and after blockade of the L-type calcium channel by adding verapamil (10 μM) to the bath, indicating the participation of transsarcolemal calcium influx on the myocardial muscle contraction (18).

**Post-pause potentiation of force.** This protocol was used to indirectly evaluate the SR function, as previously described (31). In cardiac muscle, the contractions occurring after short pauses are potentiated in the rat, post-rest cardiac muscle contractions increase in force as the rest period increases in length (22). Contractions were measured before and after pauses of stimulation with increased durations (15, 31, and 60 s) and results are expressed as relative potentiation (amplitude of post-pause contraction divided by contraction before pause).

**Western Blot Analysis**

The hearts of the control and MPR rats were homogenized, and protein (50 μg) was separated using 10% SDS-PAGE gel to quantify SR Ca²⁺-ATPase (SERCA-2a) and sodium-calcium exchanger (NCX). The low-molecular-weight proteins total phospholamban (PLB) and its phosphorylated form at serine 16 (PLB-Ser¹⁶) were separated using 15% SDS-PAGE gel. The proteins were transferred to nitrocellulose membranes (Amersham), blocked with Tris-buffered solution and 5% powdered nonfat milk, and incubated with mouse monoclonal antibodies to SERCA2a (1:1,000, Thermo Scientific, Rockford, IL), NCX (1:1,000, Thermo Scientific), PLB (1:1,000, Thermo Scientific), and PLB-Ser¹⁶ (1:5,000, Thermo Scientific). After rinsing with Tris-buffered solution, the membranes were incubated with anti-mouse IgG antibody (1:5,000, Sigma Chemical, St. Louis, MO). After thorough rinsing, the immune complexes were detected using a chemiluminescence system (ECL Plus Amersham International, Little Chalfont, UK) and film (Hyperfilm ECL International). Primary antibodies were diluted in albumin and secondary antibodies in nonfat milk (both in Tris-buffered solution). The immunoblot signals were quantified using ImageJ software (V1.56, Public do-
there was a major restriction on weight gain of offspring rats subjected to MPR, when the heart weights were indexed by the length of their tibia, no difference was found suggesting that there was no hypertrophy. Finally, the protein restriction during pregnancy caused a significant reduction in liver weight of the offspring in adulthood. However, because the body weight and tibia length also reduced, both liver-to-body weight and liver-to-tibia length ratios were similar between groups.

### Hemodynamic Parameters

Systolic blood pressure, as indirectly measured with the animals awake, was higher in the MPR group compared with control (Table 2). However, when measured with the animals anesthetized, no difference was found for arterial or intraventricular pressures (Table 2). Ventricular hemodynamic analysis only evidenced changes on the positive and negative derivatives of the left intraventricular pressure that were higher in the MPR group.

### Effects of Maternal Protein Restriction on the Contractility of the Cardiac Muscle

Although maternal protein restriction caused elevation of the systolic arterial pressure in the offspring, no significant changes were found in the force developed by the isolated papillary muscles (Fig. 1A). However, muscles from MPR rats exhibited longer time-to-peak force and time to relaxation compared with controls (Fig. 1, B and C). In contrast to the in vivo data, the positive and negative force derivatives (+dF/dt\(\max\) and \(-dF/dt\(\max\), respectively) were reduced in the MPR group (Fig. 1, D and E).

We also investigated whether maternal protein restriction altered the inotropic response of the offspring through the isoproterenol concentration-response curve (Fig. 2A). The results showed that the positive inotropic response to isoproterenol was significantly depressed in the MPR group. The lusitropic effect of isoproterenol was slightly reduced in MPR, but without statistical significance (data not shown).

To assess the functional relevance of the transsarcolemmal calcium influx on cardiac muscle contraction in the MPR

### Table 2. Hemodynamic parameters corresponding to the control animals and the young offspring of rats subjected to protein restriction during pregnancy (MPR)

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 12)</th>
<th>MPR (N = 12)</th>
</tr>
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<tbody>
<tr>
<td>Pressure indirect measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>126 ± 2</td>
<td>147 ± 2*</td>
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<tr>
<td>Pressure direct measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>103 ± 4</td>
<td>102 ± 7</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>54 ± 4</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>72 ± 4</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>102 ± 7</td>
<td>103 ± 5</td>
</tr>
<tr>
<td>LVDp, mmHg</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>+dP/dt(\max), mmHg/s</td>
<td>3,685 ± 282</td>
<td>5,678 ± 321*</td>
</tr>
<tr>
<td>-dP/dt(\max), mmHg/s</td>
<td>4,782 ± 183</td>
<td>-6,824 ± 320*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>302 ± 9</td>
<td>300 ± 12</td>
</tr>
</tbody>
</table>

