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Food restriction induces *in vivo* ventricular dysfunction in spontaneously hypertensive rats without impairment of *in vitro* myocardial contractility

K. Okoshi[1], J.R. Fioretto[2], M.P. Okoshi[1], A.C. Cicogna[1], F.F. Aragon[1], L.S. Matsubara[1] and B.B. Matsubara[1]

Departamentos de [1]Clínica Médica and [2]Pediatria, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, SP, Brasil

Abstract

Cardiac structures, function, and myocardial contractility are affected by food restriction (FR). There are few experiments associating undernutrition with hypertension. The aim of the present study was to analyze the effects of FR on the cardiac response to hypertension in a genetic model of hypertension, the spontaneously hypertensive rat (SHR). Five-month-old SHR were fed a control or a calorie-restricted diet for 90 days. Global left ventricle (LV) systolic function was evaluated *in vivo* by transthoracic echocardiogram and myocardial contractility and diastolic function were assessed *in vitro* in an isovolumetrically beating isolated heart (Langendorff preparation). FR reduced LV systolic function (control (mean ± SD): 58.9 ± 8.2; FR: 50.8 ± 4.8%, N = 14, P < 0.05). Myocardial contractility was preserved when assessed by the +dP/dt (control: 3493 ± 379; FR: 3555 ± 211 mmHg/s, P > 0.05), and developed pressure (*in vitro*) at diastolic pressure of zero (control: 152 ± 16; FR: 149 ± 15 mmHg, N = 9, P > 0.05) and 25 mmHg (control: 155 ± 9; FR: 150 ± 10 mmHg, N = 9, P > 0.05). FR also induced eccentric ventricular remodeling, and reduced myocardial elasticity (control: 10.9 ± 1.6; FR: 9.2 ± 0.9%, N = 9, P < 0.05) and LV compliance (control: 82.6 ± 16.5; FR: 68.2 ± 9.1%, N = 9, P < 0.05). We conclude that FR causes systolic ventricular dysfunction without *in vitro* change in myocardial contractility and diastolic dysfunction probably due to a reduction in myocardial elasticity.

Correspondence
J.R. Fioretto
Departamento de Pediatria
Faculdade de Medicina, UNESP
Caixa Postal 530
18618-970 Botucatu, SP
Brasil
Fax: +55-14-6822-0421
E-mail: jrf@fmb.unesp.br

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- Isolated heart
- Echocardiogram
- Langendorff preparation
- Ventricular remodeling

Introduction

Researchers have long believed that the heart is spared in the presence of undernutrition and have focused their studies on other organs and systems that are strongly affected by food restriction (FR).

Today it has been well established that the heart can be challenged by FR. Many clinical and experimental studies have shown that cardiac structure, function, and myocardial contractility are affected during FR (1-

6), with important ultrastructural changes occurring in the rat myocardium (4,6). We have previously demonstrated that FR increases myocardial hydroxyproline concentration and causes left ventricular (LV) eccentric remodeling and diastolic dysfunction (7). We have also observed that FR induces major ultrastructural changes in the myocardium of normotensive and spontaneously hypertensive rats (SHR) (4,8). If these pathological conditions, hypertension and undernutrition, coexist in the same individual, we may assume that ventricular function is markedly affected. However, there are few experiments associating undernutrition with systemic arterial hypertension (4,9,10). This association is very interesting because undernutrition is a low nutrient supply condition, while systemic arterial hypertension causes further energy consumption by increasing myocardial protein synthesis (11).

The aim of the present study was to analyze the effects of FR on *in vivo* LV systolic function and on *in vitro* LV contractility and diastolic function in a genetic model of hypertension, the SHR. To the best of our knowledge, this is the first study of SHR with FR in which the echocardiogram and isolated heart technique are used in the same animal.

Material and Methods

Study groups

All investigations were performed according to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health and were approved by the Animal Research Committee of the Medical School of Botucatu, São Paulo, Brazil.

