The Effect of Chronic Protein-Calorie Undernutrition in the Rat on Myocardial Function and Cardiac Function

DONALD O. NUTTER, THOMAS G. MURRAY, STEVEN B. HEYMSFIELD, AND ELLEN O. FULLER

SUMMARY The effect of chronic protein-calorie undernutrition (PCU) on cardiac structure, biochemistry, myocardial function, and left ventricular dynamics was studied in young, male, Long-Evans rats. Chronic PCU produced marked cachexia of the marasmic type (body weight decreased to 48% of normally nourished control rats) and cardiac atrophy (heart weight at 57% of control). Myocardial structure on light microscopy was normal and myocardial edema (dry/wet weight) was not present. An increased left ventricular DNA content (1.82 ± 0.67 BE vs. 1.25 ± 0.58 pg/mg tissue wet weight) and collagen content (70.61 ± 4.54 vs. 31.72 ± 2.44 pg/mg, P < 0.001) in the presence of normal concentrations of RNA and actomyosin suggested a decrease in myofiber size with normal contractile proteins and protein synthesis. Resting length-tension curves for left ventricular papillary muscles failed to demonstrate alterations in myocardial stiffness with PCU. Active length-tension curves demonstrated enhanced myocardial contractility in chronic PCU hearts: peak developed isometric tension at U, was 4.84 ± 0.21 vs. 3.24 ± 0.31 g/mm², P < 0.01. The in situ heart preparation in anesthetized PCU rats demonstrated bradycardia, hypotension, and a depressed cardiac output when compared to control hearts. However, cardiac output adjusted for body weight was normal (0.048 ± 0.005 vs. 0.044 ± 0.002 ml/min per g), and ventricular function curves, using stroke work index, showed a normal cardiac reserve in PCU rats. We conclude that uncomplicated chronic PCU is accompanied by cardiac atrophy, normal or enhanced myocardial contractility, and left ventricular function that has adjusted to the decrease in body mass and metabolic requirements.

PROTEIN-CALORIE undernutrition (PCU) has been found to occur in 30–40% of hospitalized patients in industrialized societies and is recognized as a major medical problem in the general population of developing nations (Bistrian et al., 1976; Hill et al., 1977). Despite the medical importance of PCU, the effects of acute starvation and chronic PCU on cardiac structure, biochemistry, myocardial contractility, and cardiovascular dynamics are incompletely defined.

Cardiac atrophy has been found in children suffering from chronic PCU (Smythe et al., 1962; Wharton et al., 1969; Piza et al., 1971), in normal adult volunteers experimentally subjected to semistarvation (Keys et al., 1947), and in rats in which chronic PCU was induced (Svoboda et al., 1966; Cohen et al., 1976). Descriptions of myocardial histopathology in both human and experimental PCU have been quite variable and have included normal myocardial structure, interstitial edema, vacuolation, myofibrillar necrosis, and scattered fibrosis (Smythe et al., 1962; Wharton et al., 1969; Piza et al., 1971; Svoboda et al., 1966; Thomas et al., 1972; Chauhan et al., 1965.) Hemodynamic measurements have revealed bradycardia, hypotension, and a decrease in both cardiac output and work in undernourished human subjects (Alleyne, 1966; Viart, 1977). The design and methodology of these studies do not answer the question as to whether cardiac pump performance in cachexia is determined by general metabolic requirements and the resultant cardiac loading conditions or whether it results from intrinsic depression of cardiac contractility with latent heart failure.

In a recent investigation of adult patients with severe undernutrition of diverse etiologies, we found cardiac atrophy to be accompanied by normal left ventricular (LV) shortening indices and ejection fraction, systemic arterial hypotension, and a depressed cardiac output (Heymsfield et al., 1978a). Furthermore, when cardiac outputs were adjusted for body mass and metabolic rate, they were actually augmented relative to height-matched normal controls of the same sex. To examine the cardiac effects of PCU in more detail, the present investigation using chronic food deprivation was undertaken in young male rats. The specific aims of the study were to define the effects of PCU on cardiac structure, biochemistry, passive and active myocardial mechanics, and in situ cardiac dynamics.
Methods

