Effects of Protein-Calorie Restriction on Mechanical Function of Hypertrophied Cardiac Muscle

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Objective - To assess the effect of food restriction (FR) on hypertrophied cardiac muscle in spontaneously hypertensive rats (SHR).

Methods - Isolated papillary muscle preparations of the left ventricle (LV) of 60-day-old SHR and of normotensive Wistar-Kyoto (WKY) rats were studied. The rats were fed either an unrestricted diet or FR diet (50% of the intake of the control diet) for 30 days. The mechanical function of the muscles was evaluated through monitoring isometric and isotonic contractions.

Results - FR caused: 1) reduction in the body weight and LV weight of SHR and WKY rats; 2) increase in the time to peak shortening and the time to peak developed tension (DT) in the hypertrophied myocardium of the SHR; 3) diverging changes in the mechanical function of the normal cardiac muscles of WKY rats with reduction in maximum velocity of isotonic shortening and of the time for DT to decrease 50% of its maximum value, and increase of the resting tension and of the rate of tension decline.

Conclusion - Short-term FR causes prolongation of the contraction time of hypertrophied muscles and paradoxical changes in mechanical performance of normal cardiac fibers, with worsening of the shortening indices and of the resting tension, and improvement of the isometric relaxation.

Key words: food restriction, myocardial function, spontaneously hypertensive rat

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Protein-calorie malnutrition (PCM) is a primary public health issue in developing countries. Even in industrialized societies, PCM is found in 30% to 40% of hospitalized patients. PCM can affect a series of organs and tissues. There is evidence in humans and especially in experimental animals that PCM can cause damage to the cardiovascular system. PCM can promote the following alterations: 1) clinical changes that vary from bradycardia to heart failure; 2) functional changes, such as reduction in cardiac output, in ventricular compliance, and in myocardial contractility; 3) biochemical changes, such as the activation of calcium-dependent proteinase, reduction of the protein synthesis and of the RNA/DNA ratio, and an increase in DNA; and 4) morphological changes, such as dilation of the cardiac chambers, atrophy and/or degeneration of myocytes, interstitial and mitochondrial edema, and increase in collagen.

Hypertrophy of myocardial cells is the most efficient mechanism for cardiac compensation in response to increased workload. Its purpose is to maintain adequate cardiac performance in response to systemic metabolic demands.

The spontaneously hypertensive rats (SHR) were developed as a genetic model of hypertension, which in many ways is similar to essential hypertension in man. The animals develop early left ventricular hypertrophy (LV), which is responsible for the maintenance of the normal cardiac function, despite the elevated systemic blood pressure (BP).

Information in the literature about the influence of different types of nutritional deficiencies on the functional behavior of the hypertrophied muscle is scarce. Yokota et al. observed that SHR fed a protein-deficient diet showed deterioration of LV function. Tabayashi et al. observed that dogs with LV hypertrophy that underwent chronic PCM had normal pump function and a reduced myocardial contractility. Olivetti et al. studying SHR with nutritional anemia, observed LV dilation and dysfunction in those animals.

Due to lack of information in the literature about the association between PCM and function of the hyper-
trophied ventricle, this study assesses the effect of a 30-day food restriction (FR) diet on the mechanical behavior of the hypertrophied cardiac muscles from SHR. This period of time was chosen because Klebanov et al. observed similar changes in mechanical function of normotensive rats when undergoing FR for 10 to 13 months or for three weeks. The hypothesis tested was that hypertrophied hearts, which need a higher nutritional supply, would present more changes with FR.

**Methods**

The 60-day-old male SHR and normotensive Wistar-Kyoto (WKY) rats were divided into two experimental groups: Group C – control, the animals were fed an unrestricted diet for 30 days; and Group R – the animals underwent FR for 30 days.

The control rats (WKYc and SHRc) received a Purina unrestricted diet (3.76% fat, 20.96% protein, 52.28% carbohydrate, 9.6% ashes, and 13.4% humidity). The animals under FR (WKYr and SHrr) received 50% of the amount of food of the control rats. The animals were kept in individual cages at a room temperature of 23°C. The weight and BP measured at the tail of the animals, were determined at the onset of the experiment and before sacrifice.