Five-month-old male SHR were fed a control or a restricted diet for 90 days. The control group (N = 23) had free access to regular rat chow (Purina Labina®, São Paulo, SP, Brazil), and its consumption was measured daily. The animals subjected to FR (N = 23) received 50% of the amount of chow consumed by the control group on the previous day. Water was provided *ad libitum*. The rats were housed in individual cages at room temperature (23ºC) on a 12-h light/dark cycle. Body weight was measured once a week and blood pressure was recorded at the beginning of the protocol and before sacrifice using the indirect tail-cuff technique (12).

Echocardiographic study

At the end of the 90-day FR experimental protocol an echocardiogram was obtained for 14 animals from each group to evaluate heart structure and function using a commercially available SONOS 2000 echocardiographic machine (Hewlett-Packard Medical Systems, Andover, MA, USA) equipped with a 7.5-MHz phased array transducer. Imaging was performed with a 60º sector angle and 3-cm imaging depth. Rats were anesthetized by intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg), their chest was shaved and the animals were placed in left lateral decubitus. Two-dimensionally targeted M-mode echocardiograms were obtained from short-axis views of the LV at or just below the tip of the mitral valve leaflets, and recorded on a black-and-white thermal printer (SONY UP-890MD) at a sweep speed of 100 mm/s. All LV tracings were measured manually with a caliper by the same observer, and according to the leading-edge method of the American Society of Echocardiography (13). Data are reported as the mean of at least five consecutive cardiac cycles. LV end-diastolic diameter (LVDD) and LV wall thickness (LVWT) were measured at maximum diastolic diameter, and the LV end-systolic diameter (LVSD) was measured at maximum anterior motion of the posterior wall. The LVWT/LVDD ratio was used to assess geometric variation of the chamber. LV systolic function was assessed by calculating the frac-

tional shortening index ((LVDD-LVSD)/LVDD x 100).

Intra-observer (K.O.) variability was calculated by reading the M-mode tracings twice in a blind fashion (mean ± SD; LVDD: 1.31 ± 1.55; LVSD: 2.49 ± 1.91; LVWT: 2.60 ± 1.56%).

Left ventricular study - Langendorff preparation

The *in vitro* study was performed on 9 animals from each group after the echocardiogram. Hearts from four animals were frozen and stored for later morphological study. One heart was eliminated from the *in vitro* study because it did not reach stability. The hearts were studied using a modified Langendorff preparation procedure as previously described (7). Briefly, rats were anesthetized with sodium thiopental (50 mg/kg, *ip*) and heparinized (1,000 IU). The chest was entered by median sternotomy under artificial ventilation. The ascending aorta was isolated and cannulated for retrograde perfusion with filtered oxygenated Krebs-Henseleit solution maintained at constant temperature and perfusion pressure, 37ºC and 75 mmHg, respectively. The Krebs-Henseleit solution, gassed with 95% oxygen-5% carbon dioxide, pH 7.3-7.4, had the following composition: 115 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.15 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, and 8 mM mannitol. The heart was removed quickly from the chest and attached to the perfusion apparatus (model 830; Hugo Sachs Elektronic, Grunstasse, Germany). The pulmonary artery was cut to vent the right ventricle during systole, the left atrial appendage was removed, and a latex balloon (7 mm in length) was placed inside the LV via the mitral valve orifice. The balloon was already attached to a plastic cannula connected to a three-way stopcock through which the balloon was filled with saline solution or emptied; ventricular pressure was measured using a P23XL transducer and a polygraph (model 40-9800-20 Windograph; Gould, Valleyview, OH, USA). Once the heart developed stable isovolumetric contractions (maintenance of systolic and diastolic pressure at a certain intraventricular volume), the balloon volume was increased in 20-µl increments over an end-diastolic pressure range of 0-25 mmHg. Pressure and volume within the balloon were recorded after each increase and corresponded to the LV pressure and volume, respectively. The volume at zero end-diastolic pressure reflects unstressed ventricular volume (V0), which was used as an index of chamber size. To ensure stability of the preparation, two or three data sets were recorded. All hearts were paced from the right atrium at 230 beats/min using an artificial pacer (model 79232; Hugo Sachs Elektronic).

