Ventricular remodeling and diastolic myocardial dysfunction in rats submitted to protein-calorie malnutrition

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Received 21 May 2001; accepted in final form 19 November 2001

Fioretto, José R., Susana S. Querioz, Carlos R. Padovani, Luiz S. Matsubara, Katashi Okoshi, and Beatriz B. Matsubara. Ventricular remodeling and diastolic myocardial dysfunction in rats submitted to protein-calorie malnutrition. Am J Physiol Heart Circ Physiol 282: H1327–H1333, 2002. First published November 29, 2001; 10.1152/ajpheart.00431.2001.——The effects of protein-calorie malnutrition (PCM) on heart structure and function are not completely understood. We studied heart morphometric, functional, and biochemical characteristics in undernourished young Wistar rats. They were submitted to PCM from birth (undernourished group, UG). After 10 wk, left ventricle function was studied using a Langendorff preparation. The results were compared with age-matched rats fed ad libitum (control group, CG). The UG rats achieved 47% of the body weight and 44% of the left ventricular weight (LVW) of the CG. LVW-to-ventricular volume ratio was smaller and myocardial hydroxyproline concentration was higher in the UG. Left ventricular systolic function was not affected by the PCM protocol. The myocardial stiffness constant was greater in the UG, whereas the end-diastolic pressure-volume relationship was not altered. In conclusion, the heart is not spared from the adverse effects of PCM. There is a geometric alteration in the left ventricle with preserved ventricular compliance despite the increased passive myocardial stiffness. The systolic function is preserved.

Methods

All experiments were conducted in accordance with the university’s Animal Use Committee and the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, Revised 1985). Forty-two 10-wk-old male Wistar rats were divided into two experimental groups: undernourished (UG, n = 20) and control (CG, n = 22).

PCM Two-Phase Induction Protocol

Phase 1. From birth to 21 days, the number of animals per litter was increased to 10 and the rats were restricted to 4 h of suckling daily, always at the same time of day. Newborn rats in the control group (CG) were maintained at a maximum of five animals per litter and were fed uninterrupted. Mothers of both groups received chow and water ad libitum. During the breast-feeding restriction phase, it was possible that competition caused the rats to achieve different nutritional status. Therefore, only UG animals that achieved a body weight 2 SD below the mean body weight of the CG rats were used in the next phase.

Phase 2. From 21 days to 10 wk old, the rats were housed in individual wire cages with raised wire floors to avoid coprophagy (4, 7). Dietary restriction was maintained by limiting the amount of chow (Purina Labina, São Paulo, Brazil) to 70% of the control group. Left ventricular systolic and diastolic function was assessed using an isolated heart preparation. Additional studies were performed in the isolated left atrium (7, 30), whole heart (14, 21, 34, 39, 52), and isolated atria (22, 23), and in dogs (3). Children (5, 24, 37, 40), and adults (18) supporting the concept of preserved systolic function. Additionally, Savabi and Kirsch (39) observed enhanced and depressed contractile function with the same preparation. It has to be pointed out that few reports have assessed diastolic function in PCM with the same preparation. Differences in the heart on the time the aggression is imposed from birth.

Because very young animals have rapid body and organ growth, we hypothesized that PCM from birth would have a greater effect on the heart. Accordingly, the aims of this study were 1) to study systolic and diastolic function of the left ventricle (LV) and 2) to analyze morphometric and biochemical characteristics contributing to myocardial dys-function in the heart of young rats submitted to PCM from birth.

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Brazil) provided to the UG rats to 50% of that consumed by the age-matched control rats.

LV Study-Langendorff Preparation

On the day of the functional study, animals were weighed, tail cuff systolic blood pressure was recorded, and blood samples were taken for biochemical analysis. Nine rats from the CG and 10 from UG were randomly chosen to study LV function. The hearts were studied using a modified Langendorff preparation as previously described (6, 26). Briefly, the animals were anesthetized with thiopental sodium (50 mg/kg ip) and heparinized (1,000 IU ip). 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 (37°C and 75 mmHg, respectively). The Krebs-Henseleit solution, gassed with 95% oxygen-5% carbon dioxide, 7.3–7.4 pH, had the following composition (in mM): 115 NaCl, 5.4 KCl, 1.2 MgSO₄, 1.25 CaCl₂, 1.15 NaH₂PO₄, 25 NaHCO₃, 11 glucose, and 8 mannitol. After cannulation, the entire heart was quickly removed from the chest and attached to the perfusion apparatus (model 830; Hugo Sachs Electronic, Grunstasse, Germany). The pulmonary artery was cut to vent the right ventricle during systole, and after the removal of the left atrial appendage, a latex balloon (7-mm length) was placed into the LV via the mitral valve oriﬁce. The proximal end of the balloon had been previously 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). Once the heart developed stable isovolumetric contractions, balloon volume was increased in 10-μl increments over an end-diastolic pressure range of 0–25 mmHg. Pressure and volume within the balloon were recorded after each incremental 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. Typically, 8–10 pressure-volume points were obtained from each series of measurements; two or three data sets were recorded to ensure preparation stability. All hearts were paced from the right atrium at 250 beats/min using an artiﬁcial pacemaker (model 792232; Hugo Sachs Electronic). Completion of the entire procedure required no more than 20 min, thereby minimizing the risk of myocardial edema.