The study of the mechanical function was performed using the papillary muscles of the LV. The muscles were obtained in the following way: the rat was decapitated, the chest opened, heart rapidly removed and put into the Krebs-Henseleit solution, at a temperature of 28°C. This solution had been previously oxygenated for 10 minutes with 95% oxygen (O2) and 5% carbon dioxide (CO2). After dissecting the right ventricle (RV) and cutting the interventricular septum, the LV was divided into two parts, each one containing one papillary muscle. These muscles were then carefully dissected in a chamber containing the Krebs-Henseleit solution, adequately oxygenated and warmed to 28°C. Two stainless steel rings were attached to the extremities of the papillary muscles, which were then rapidly transferred to a glass chamber containing the Krebs-Henseleit solution, constantly oxygenated with 95% O2 and 5% CO2, and kept at 28°C, with the use of a circulating bath. The papillary muscles were kept in the vertical position inside the glass chamber. The inferior ring was linked to a stainless steel wire of 0.031 cm diameter, connected to a stainless steel wire similar to the former and connected to a parallel platinum electrodes that were coupled with an electric stimulator programmed to release stimuli in square waves of 5 ms, with an approximately 10% higher voltage than the minimum necessary to cause a maximum mechanical response in the muscle.

The composition of the Krebs-Henseleit solution, in millimoles per liter, was: 118.5 NaCl; 4.69 KCl; 2.52 CaCl2; 1.16 MgSO4; 1.18 KH2PO4; 5.50 glucose; and 25.88 NaHCO3. The partial O2 pressure was kept between 550 and 600 mmHg.

After a period of 60 minutes in which the muscles contracted only against the preload (isotonic contraction), the lever was prevented from moving with an additional load (afterload) put at the extremity of the short arm of the lever. In isometric contraction, the muscle was progressively stretched until the developed tension reached its maximum value (the diastolic length of the muscle fiber associated with the peak isometric developed tension was designated Lmax). After reaching Lmax, the muscle underwent isotonic contraction again. Then, a new Lmax was determined. The experiment began after 15 min of stable isometric contraction. Unstable preparations or those with an unsatisfactory performance were not used. The following isometric parameters were measured: peak developed tension (DT); resting tension (RT); time to peak tension (TPT); maximum rate of tension development (+dT/dt); maximum rate of tension decline (-dT/dt); and period of time necessary for a 50% reduction in the peak developed tension (RT50).

After the end of the isometric record, the muscle underwent isotonic contraction, contracting against the smallest total load (preload plus afterload) capable of keeping the muscle resting length equal to Lmax. The following isotonic parameters were measured: peak shortening (PS), time to peak shortening (TPS); maximum rate of isotonic shortening (+dL/dt); and maximum rate of relaxation (-dL/dt).

The mechanical parameters were recorded using the Gould 2200 S polygraph, with a paper speed of 100 mm/s.

The values of DT, RT, +dT/dt, -dT/dt were normalized for the cross sectional area (CSA) of the muscle. The values of velocity of isotonic shortening and relaxation were divided by the length of the muscle Lmax.

The parameters used to individually characterize the papillary muscles were: length (mm), weight (mg), and CSA (mm²). The \textit{in vitro} length, Lmax, was measured with pachymeter. The portion of muscle between the steel rings was blotted dry and weighed. Because the papillary muscle has a geometrical, uniform and cylindrical shape and specific gravity of 1.0, the SA was calculated by dividing the muscle weight by its length. The LV wet weight normalized to the body weight of the rat at the moment of the sacrifice (LV/FW) was used as an index of LV hypertrophy.
Statistical analysis – The values obtained are shown as mean±standard deviation (Tables I, II, III). The comparisons between the groups were made by analysis of variance, complemented by the Tukey test. The level of significance was considered 5%.

**Results**

Table I shows the body parameters and the BP of the animals. The initial weights (IW) and the final weights (FW) of the WKY rats were greater than those of the SHR. The rats on the unrestricted diet had body weight gain (WKYC: from 237±19g to 309±21g; SHRc: from 205±15g to 279±22g); the animals undergoing FR decreased the body weight (WKYr: from 288±49g to 199±6g; SHRr: from 193±17g to 168±31g). While the normal diet increased the ratio between FW and IW in the WKY rats (1.31±0.09) and in the SHRc (1.37±0.13), the FR reduced the ratio FW/IW in the WKYr rats (0.70±0.12) and in the SHRr (0.87±0.08). BP and the LV/FW ratio, higher in the SHRc than in the WKYc rats, remained unchanged with FR. The CSA of the WKYc was higher than in the SHRc group; FR did not change CSA in any animal group. Lmax was the same for the SHRc and WKYc rats. FR caused reduction of Lmax in WKYr, but did not change this variable in the SHRr.