Diastolic function was analyzed by measuring or calculating the following variables: maximal rate of decrease in LV pressure (-dP/dt, index of myocardial relaxation), percent variation in LV volume required to increase diastolic pressure from 0 to 25 mmHg (ΔV_{25}, index of LV compliance), and percent myocardial strain caused by a diastolic stress of 25 g/cm^2 (ε_{25}, index of myocardial elasticity). Myocardial elasticity was calculated as follows (14,15):

$$\text{stress} = (1.36 \times LVP \times V^{2/3})/[(V + 0.943 \times LVW)^{2/3} - V^{2/3}]$$

$$\text{strain} = \{[V^{1/3} + (V + 0.943 \times LVW)^{1/3}]/[V0^{1/3} + (V0 + 0.943 \times LVW)^{1/3}] - 1\} \times 100$$

where LVP is LV pressure (mmHg), V is chamber volume (ml), LVW is left ventricular weight (g), and V0 is volume (ml) at a diastolic pressure of 0 mmHg.

Contractile function was assessed on the basis of the following variables: maximal rate of rise in LV pressure (+dP/dt); developed pressures at diastolic pressures of 0 and 25 mmHg (P_{iso} 0 and P_{iso} 25, respectively); and developed stress at diastolic pressures of

0 and 25 mmHg (DS-0 and DS-25, respectively). Developed stress was calculated using the same formula as described above.

Statistical analysis

Data are reported as means ± SD. Differences between groups were determined by the unpaired two-tailed Student *t*-test, with the level of significance set at $P < 0.05$.

Table 1. General characteristics of rats and echocardiographic data.

	Control	Food restriction
BWi (g)	350 ± 20	345 ± 14
BW (g)	380 ± 33	258 ± 22*
HR (*in vivo*, bpm)	268 ± 43	240 ± 24
SBP (mmHg)	190 ± 24	196 ± 21
LVDD (mm)	7.03 ± 0.46	6.96 ± 0.45
LVSD (mm)	2.91 ± 0.70	3.44 ± 0.47*
LVWT (mm)	1.97 ± 0.14	1.64 ± 0.17*
LVDD/BW (mm/kg)	18.7 ± 1.2	27.2 ± 3.2*
FS (%)	58.9 ± 8.2	50.8 ± 4.8*

Data are reported as means ± SD for 14 animals in each group. BWi = body weight at the beginning of the food restriction (FR) protocol; BW = body weight at the end of the FR protocol; HR = heart rate; SBP = tail-cuff systolic blood pressure; LVDD = left ventricular diastolic diameter; LVSD = left ventricular systolic diameter; LVWT = left ventricular wall thickness; FS = left ventricular fractional shortening.
*P < 0.05 *vs* control (Student *t*-test).

Table 2. Structural study of the left ventricle in an isolated Langendorff heart preparation.

	Control	Food restriction
LVW (g)	1.11 ± 0.11	0.73 ± 0.07*
V0 (ml)	0.25 ± 0.04	0.23 ± 0.03
LVW/BW (g/kg)	2.98 ± 0.13	2.98 ± 0.28
V0/BW (ml/kg)	0.67 ± 0.10	0.96 ± 0.17*

Data are reported as means ± SD for 9 animals in each group. LVW = left ventricular weight; V0 = left ventricular unstressed volume; BW = body weight.
*P < 0.05 *vs* control (Student *t*-test).

Results

There were no differences in initial body weight (BWi) between the experimental groups. FR decreased body weight (BW) with no effect on tail-cuff systolic blood pressure or *in vivo* heart rate (Table 1).

The echocardiographic study (Table 1) showed that FR did not change LVDD, increased LVSD, and reduced LVWT. Normalization of LVDD to BW showed that FR increased this ratio. Undernutrition decreased the LVWT/LVDD ratio and LV systolic performance assessed by the fractional shortening index.