Values for indirect estimation of the systolic arterial pressure (SAP) and direct measurement of SAP, diastolic arterial pressure (DAP), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVDp), maximal rate of pressure rise (+dP/dt\(\max\)) and decline (–dP/dt\(\max\)), and heart rate (HR) are expressed as means ± SE. *P < 0.05, MPR vs. control. Data analyzed by means of unpaired Student’s t-test.

### RESULTS

#### Body Weight Gain and Heart Weight

Maternal protein restriction caused a delay in the weight gain of the offspring (Table 1). The birth weight was lower in the MPR. Upon weaning, on day 21, the body weight was still lower in the MPR group, remaining until day 90. Furthermore, analysis of heart weight and its left and right chamber as absolute value indicated significant decrease in the MPR group compared with controls, while heart weight and left ventricular weight indexed to body weight were increased. However, since

### Table 1. Biometric data corresponding to the control animals and the young offspring of rats subjected to protein restriction during pregnancy (MPR)

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 24)</th>
<th>MPR (N = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth, g</td>
<td>7.36 ± 0.09</td>
<td>3.51 ± 0.07*</td>
</tr>
<tr>
<td>21 days, g</td>
<td>64.5 ± 1.3</td>
<td>42.4 ± 2.3*</td>
</tr>
<tr>
<td>90 days, g</td>
<td>357.8 ± 7.7</td>
<td>267.2 ± 2.7*</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>1,032 ± 24</td>
<td>874 ± 23*</td>
</tr>
<tr>
<td>Heart-to-body weight ratio, mg/g</td>
<td>2.82 ± 0.05</td>
<td>4.08 ± 0.09*</td>
</tr>
<tr>
<td>Heart-to-tibia length ratio, mg/mm</td>
<td>26.84 ± 0.37</td>
<td>26.67 ± 0.54</td>
</tr>
<tr>
<td>Right ventricle, mg</td>
<td>232 ± 8.7</td>
<td>188 ± 10.9*</td>
</tr>
<tr>
<td>Right ventricle-to-body weight ratio, mg/g</td>
<td>0.60 ± 0.02</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>Right ventricle-to-tibia length ratio, mg/mm</td>
<td>5.82 ± 0.19</td>
<td>5.98 ± 0.35</td>
</tr>
<tr>
<td>Left ventricle, mg</td>
<td>586 ± 13</td>
<td>466 ± 20*</td>
</tr>
<tr>
<td>Left ventricle-to-body weight ratio, mg/g</td>
<td>1.48 ± 0.04</td>
<td>1.85 ± 0.15*</td>
</tr>
<tr>
<td>Left ventricle-to-tibia length ratio, mg/mm</td>
<td>14.70 ± 0.22</td>
<td>14.79 ± 0.64</td>
</tr>
<tr>
<td>Liver, mg</td>
<td>13,949</td>
<td>6,339*</td>
</tr>
<tr>
<td>Liver-to-body weight ratio, mg/g</td>
<td>0.22 14.79</td>
<td>6,824*</td>
</tr>
<tr>
<td>Liver-to-tibia length ratio, mg/mm</td>
<td>36.6 ± 1.4</td>
<td>32.6 ± 2.2</td>
</tr>
<tr>
<td>Liver-to-tibia length ratio, mg/mm</td>
<td>333 ± 23</td>
<td>331 ± 17</td>
</tr>
</tbody>
</table>

Values expressed as means ± SE. *P < 0.05, MPR vs. control. Data analyzed by means of unpaired Student’s t-test.

### Drugs Used

The following drugs were used: heparin (Roche Q.F.S.A., Brazil), anhydrous caffeine (B. Herzog, Brazil), urethane, bovine serum albumin, and verapamil (Sigma Chemical). The other reagents used were analytical grade and purchased from Sigma and Merck (Germany) or Reagen (Brazil).