Measured LV volume (LVV) was adjusted to account for the volume contributed by the balloon material. Balloon volume was calculated from the weight of the balloon divided by latex density (0.898 g/ml). Because the weight of the balloon was 0.0215 g, the volume added to each diastolic volume was 0.024 ml.

After hemodynamic data were recorded, the heart was detached, the atria and great vessels were removed, and the ventricles were separated and weighed.

Diastolic Function Indexes

Myocardial relaxation. The active myocardial relaxation phase was assessed by the maximum rate of ventricular pressure fall (−dP/dt; mmHg/s).

Passive myocardial and ventricular stiffness. To assess myocardial diastolic stiffness for different LV weights and size hearts, stress (σ; g/cm²) and strain (ε; %) for the LV midwall were calculated assuming the LV to be a thick-walled sphere. The equations were as follows (49)

\[
\sigma = [1.36 \times LVP \times LVV^{3/2} - LVV^{2}] \quad (1)
\]

\[
\epsilon = ([LVV^{1/3} + (LVV + 0.943 \times LVW)^{1/3}] - 1) \times 100 \quad (2)
\]

where LVV is LV volume (ml), V₀ is LV at an end-diastolic pressure of 0 mmHg, LVW is LV weight (g), and LVP is either LV end-diastolic or peak isovolumetric pressure (mmHg).

Assuming that passive myocardial stress-strain relations are exponential in nature (27, 31), the relationship can be expressed as: \(\sigma = A + B e\), where σ and e are stress and strain, respectively; e is the natural logarithm; A is the y-axis intersection and B is the myocardial stiffness constant (K_MO). With a log transformation, log (σ) = A + Be. Thus B (K_MO) can be determined from the slope of the relationship between log (σ) and e.

Passive LV stiffness was analyzed by the end-diastolic pressure-volume relationship. Before the two groups were compared, V₀ was subtracted from all subsequent volumes (V − V₀) in each experiment so as to have all pressure-volume curves passing through the graph’s origin. In this way, curve deviations to the right or left reﬂect changes in the mechanical behavior of the chamber. LV passive stiffness was calculated using the equation: \(P = a + bV\), where P and V are the end-diastolic pressure and volume, respectively; a is the natural logarithm; a is y-axis intersection and b is the LV stiffness constant (K_V). With a log transformation, log (P) = a + bV. Thus b (K_V) can be determined from the slope of the log-to-volume relationship.

Systolic Function Indexes

Parameters assessed to evaluate systolic function are those most commonly utilized in this preparation (6, 21, 38, 46): 1) slope of end-systolic pressure-volume normalized relationship (ESPVRN; mmHg·ml⁻¹·g⁻¹) [volume was normalized to LVW as suggested by Suga et al. (44) and the mean ESPVRN curve was obtained; steeper slope indicates increased myocardial contractility]; 2) maximum rate of ventricular pressure rise (+dP/dt; mmHg/s); and 3) maximum developed pressure (DP_max; mmHg), corresponding to the difference between peak systolic pressure and diastolic pressure with the LV balloon ﬁlled to develop an end-diastolic pressure of 25 mmHg.

Biochemical and Morphometric Study

The animals assigned solely for morphometric and biochemical studies (CG, n = 12 and UG, n = 10) were anesthetized, and blood samples were taken for hematocrit and hemoglobin measurements. The hearts were then removed, the atria and great vessels were dissected free, and coronal sections of the left and right ventricles were ﬁxed in 10% buffered formalin and embedded in parafﬁn blocks. Four-micrometer-thick sections were cut from the blocked tissue and stained with hematoxylin-eosin. Transverse sections from the middle portion of the LV were used to determine the myocyte cross-sectional area (MSA, μm²) for a minimum of 100 myocytes in ﬁve hearts from both experimental groups, as previously described (26). The MSA was used as an indicator of cell size.

