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Food restriction impairs myocardial inotropic response to calcium and β-adrenergic stimulation in spontaneously hypertensive rats

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Abstract

Although long-term food restriction (FR) has been shown to induce cardiac remodeling and dysfunction, there are few data on the effects of FR on pressure-overloaded hearts. The aim of this study was to examine the effects of FR on cardiac muscle performance during inotropic stimulation in the myocardium of spontaneously hypertensive rats (SHRs). Male 60-day-old SHRs were subjected to FR for 90 days. Food-restricted animals received 50% of the ad libitum amount of food consumed by the control group. Myocardial function was studied in isolated left ventricular papillary muscle under isometric contraction in basal condition (1.25 mmol/L extracellular Ca^{2+} concentration) and after 3 inotropic maneuvers: (1) at postrest contraction of 30 seconds, (2) at extracellular Ca^{2+} concentration of 5.2 mmol/L, and (3) after β-adrenergic stimulation with 10^{-6} mol/L isoproterenol. At basal condition, time from peak tension to 50% relaxation was greater in the food-restricted group ($P < .05$). Inotropic stimulation with postrest contraction and isoproterenol promoted a significant lower increase of developed tension, maximum rate of tension development, and maximum rate of tension decline in the food-restricted compared to the control group. The elevation of extracellular Ca^{2+} concentration induced a lower increase of developed tension, maximum rate of tension development, and time from peak tension to 50% relaxation in the food-restricted than in the control group. In conclusion, long-term FR promotes impairment of myocardial inotropic response to calcium and β-adrenergic stimulation in SHRs.

Keywords: Inotropic stimulation; Hypertrophy; Myocardial contractility; Spontaneously hypertensive rat; Undernutrition

Abbreviations: BW, body weight; DT, developed tension; +dT/dt, maximum rate of tension development; −dT/dt, maximum rate of tension decline; FR, food restriction; L_{max}, muscle length associated with maximal developed tension; LV, left ventricle; LVW, left ventricular weight; PRC, postrest contraction; RT, resting tension; RT_{50}, time from peak tension to 50% relaxation; RV, right ventricle; RVW, right ventricular weight; SR, sarcoplasmic reticulum; TPT, time to peak tension.

1. Introduction

Food restriction increases rat maximum lifespan and retards or prevents a broad spectrum of age-related pathophysiologic changes such as loss of skeletal muscle mass, diabetes, hypertension, and cancer [1-4]. Although FR is beneficial to health, our previous work [5-11] and findings

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from other investigators [12-14] have shown that dietary restriction induces cardiac remodeling and depresses cardiac and myocardial performance. In young and mature normotensive Wistar Kyoto rats subjected to long-term FR, myocardial dysfunction was associated with striking ultrastructural alterations, and calcium handling and β-adrenergic system changes [8,9,15,16].

Few experiments have evaluated heart functional properties under pressure overload and FR [17-19]. Tabayashi et al [18] have observed a depressed myocardial contractility in protein-calorie malnourished dogs subjected to left ventricular pressure overload with preserved cardiac pump function. We previously found that FR of 50% less of than the amount consumed by the control group for 90 days induces in vivo and in vitro ventricular dysfunction in SHRs [11]. We also showed that mechanical function of left ventricular (LV) papillary muscles was depressed in food-restricted SHRs [20].

The aim of this study was to evaluate the effects of extracellular calcium concentration changes, β-adrenergic stimulation, and postrest contraction (PRC) on hypertrophied myocardium function to further identify mechanical activity abnormalities. In this study we tested the hypothesis that SHRs subjected to FR present impaired contractile response to inotropic stimulation, which is related to calcium transient and β-adrenergic system changes.

2. Methods and materials

2.1. Animal model and experimental protocol

All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and were approved by the Animal Ethics Committee of the Faculdade de Medicina de Botucatu, UNESP, São Paulo, Brazil.

Sixty-day-old spontaneously hypertensive male rats (SHRs) were fed a control or a food-restricted diet for a 90-day period. Both groups (control group SHR-C, n = 8) were fed Purina rat chow (3.76% fat, 20.96% protein, 52.28% carbohydrate, 9.60% fiber, and 13.40% humidity) and water ad libitum. Food consumption for the control group was measured daily and was used to calculate the amount of food for the restricted group. The rats subjected to FR (SHR-FR, n = 9) received 50% of the amount consumed by the control group.

All rats were housed in individual cages in a room maintained at 23°C and kept on a 12-hour light-dark cycle. Body weight (BW) and systolic arterial pressure of rats were evaluated at the beginning and at the end of experiment. Blood pressure was measured by the indirect tail-cuff technique [21]. Body weight, LV weight (LVW), and right ventricular (RV) weight (RVW) were measured, and LVW/BW and RVW/BW ratios were used as indexes of ventricular hypertrophy.

2.2. Functional study

At the end of the experimental period, myocardial intrinsic contractile performance was evaluated in isolated LV papillary muscle preparations as previously described [9,15]. This preparation allows us to measure the ability of the cardiac muscle to shorten and develop force, independently of influences that can modify in vivo mechanical performance of the myocardium, such as heart rate, preload, and afterload.

The rats were anesthetized (pentobarbital sodium, 50 mg/kg, IP) and decapitated. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. Left ventricular anterior or posterior papillary muscle was dissected free, mounted between 2 spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution at 28°C and oxygenated with a mixture of 95% O_2 and 5% CO_2 (pH 7.38). The composition of the Krebs-Henseleit solution in mmol/L was as follows: 118.5 NaCl, 4.69 KCl, 1.25 $CaCl_2$, 1.16 $MgSO_4$, 1.18 KH_2PO_4, 5.50 glucose, and 25.88 $NaHCO_3$. The spring clips were attached to a Kyowa model 120T-20B force transducer and a lever system, which allowed for muscle length adjustment. Preparations were stimulated 12 times per minute at a voltage of 10% above the threshold value.

After a 60-minute period, during which the preparations were permitted to shorten while carrying light loads, muscles were loaded to contract isometrically and stretched to the apices of their length-tension curves. After a 5-minute period, during which preparations performed isotonic contractions, muscles were again placed under isometric conditions, and the apex of the length-tension curve (L_{max}) was determined. A 15-minute period of stable isometric contraction was imposed before the experimental period. One isometric contraction was then recorded for later analysis.

The following parameters were measured from isometric contraction: peak developed tension (DT, g/mm^2), resting tension (RT, g/mm^2), time to peak tension (TPT, milliseconds), maximum rate of tension development (+dT/dt, g/mm^2 per second), maximum rate of tension decline (−dT/dt, g/mm^2 per second), and time from peak tension to 50% relaxation (RT_{50}, milliseconds).

The mechanical performance of the papillary muscle was evaluated in basal condition and after inotropic stimulation with the following agents:

1. Postrest contraction of 30 seconds;
2. Increase in extracellular Ca^{2+} concentration from 1.25 mmol/L to 5.2 mmol/L; and
3. Addition of the β-adrenergic agonist isoproterenol (10^{-6} mol/L) to the nutrient solution.

After registering a papillary muscle contraction at 5.2 mmol/L extracellular calcium concentration, nutrient solution was changed to 1.25 mmol/L extracellular calcium concentration, and the preparation was allowed to stabilize for 15 minutes before adding isoproterenol.