The *in vitro* study (Table 2) showed that FR reduced LVW in proportion to BW reduction. The volume at zero mmHg LV diastolic pressure (V0) was not changed after FR. Undernutrition reduced both the LVW/V0 and LVWT/LVDD ratios, indicating the occurrence of eccentric ventricular remodeling (Figure 1). FR did not change +dP/dt, P_{iso} 0 and P_{iso} 25 (Table 3). Food-restricted animals showed increased developed stress in both diastolic pressures (zero and 25 mmHg). LV relaxation, evaluated by the -dP/dt index, was not affected by FR (Table 3). However, the ε_{25} and ΔV_{25} values respectively showed that FR reduced myocardial elasticity and LV compliance (Figure 2).

Discussion

The undernutrition protocol used in this experiment was based on the fact that human food deprivation is generally the result of a deficiency of all diet components (16). Investigators have been using different reduction levels of total chow amount without specific deficiencies (6,17-19). Our undernutrition protocol was effective by causing a marked body weight reduction. This study did not include a control group, preventing conclusions about the impact of hypertension on cardiac remodeling in normotensive animals.

FR did not change tail-cuff systolic blood

pressure, in agreement with data reported by Gradin and Persson in a study on SHR (17). However, Overton et al. (20) found a reduction in blood pressure which was explained by diminished sympathetic support. As was also the case in the present study, these investigators did not observe FR interference with heart rate. Since we found no changes in tail-cuff systolic blood pressure or heart rate, we may assume that these parameters had no influence on the *in vivo* results.

FR did not alter LVDD but decreased LVWT; consequently, the LVWT/LVDD ratio was decreased in FR rats. In undernourished children and patients with anorexia nervosa, echocardiographic studies have always shown lower LVDD and LVWT (1,2,21,22). In our laboratory, LVDD and LVWT reduction was seen in normotensive rats submitted to the same FR protocol. This highlights the question concerning the influence of hypertension on ventricular cavity remodeling in rats submitted to FR. The absence of a reduction in LV chamber size suggests an inability to adapt to hemodynamic load when undernutrition restricts myocardial growth. This was confirmed by the decrease in ejection index found in the *in vivo* study. The lower LVWT/LVDD ratio indicates that FR caused a change in the shape of the ventricular cavity, showing an eccentric remodeling.

The *in vitro* study confirmed the structural findings obtained *in vivo*; FR did not alter V0, but decreased LVW. Consequently, the LVW/V0 ratio was lower in FR rats, indicating eccentric remodeling.

FR decreased the ability of the LV to eject, which may have been caused by the reduction in myocardial contractility and/or cardiac load changes. The M-mode echocardiogram is an efficient method for *in vivo* studies, which, however, can be used only in the study of myocardial contractility. The study of the isolated heart from the same animal allowed us to gain a better understanding of the *in vivo* changes. We did not observe changes in +dP/dt or P_{iso} 0, and P_{iso} 25 values in FR rats, a fact that may be interpreted as the maintenance of myocardial contractility (23,24) since cardiac load

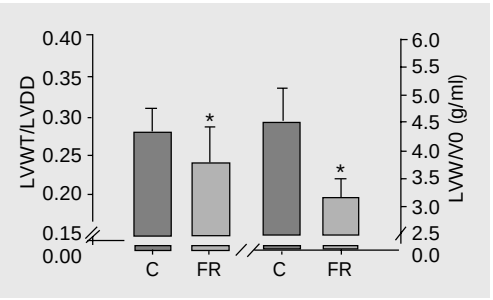

Figure 1. Ratio of left ventricular wall thickness (LVWT) to diastolic diameter (LVDD) and ratio of left ventricular weight (LVW) to volume (V0). Data are reported as means ± SD for 9 animals in each group. C = control group; FR = food-restricted group. Note that both ordinates and abscissa are discontinuous. *P < 0.05 compared to control animals (Student *t*-test).