The V₀-to-body weight (μl/g) ratio was used as the parameter for normalized ventricular chamber size and LVW/V₀ (mg/μl) was used as the marker for modiﬁed ventricular
geometry. Also, LVW and right ventricular weight (RVW) were normalized to body weight. Myocardial edema was determined by analysis of the wet-to-dry LVW ratio. Myocardial hydroxyproline concentration (HOP; μg/mg), a marker for myocardial collagen, was measured in left and right ventricle apex tissue from 10 rats per group according to the methodology of Switzer (45). The tissue was dried for 4 h at 40°C using a SpeedVac concentrator SC 100 attached to a refrigerated condensation trap (TR 100) and vacuum pump (model VP 100; Savant Instruments, Farmingdale, NY). Tissue dry weight was determined, and the samples were hydrolyzed overnight at 110°C with 6 N HCl (1 ml/10 mg dry tissue). An aliquot of 50 μl of hydrolyzate was transferred to an Eppendorf tube and dried in the SpeedVac concentrator. Deionized water (1 ml) was added and the sample was transferred to a glass tube with a Teflon screw cap. To maintain constant pH, 1 ml of potassium borate buffer (pH 8.7) was added and the sample was oxidized with 0.3 ml of chloramine T solution at room temperature for exactly 10 s. The solution was then saturated with 1.5 g of KCl, 1 ml of 3.6 mol/l sodium thiosulfate and thoroughly mixing for 10 s. The solution was then saturated with 1.5 g of KCl, and the tubes were capped and heated in boiling water for 20 min. After being cooled to room temperature, the aqueous layer was extracted with 2.5 ml of toluene. Toluene extract (1 ml) was transferred to a 12 × 75-mm test tube, and 0.4 ml of Ehrlich’s reagent was added and left for 30 min to allow color to develop. Absorbencies were read at 565 nm against a reagent blank. Deionized water and 20 μg/ml HOP were used as blank and standard, respectively.

Statistical Analysis

Variable distribution was determined for group comparisons. If the distribution was normal, Student’s t-test was used and data were expressed as means ± SD. When data were nonnormally distributed, the Mann-Whitney method was used, and data were expressed as medians (range) (42). Differences were considered significant at a P < 0.05.

RESULTS

Undernourished animals demonstrated marked cachexia, judging by complete absence of adipose tissue. PCM protocol did not cause death or result in detectable peripheral edema.

Morphometric, Biochemical, and Tail Cuff Systolic Blood Pressure Data

Data from the body weight, MSA, and LVW and RVW (normalized to body weight) are presented in Table 1. Undernourished rats achieved 47% body weight, 44% LVW, and 51% RVW of the values observed in control animals. Also, MSA was significantly smaller in the UG compared with CG. Heart and body weight decreased proportionally, so that neither LVW-to-body weight or RVW-to-body weight ratios differed significantly between UG and CG, indicating that the heart was not spared in malnutrition.

Myocardial HOP concentration from both ventricles was higher in the UG, consistent with increased myocardial collagen concentration secondary to PCM (Fig. 1). The UG animals did not develop anemia because hematocrit (CG: 39.2 ± 2.1%; UG: 40.6 ± 4.0%; P > 0.05) and hemoglobin (CG: 14.4 ± 0.85 g/dl; UG: 15 ± 1.3 g/dl; P > 0.05) values were not statistically different from CG. Also, no significant difference in tail cuff systolic blood pressure (CG: 135 ± 15 mmHg; UG: 137 ± 15 mmHg; P > 0.05) or wet-to-dry LVW ratio (CC: 4.16 ± 0.16; UG: 3.26 ± 0.88, P > 0.05) was observed between the groups.

In Fig. 2A, the LV unstressed volume (V0) was significantly less in the UG compared with CG, apparently indicating decreased LV chamber size in malnutrition. However, V0 normalized to body weight was higher in UG, actually showing relative enlargement of the LV chamber in UG rats (Fig. 2B). Also, a modification of LV chamber geometry (eccentric remodeling) was observed because the LVW-to-V0 ratio was smaller in the UG, indicating a disproportionate reduction of LV mass in relationship to volume (Fig. 2C).

LV Function

Systolic function. Table 2 shows the functional systolic parameters obtained in isolated heart prepara-
tion. Myocardial contractility as measured by ESPVNR and +dP/dt was not influenced by malnutrition; however, a significant decrease in the DP_max index was noted for UG hearts.