### Data Analysis and Statistics

All the results are expressed as means ± standard error of the mean (SE), and all data passed by D’Agostino and Pearson’s normality test. Therefore, inferential analysis was made by parametric tests such as unpaired Student’s t-test and one-way or two-way ANOVA. When the results on ANOVA were statistically significant, the Bonferroni post hoc test was used to compare individual means. The significance value was set to P value < 0.05. The data were analyzed and the graphics plotted using the software GraphPad Prism (version 5.0, GraphPad Software).

### Main

As an internal control, the same membrane was used to measure the expression of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) using mouse monoclonal antibody to GAPDH (1:5,000, Abcam, Cambridge, MA). After rinsing, the membrane was incubated with anti-mouse antibody (1:5,000, Ensaio designers, Hines Drive, Ann Arbor, MI). The phosphorylation level of PLB was estimated by the calculation of the PLB-Ser16/total PLB ratio. All the reagents used in Western blot transfer were purchased from Sigma Chemical.

### RESULTS

#### Body Weight Gain and Heart Weight

Maternal protein restriction caused a delay in the weight gain of the offspring (Table 1). The birth weight was lower in the MPR. Upon weaning, on day 21, the body weight was still lower in the MPR group, remaining until day 90. Furthermore, analysis of heart weight and its left and right chamber as absolute value indicated significant decrease in the MPR group compared with controls, while heart weight and left ventricular weight indexed to body weight were increased. However, since
Control (11) observed a reduction in post-rest contractions, suggesting a transitory effect to the pause period. However, in the MPR group we noted a greater effect was noted in the MPR group, suggesting that changes might be related to impairment of the SR, as suggested by the data obtained through contractions without verapamil (Fig. 3, right). A greater effect was noted in the MPR group compared with control. However, after treatment with verapamil, these twitches decreased in both groups to the same level. When comparing the difference between post-rest contraction with or without verapamil (Fig. 3, right), a greater effect was noted in the MPR group, suggesting that was an increased participation of transsarcolemmal calcium influx in myocardial mechanics.

Additionally, as shown in Fig. 2B, the post-pause contraction protocol evidenced that the papillary muscles from control rats exhibit significant potentiation of force, directly proportional to the pause period. However, in the MPR group we observed a reduction in post-rest contractions, suggesting a reduction in sarcolemmal calcium influx in isolated papillary muscles.

The results described show that although maternal protein restriction did not alter the force developed by the isolated papillary muscles of the offspring, it promoted increased contraction-relaxation cycle duration and depressed time derivative of force, and reduced response to β-AR stimulation. Those changes might be related to impairment of the SR, as suggested by the data obtained through contractions after short pauses of stimulation (22). The amounts of some of the main proteins

Fig. 1. Effects of maternal protein restriction (MPR) on the myocardial mechanics in adult offspring. Parameters of developed force (A), time to peak force (B) and to relax 50% of developed force (C), and maximal positive (+dF/dtmax) and negative (−dF/dtmax) rates of force development (D, E) were assessed in isolated papillary muscles from control (n = 11) and MPR group (n = 8). Bars represent means ± SE, and numbers of animals are in the parentheses. *P < 0.05, MPR vs. control, by unpaired Student’s t-test.

Fig. 2. Myocardial inotropic response to isoproterenol and pause of stimuli. Effects of increasing concentrations of isoproterenol on the developed force of isolated papillary muscles from control and MPR groups (A). Inotropic effect is evidenced by the percentage of force change in relation to baseline conditions. B: effect of increasing pause durations (in s) on the relative contraction developed by papillary muscles. Post-pause contractions are expressed as a ratio between the contractions before and after the respective pause. The results are expressed as means ± SE, and numbers of animals are in the parentheses. *P < 0.05, MPR vs. control, by two-way ANOVA followed by Bonferroni post hoc test.

Fig. 3. Effects of MPR on the contraction dependent on transsarcolemmal calcium influx. Left: the first contraction obtained after 10 min without stimulation in calcium-free solution containing caffeine (5 mM) followed by calcium replacement and stimuli restart was assessed in papillary muscles from control and MPR group and represented as the percentage of the respective steady-state twitch. KHS: Krebs-Henseleit solution. Protocols were performed also in the presence of verapamil (Verap) 10 μM to block L-type calcium channels. Right: represents the contraction dependent on transsarcolemmal calcium influx that was sensible to blockage with verapamil, that is, the difference (delta) between that contraction without and with verapamil incubation. The results are expressed as means ± SE, and numbers of animals are in the parentheses. *P < 0.05 by 2-way ANOVA followed by Bonferroni post hoc test, and #P < 0.05 by unpaired Student’s t-test.
involved in the regulation of calcium homeostasis, and thus in myocyte contractility, were assessed through Western blot (Fig. 4). Corroborating the functional data, MPR promoted reduction of cardiac SERCA-2a protein content in offspring. In addition, although total PLB was decreased, its phosphorylation level evaluated by the PLB-Ser16/total PLB ratio was slightly reduced. Finally, the protein expression of NCX was increased in the MPR group compared with control.