The mechanical data are shown in tables II and III. DT, +dT/dt, RT, +dT/dt, and -dT/dt were significantly higher in SHRe than in WKYe rats. While RT and (Lmax-PS)/Lmax were higher in the WKYe rats than in the SHRc, TPT, -dT/dt, PS and TPS did not differ in the two groups of animals. In WKYe rats, FR caused elevation of RT, -dT/dt and (Lmax-PS)/Lmax, reduction of RT, PS, and +dT/dt and did not change DT, TPT, +dT/dt, TPS and -dT/dt. In the SHR group, FR significantly elevated TPT and TPS, but did not change the other isometric and isotonic variables.

**Discussion**

The results of this study show that restriction of protein-calorie ingestion caused restriction in weight gain of the body and of the LV of the SHR and WKY rats. However, the LV/FW ratio in the normotensive and hypertensive animals did not change with FR, when compared to that in the rats on a normal diet (Table I). The maintenance of the LV/FW ratio after FR, in both animal groups, suggest that there was not a major loss of mass in the SHR hearts, which, theoretically, would need a greater nutritional supply because of the hypertrophic process. However, the normalization of LV weight in relation to the body weight of the animal, a measurement often used for assessing the degree

### Table I - Morphological parameters and systolic blood pressure of SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>WKY (n=11)</th>
<th>SHR (n=10)</th>
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<tbody>
<tr>
<td>IW (g)</td>
<td>237±19a</td>
<td>288±49c</td>
</tr>
<tr>
<td>FW (g)</td>
<td>309±21a</td>
<td>205±15a</td>
</tr>
<tr>
<td>FW/IW</td>
<td>1.31±0.09</td>
<td>0.70±0.12</td>
</tr>
<tr>
<td>LV (mg)</td>
<td>0.63±0.06</td>
<td>0.43±0.05</td>
</tr>
<tr>
<td>LV/FW</td>
<td>2.03±0.13</td>
<td>2.15±0.15</td>
</tr>
<tr>
<td>ISP (mmHg)</td>
<td>133±8</td>
<td>128±8</td>
</tr>
<tr>
<td>ESP (mmHg)</td>
<td>133±8</td>
<td>129±11</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>0.92±0.19</td>
<td>0.84±0.25</td>
</tr>
<tr>
<td>Lmax (mm)</td>
<td>6.32±0.38</td>
<td>5.37±1.01</td>
</tr>
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Mean values ± standard deviation; n- number of papillary muscles; C- normal diet; R- food restriction; IW- initial body weight; FW- final body weight; LV- left ventricle; ISP- initial systolic pressure; ESP- end systolic pressure; CSA- cross sectional area; Lmax- length at rest associated with peak developed tension; g- gram; mg- milligram; mmHg- millimeter of Hg; mm²- square millimeter; mm- millimeter; the groups of animals identified by different letters are significantly different (ANOVA and Tukey, p<0.05).

### Table II - Mechanical parameters obtained in isometric contraction

<table>
<thead>
<tr>
<th></th>
<th>WKY (n=11)</th>
<th>SHR (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT (g/mm²)</td>
<td>7.15±1.42</td>
<td>7.95±2.04</td>
</tr>
<tr>
<td>RT (g/mm²)</td>
<td>0.94±0.49</td>
<td>1.43±0.41</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>194±15</td>
<td>192±30</td>
</tr>
<tr>
<td>+dT/dt (g/mm²/s)</td>
<td>63±18</td>
<td>73±24</td>
</tr>
<tr>
<td>-dT/dt (g/mm²/s)</td>
<td>18±4</td>
<td>27±6</td>
</tr>
<tr>
<td>RT (ms)</td>
<td>259±22</td>
<td>219±67</td>
</tr>
</tbody>
</table>