Diastolic function. The active myocardial relaxation phase was not affected by PCM as assessed by –dP/dt (CG, 1,798 ± 209 mmHg/s; UG, 1,887 ± 216 mmHg/s; P > 0.05). Passive myocardial stiffness was significantly increased in malnutrition. The full range of the mean diastolic stress-strain curve of the UG rats was significantly shifted to the left compared with the control curve (Fig. 3). However, the weight-normalized mean end-diastolic pressure-volume relationship (V – V₀) curves from both groups did not show significant deviation (Fig. 4A); this indicated preserved mechanical LV chamber behavior in malnutrition despite increased myocardial stiffness. The weight-normalized volume-end-diastolic pressure relationship is graphed in Fig. 4B.

DISCUSSION

This study differs from previous reports in that PCM was induced from birth and the animals were studied while they were young adults. We have demonstrated that the PCM protocol caused structural alteration (ventricular eccentric remodeling) and myocardial diastolic dysfunction (increased passive stiffness) with preserved LV compliance.

Morphometry, Biochemistry, and Hemodynamics

Morphometric data showed that the cardiac muscle mass growth was impaired to the same extent as body

### Table 2. Isolated heart systolic function indexes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ESPVNR, mmHg ml⁻¹ g⁻¹</th>
<th>+dP/dt, mmHg/s</th>
<th>DP_max, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>453 (188–775)</td>
<td>3,006 ± 224</td>
<td>143 ± 13.8</td>
</tr>
<tr>
<td>Undernourished</td>
<td>10</td>
<td>411 (298–616)</td>
<td>3,012 ± 242.6</td>
<td>130 ± 7.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD and medians (range) for nonnormally distributed data (ESPVNR, end-systolic pressure-volume normalized relationship); n = number of rats per group. +dP/dt, maximum rate of ventricular pressure rise; DP_max, maximum developed pressure. *P < 0.01 by Student’s t-test.

Fig. 2. Indexes of left ventricular (LV) chamber size and geometry. A: LV unstressed volume (V₀). B: unstressed volume normalized to body weight (V₀/body wt). C: LV weight normalized to unstressed volume (LVW/V₀). Vertical bars represent means ± SD. *P < 0.01 compared to CG using Student’s t-test.

Fig. 3. Mean end-diastolic stress-strain relationships for the CG (n = 9) and undernourished group (UG, n = 10). The mean curve of UG rats (○) was found to be significantly shifted to the left compared with the CG (●). Bars represent means ± SD for average stress and strain corresponding to end-diastolic pressures of 5, 10, 15, 20, and 25 mmHg. K_MIO, myocardial passive stiffness constant. *P < 0.01 compared with CG using Student’s t-test.
weight, supporting the concept that the heart is not spared in chronic PCM. This concept was first described by Keys et al. in 1947 (20), and it was later validated by others (19, 25, 33, 47); however, in opposition to these studies, cardiac sparing has been observed with shorter duration PCM protocols limited to hours (14, 30, 39) or days (30). The rationale is that a normally developed heart and body subjected to starvation will undergo cachexia and atrophy in a presumably different fashion from the growth retardation caused by chronic malnutrition. In an individual of normal weight starved for a relatively short time, the heart would be relatively spared because of preferential catabolism of other tissues, such as fat or glycogen reserves. However, in chronic PCM due to stunted growth, the heart will never attain normal size because the smaller body will not place sufficient demands on the heart to induce additional cardiac growth.

Cardiac sparing during PCM has been reported in association with an anemia-induced hypermetabolic state (11, 18, 24, 30). In anemia, the necessity to maintain high cardiac output reduces LV muscle catabolism so that LV mass is maintained longer than body weight (3, 18, 35). Another factor that could potentially cause LV chamber structural changes during PCM is systolic blood pressure variation. There is a well-established relationship between concentric LV hypertrophy and pressure overload (31). As a corollary, the blood pressure reduction sometimes associated with PCM (15, 41) might inhibit LV hypertrophy. Our results allowed us to exclude hypotension and anemia as a source of LV morphometric modification because systolic blood pressure, hematocrit, and hemoglobin values were not statistically different between the groups.

To the best of our knowledge, this is the first study identifying eccentric LV chamber remodeling in rats submitted to PCM from birth. This architectural alteration was caused by a disproportionate mass reduction in relationship to LV volume. Several authors have tried to explain LV wall thinning induced by malnutrition. These explanations included a failure to deliver the metabolic requirements to the myocardium (41), a decrease in the synthesis of myofibrillar component (36), and an increase in myofibrillar protein catabolism (51). LV chamber enlargement and wall thinning has also been described in dogs (2, 3) and humans (34, 37) with malnutrition.