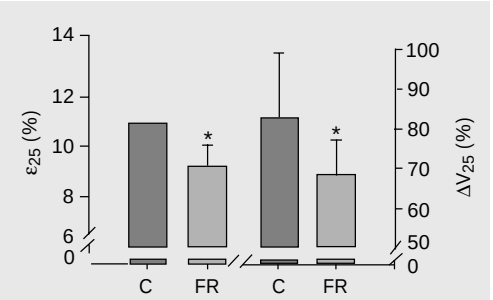

Figure 2. Left ventricular diastolic function evaluated in the isolated heart. Data are reported as means ± SD for 9 animals in each group. C = control group; FR = food-restricted group; ε_{25} = percentage of myocardial strain caused by a diastolic stress of 25 g/cm^2; ΔV_{25} = percentage of variation in ventricular volume required to increase diastolic pressure from 0 to 25 mmHg. Note that both ordinates and abscissa are discontinuous. *P < 0.05 compared to control animals (Student *t*-test).

Table 3. Functional study of the left ventricle in an isolated Langendorff heart preparation.

	Control	Food restriction
Systole		
+dP/dt (mmHg/s)	3493 ± 379	3555 ± 211
P_{iso} 0 (mmHg)	152 ± 16	149 ± 15
P_{iso} 25 (mmHg)	155 ± 9	150 ± 10
DS-0 (g/cm^2)	104 ± 19	136 ± 19*
DS-25 (g/cm^2)	174 ± 19	217 ± 22*
Diastole		
-dP/dt (mmHg/s)	2090 ± 234	1986 ± 246

Data are reported as means ± SD for 9 animals in each group. +dP/dt = rate of rise of ventricular pressure; P_{iso} 0 and P_{iso} 25 = developed pressure at diastolic pressure of zero and 25 mmHg, respectively; DS-0 and DS-25 = developed stress at diastolic pressure of zero and 25 mmHg, respectively; -dP/dt = rate of decrease of left ventricular pressure.
*P < 0.05 *vs* control (Student *t*-test).

and heart rate were controlled in the study of the isolated isovolumetrically beating heart. We do not think that the increase of developed stress in FR rats is an indication of improved systolic performance because this index is inappropriate for the evaluation of myocardial contractility when the LV shows eccentric remodeling (7). However, an increased developed stress with unchanged systolic pressure may help understand the mechanism by which ventricular performance was impaired in the *in vivo* study on FR rats. Since both groups had the same *in vivo* arterial systolic pressure and undernutrition caused eccentric remodeling, we assumed that the FR rats presented a higher *in vivo* afterload. This increased afterload may explain the ventricular ejection impairment even when the inotropic state is unchanged. One might ask if the *in vivo* preload condition would favorably affect LV performance of FR rats, i.e., if for a given diastolic pressure the preload is higher in an eccentrically shaped ventricle. Consequently, preload reserve would be recruited in these hearts, helping systolic function. However, we believe that this was not the case in our study. We analyzed all hearts in the same diastolic pressure range, and systolic performance was the same in both groups.

FR caused a decrease in myocardial elasticity and ventricular compliance. In our previous studies, we observed that FR increased myocardial hydroxyproline concentration in adult and young normotensive rats (3,7), possibly explaining the alterations found in the diastolic property. It was interesting to observe that the increase in myocardial stiffness and the consequent reduction in ventricular compliance occurred in the presence of eccentric remodeling. This remodeling, caused by a disproportional reduction in LV weight (or wall thickness) compared to LV volume (or diastolic diameter), might have led to increased ventricular compliance (25).

In summary, SHR submitted to FR for 90 days showed a reduction in LV wall thickness, eccentric remodeling, *in vivo* systolic performance depression, and changes in passive diastolic properties, with no changes in tail-cuff systolic blood pressure, heart rate, LV diastolic diameter, or myocardial contractility.

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