Mean values ± standard deviation; WKY- Wistar-Kyoto rats; SHR- spontaneously hypertensive rats; n- number of papillary muscles; C- normal diet; R- food restriction; DT- peak developed tension; RT- resting tension; TPT- time to peak tension; +dT/dt- maximum rate of tension development; -dT/dt- maximum rate of tension decline; RT, period of time necessary for the DT to reduce 50% of its maximum value; g/mm²- gram per square millimeter; ms-millisecond; g/mm²/s- gram per square millimeter per second; the groups of animals identified by different letters are significantly different (ANOVA and Tukey, p<0.05).
of cardiac hypertrophy\textsuperscript{12-14}, can result in interpretational errors when the animals lose weight during the experimental process. That is why many authors working with malnutrition\textsuperscript{6,12} have introduced an additional group of animals, younger and with body weight comparable to that of\textsuperscript{10} FR. In this case, since the animal groups have the same body weight, the comparison of the LV/FW ratios allows the adequate assessment of the cardiac muscle mass in the animals on FR. In this study, as the SHR developed LV hypertrophy during the process of maturation, it was not possible to use younger SHR with body weight equivalent to that of the malnourished rats, because there was not time enough for the younger ones to develop cardiac hypertrophy.

FR caused a delay in the time of contraction of the isolated hypertrophied cardiac muscles of the SHR. This could be seen by the increase of the TPT and TPS (Tables II and III). These results were different from those obtained in the normal muscles of the WKY rats, which showed worsening of the RT and of the parameters derived from the shortening phase and improvement of the isometric relaxation (Tables II and III). The mechanisms responsible for the changes in the myocardial mechanical function resulting from FR remain unknown\textsuperscript{10}.

Studies analyzing the effects of protein-calorie malnutrition (PCM) upon the mechanical performance of the hypertrophied cardiac muscles are scarce. The results showed that changes in the composition or in the amount of food ingested caused LV dysfunction \textsuperscript{5,9}. Yokota et al\textsuperscript{7} studied SHR receiving a low-protein diet (10% protein) for 2 and 4 weeks, and assessed the LV function by means of hemodynamic parameters, such as dI/dtmax/integrated isometric pressure and LV end diastolic pressure (LVEDP). Tabayashi et al\textsuperscript{a} analyzed dogs with aortic stenosis fed on a low protein-calorie diet for two weeks. The ventricular function was studied using the mean velocity of circumferential fiber shortening and using the relation between the parietal tension and the end-systolic diameter. Olivetti et al\textsuperscript{b} studied 4-week-old SHR undergoing nutritional anemia induced by iron and copper deficiency for 12 weeks. The LV behavior was assessed using LVEDP, LV end-systolic pressure, and +dL/dt. From all these studies, the variables used in the assessment of ventricular function were influenced by changes in preload, afterload, heart rate, and myocardial contractility. Changes in the loads and heart rate can alter ventricular function, independently of cardiac inotropism, which hinders the analysis of the behavior of the contractile status of the cardiac muscle. The utilization of the isolated papillary muscle allows the assessment of myocardial contractility, because the other variables influencing the cardiac performance can be fixed\textsuperscript{15}.

Unlike the results of the SHR group, FR caused contradictory changes in myocardial function of the WKY rats, with improvement of the relaxation phase and worsening of the resting tension and of the indices of muscle shortening. We could not find an explanation for the diverging results observed in the WKY rats. Contradictory data that assess cardiac mechanics have also been observed in other experimental studies. Recently, Hoit et al\textsuperscript{16}, studying monkeys with hypertension and LV hypertrophy, observed paradoxical data, such as depression of the indices of contractility dependent on velocity and preservation of contractility when assessed through parameters derived from force. Previous publications\textsuperscript{5,10,17-19} studying the relation between PCM and cardiac function of normal muscles showed that cardiac performance could remain unaltered, decrease, or improve with FR.

In conclusion, this study shows that FR of 50% of a normal diet for 30 days causes prolongation in the time of contraction of the hypertrophied muscles of SHR. In normal hearts of WKY rats, this diet causes contradictory changes in myocardial performance, resulting in worsening of the shortening phase and of the resting tension and improvement in isometric relaxation. The results obtained do not allow us to conclude that hypertrophied muscles suffer the effects of FR more intensely than do the normal heart muscle.

\textbf{Acknowledgements}

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