Another interesting finding was the increased myocardial HOP concentration in malnourished rats. Although our results do not allow a definitive conclusion, we would speculate that increased collagen synthesis might be due to local neurohormonal activation (8); however, another possibility is that chronic malnutrition has less of an affect on interstitial compartment development than impairment of myocyte compartment development because collagen is highly resistant to degradation and its turnover is relatively slow compared with other myocyte proteins (8).

LV Function

Systolic function. Instead of the expected systolic dysfunction in chronic PCM protocol, we found preserved myocardial contractility while using ESPVNR and \( \frac{dP}{dt} \) indexes. Similar findings were reported in studies performed on humans submitted to prolonged PCM protocols (13, 20). \( \frac{dP}{dt} \) analysis, however, revealed depressed contractility. This result should be interpreted with caution. According to many authors, myocardial contractile function is better assessed using indexes that consider myocyte-generated force.
(stress) and muscle deformation (strain) instead of developed pressure (9, 29, 48, 50). While using $DP_{\text{max}}$ to assess systolic function in isovolumetric preparation, Murad and Tucci (28) demonstrated that $DP_{\text{max}}$ might induce a misinterpretation on contractile function of the concentrically hypertrophied ventricle. This was explained by a rearrangement of Laplace’s equation so that $P = 2 \sigma / h^{1/2} R_i^{-1}$, where $P$ is the intraventricular pressure, $\sigma$ is wall stress, $R_i$ is LV internal radius, and $h$ is wall thickness. It can be seen that, for a given stress value, $P$ depends on the $h$-to-$R_i$ ratio. Because $h$ depends on myocardial mass and $R_i$ depends on chamber volume, it can be assumed that $DP_{\text{max}}$ directly depends on the mass-to-volume ratio. Therefore, developed pressure will increase in association with concentric hypertrophy even without true enhancement of myocardial contractility. Applying the previous assumption to eccentric remodeling, we could expect a false reduction in $DP_{\text{max}}$ because of the disproportional decrease of ventricular mass related to volume, as was observed in this study. Therefore, at this point, we have concluded that systolic function was not adversely affected in our UG animals.

**Diastolic function.** Various studies have separately assessed passive properties of cardiac muscle (30) and the LV chamber (2, 3), as well as the active relaxation phase (19, 21, 39); however, none has evaluated all these indexes in the same animal model as was done in this study.

We have demonstrated a significant shift to the left of the stress-strain relationship curve in the UG (Fig. 3), indicating increased myocardial stiffness. Many factors can interfere with passive myocardial mechanical behavior such as ischemia, muscle edema, and increased myocardial collagen concentration (31, 48). In our preparation, hypoxia was prevented and edema minimized by the short exposure time of the isolated heart to the apparatus. Also, both groups were subjected to similar experimental conditions. We therefore concluded that the increased myocardial stiffness was due to the relative increase in collagen concentration.

Interestingly, despite myocardial diastolic dysfunction demonstrated by the increased passive stiffness, LV chamber compliance was preserved as reflected by the end-diastolic pressure-volume relationship. Preserved LV chamber function was also described in undernourished dogs when the diastolic pressure-diameter relationship was assessed using ultrasound crystals (3). It is necessary to consider that LV compliance depends on pericardial constraints, ventricular interaction, myocardial properties, and chamber geometry (16, 17). Pericardium and ventricular interaction can be disregarded because, in our case, the pericardium was removed and the right ventricle worked without volume. Therefore, we assumed that preserved diastolic chamber function was the result of compensatory eccentric ventricular remodeling offsetting the increased myocardial passive stiffness. Although we have assumed that eccentric remodeling is a compensatory mechanism, it has to be pointed out that this type of architectural modification can be an early manifestation of cardiac dysfunction (5, 34, 37). Therefore, further studies are still required to determine how long this adaptation will be able to maintain normal diastolic function in the face of increased myocardial stiffness.

Despite limitations in extrapolating results from experimental studies into clinical practice, it seems relevant to suggest that increased myocardial stiffness may be responsible for the poor tolerance to volume administration in children during nutritional recovery; in this situation, congestive heart failure is common (37).

From the experimental data, it was concluded that 1) the heart is not spared from the adverse effects of PCM; 2) there is a geometric alteration in the LV (eccentric remodeling) determined by a disproportional reduction of LV mass in relationship to volume; 3) there is diastolic dysfunction of the myocardium (increased passive stiffness), probably due to the increased collagen concentration in the tissue, with unchanged LV compliance; and 4) systolic function is preserved.

We thank E. Jamas, V. M. Souza, and N. M. Ferreira for expert technical assistance and Colin Knaggs for text revision.

**REFERENCES**