**DISCUSSION**

The present results suggest that MPR promotes changes in the myocardial mechanics of the young adult offspring, associated with reduction of the SR function, and increased participation of the L-type calcium channels, with altered expression of important calcium-handling proteins. These data, together with the elevation in the blood pressure exhibited by those animals, reinforce the current suggestion that MPR predisposes the offspring to cardiovascular diseases in young adult life. These data are relevant because cardiovascular diseases are among the main causes of morbidity and mortality worldwide and the incidence of MPR is still significant in several countries, the developing ones in particular.

This study used the model of experimental qualitative malnutrition induced by the regional basic diet (RBD) described by Teodósio et al. (30). RBD is based on a nutrition survey that reproduces in rats a situation similar to the protein malnutrition that still exists in certain areas of Northeastern Brazil. Low birth weight is a significant feature exhibited by the offspring of mothers subjected to protein restriction during pregnancy. In this study, the body weight in adulthood for the offspring of rats subjected to protein restriction was lower compared with the control group, despite the offspring having been fed a normal diet after birth. Many authors suggest that a low birth weight due to inadequate nutrient supply during fetal development might program the offspring to develop a predisposition for cardiovascular diseases, such as systemic arterial hypertension, in adulthood (1, 3, 15, 17, 25). Our data confirm those reports, as we found elevation of blood pressure in the young adult offspring of female rats subjected to protein restriction. In the case of the offspring of mothers with low-protein, energy-restricted diets, hypertension seems to be caused by several mechanisms, including increased sympathetic activity (3), increased oxidative stress and activation of the renin-angiotensin system (12, 13), and retention of water and sodium by the kidneys, with reduction in the number of nephrons (32, 33). The fact that we did not find a significant difference in the blood pressure between the MPR and control rats under anesthesia might be accounted for by the depression of the central nervous system induced by urethane (26). However, hemodynamic analysis provided some relevant data, to wit, the increase of the positive and negative time derivatives of intraventricular pressure in the MPR group, which suggests increased cardiac inotropism. Several factors might regulate the cardiac contractility, including neurohumoral and intrinsic factors. In fact, previous studies demonstrated that sympathetic activity is increased in the offspring of rats subjected to protein restriction during pregnancy (3, 5), which might account for the increased positive and negative derivatives of intraventricular pressure found in the MPR rats. Based on those findings, we sought to investigate possible changes in the contractile machinery and/or in the proteins that regulate cardiac contractility in the MPR group.

Although maternal protein restriction did not alter significantly the isometric force of the isolated papillary muscles of the young adult offspring, it promoted reduction of the first derivative of force as well as increases the time to peak of force and time to relaxation, indicating impairment on both contraction and relaxation processes. Furthermore, also contractile response to β-AR stimulation with isoproterenol was reduced.
in the MPR group. These data apparently contrast to that normal systolic pressure and increased time derivative of ventricular pressure. However, if considering that sympathetic activity is increased in the MPR rats, it is reasonable that baseline cardiac pump function might be preserved despite myocardial dysfunction and downregulation of \( \beta \)-AR-mediated in vitro responses. This paradox, well described for the rat model of chronic \( \beta \)-AR activation due to sympathetic hyperactivity (23), could be attributed to enhanced myocardial noradrenaline release and circulating catecholamines, reduced cardiac vagal tone, and other inotropic mechanisms costimulated by sympathetic tone in vivo. The mechanical dysfunction as assessed in vitro results, in turn, from the intrinsically contractile capability of the cardiac muscle, being commonly related to changes in the calcium handling and/or sensitivity of the contractile machinery to myoplasmic calcium.

In fact, upon analyzing the SR function via potentiation of force after short pauses, we found a reduction of that response in the papillary muscle of the MPR rats. Corroborating the functional data, we observed here a significant reduction on protein content of SERCA-2a and PLB phosphorylation at Ser16, and an overexpression of NCX. When phosphorylated, the regulatory protein PLB relieves the inhibition of SERCA-2a. In fact, the decreased PLB phosphorylation, as seen in different rat models of cardiac remodeling, has also been described as contributing to SR uptake dysfunction (6, 10). Moreover, the overexpressed NCX may intensify the Ca\(^{2+}\) withdrawal through the membrane, reducing calcium available to reuptake and storage by the SR (6). Tappa and colleagues (29) found reduction of the protein expression of SERCA-2a in the neonate offspring of rats subjected to protein restriction during pregnancy, and suggested that calcium uptake by the SR would be impaired. Therefore, we found that the alteration in SERCA-2a expression described by Tappa et al. (29) in neonates might persist into adulthood, as observed in this study.

Another relevant point of our study was the investigation of possible alteration in the inotropic response of the papillary muscles of the young MPR rats to \( \beta \)-AR stimulation. The results showed that the positive inotropic effect of isoproterenol was significantly reduced in the MPR. Those findings corroborate the current literature when studying adult rats followed prenatal protein restriction. Fernandez-Twinn and colleagues (11) found attenuation of the \( \beta \)-adrenergic chronotropic responsiveness in vivo and reduced expression of the adrenergic signaling pathway components in cardiac tissue from young adult animals (3 mo old) born from rats fed a low-protein diet during pregnancy and lactation. However, Fernandez-Twinn and colleagues (11) assessed the pressure response to isoproterenol in vivo, while we assessed the inotropic response of papillary muscles in vitro. As stated above, it is well known that an increase of sympathetic tone, that was observed in the offspring after intrauterine malnutrition (3, 5), induces marked desensitization of \( \beta \)-AR (7).

In addition to changes in the calcium uptake by the SR and inotropic response, the results relative to the contractions dependent only on extracellular calcium that can be blocked by verapamil suggest that the calcium influx through the L-type channels was greater in the heart of the MPR rats. Notwithstanding, in the study conducted by Tappa and colleagues (29), intrauterine malnutrition promoted reduction of cardiac L-type channel expression in neonate rats. As we assessed the cardiac function of young adult rats, we can speculate that the increase in the calcium influx or the activity of the L-type channels might result from a compensatory mechanism seeking to preserve the heart contractile function. Further evidence for such a compensatory mechanism is provided by the increase in the protein expression of NCX found in the hearts of the MPR rats in this study, to prevent calcium overload when the SERCA-2a function is decreased. Such “compensatory actions” might play a relevant role when the blood pressure is elevated (as in the case of the MPR animals) to preserve the cardiac output. However, in the long run, such actions might result in considerable structural alterations, with consequent cardiac dysfunction. Therefore, this point gives further support to the idea that MPR might be a risk factor for cardiovascular diseases in the offspring in adulthood. In fact, a previous report has demonstrated that the intrauterine protein restriction leads to changes in the expression patterns of profibrotic genes and discrete structural abnormalities of vessels and hearts in offspring rats (21). Moreover, in this report, the myocardial expression and deposition of collagens was more prominent in rats born from mothers subjected to protein restriction during pregnancy (21).

To conclude, we suggest that young animals born from female rats fed a low-protein diet during pregnancy exhibit elevated blood pressure, changes in the contractile mechanics of the cardiac muscle, reduced \( \beta \)-AR contractile response, and altered expression of important proteins for calcium handling in the cardiomyocyte. Corroborating the fetal programming theory, our findings reinforce the hypothesis that maternal malnutrition is related to increased risk for cardiovascular diseases in offspring in young adult life, not only for hypertension, but also for cardiac dysfunction.

**Future Perspectives**

Our proposal was to describe that the intrauterine malnutrition induced a significant dysfunction of myocardial contractility in the offspring when assessed by in vitro assays. This, in turn, may be either a sign of direct damage caused by malnutrition, or an early signal secondary to the sympathetic hyperactivity, oxidative stress, and hypertension, and that can potentially induce late heart failure. As a result, it remains to be determined, by a time-course study, if these changes could indeed be associated with heart failure. Additionally, some proof of concept studies by using antioxidant or antihypertensive agents are required to address the reversibility of myocardial dysfunction in this context.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

REFERENCES