One hundred and twenty-eight young (2.5 months), male, Long-Evans rats were acclimated to our animal facility for 1 week and then randomly assigned into four experimental groups: (1) starvation, \( n = 9 \); (2) starvation controls, \( n = 9 \); (3) chronic PCU, \( n = 55 \); and (4) chronic PCU controls, \( n = 55 \). The starved animals received no food for 7 days but had free access to water. On the 8th day they were killed for the study of myocardial mechanics, and their LV papillary muscles were used. Their controls received Purina rat chow and water ad libitum for 7 days and were then studied in the same manner. The PCU rats received 10 g of rat chow daily with water ad libitum for 6 weeks prior to their use in the study of either papillary mechanics, and their LV papillary muscles were used. Their controls received Purina rat chow and water ad libitum for 6 weeks and were then studied in the same manner. The PCU controls received 10 g of rat chow and water ad libitum for 6 weeks and were then studied in the same manner. The PCU rats, afforded the PCU rats approximately 33% of their gross energy requirements, 66% protein, 30% fat, and normal or nearly normal minimal levels of the B complex vitamins, vitamin D, calcium, phosphorus, and magnesium (Warner and Brever, 1972). The PCU controls, fed ad libitum for 6 weeks, also were used to study papillary muscle mechanics and heart hemodynamics in situ. The food intake of these control rats averaged 36 g daily. All rats were housed in individual cages in a room where temperature was maintained at 23°C and where they were exposed to alternating 12-hour light and dark intervals. The starved rats and their controls were weighed daily; the chronic PCU rats and their controls were weighed three times per week. The rats were anesthetized prior to study with pentobarbital sodium (50 mg/kg, ip).

Papillary Muscle Studies

Studies of active and passive myocardial mechanics were performed on left ventricular papillary muscles. Following a midsternal thoracotomy, the heart was excised, placed in oxygenated physiological buffer, and the small, anterolateral left ventricular papillary muscle was dissected free and mounted in a vertical, temperature-controlled muscle bath that contained a modified Krebs-Henseleit buffer, and the small, anterolateral left ventricular papillary muscle was dissected free and mounted in a vertical, temperature-controlled muscle bath that contained a modified Krebs-Henseleit buffer solution gassed with 95% oxygen and 5% carbon dioxide at a temperature of 30°C. The concentrations of bath constituents in mM were: deoxyglucose, 16.6; NaCl, 118.5; KCl, 4.7; CaCl\(_2\), 2.5; MgSO\(_4\), 1.2; KH\(_2\)PO\(_4\), 1.2; and NaHCO\(_3\), 25.0. The holders used to clamp the specimens in place could be adjusted to permit calibrated length changes and contained bipolar field electrodes. The opposite end of the muscle was attached by an inelastic suture to an isometric force transducer (Statham, model UC2) mounted above the bath. Muscles were stimulated at 20 times per minute with a Grass stimulator (model SD9) using monophasic pulses of 10-msec duration that exceeded threshold voltage by 25%. Calibrated isometric tension and the rate of tension development, obtained with an active differentiator circuit, were monitored and photographically recorded (Electronics for Medicine, model DR8). The muscles were preloaded at 1 g and allowed to contract for a 1-hour equilibration period. The length corresponding to the peak of the length-active tension relationship (\( L_{\text{max}} \)) was then determined by progressively stretching the specimen from its unloaded (zero force) length to the point where active tension plateaued. The muscle length at \( L_{\text{max}} \) was measured with a telescope cathetometer, and the length was adjusted to 84% of \( L_{\text{max}} \). Length-tension curves were then performed by stretching the muscle from 84% of \( L_{\text{max}} \) to 106% of \( L_{\text{max}} \) in 2% increments. Bath gas tensions (\( P_O_2 \) and \( P_CO_2 \)) and pH were determined before and after the length-tension curves with a Radiometer blood gas analyzer.

The variables describing isometric contraction and resting tension in papillary muscles were obtained after 2 minutes of equilibration at each length and were normalized for muscle cross-sectional area. Previous analysis of papillary muscle isometric tension data in our laboratory has shown no correlation between peak developed tension and the total cross-sectional area of the muscle below an area of 1.2 mm\(^2\). For this reason, in the present study, results from papillary muscles whose cross-sectional area exceeded 1.2 mm\(^2\) were excluded. To provide body weight-matched controls for the PCU rats, papillary muscle studies also were performed in six young (2 months), lean, normally nourished, control rats.

In Situ Hemodynamic Studies

Rats used for hemodynamic studies were anesthetized with pentobarbital sodium (50 mg/kg, ip), intubated with a tracheal cannula, and ventilated with 100% oxygen, using a volume respirator (Harvard Apparatus rodent respirator, model 681). Arterial blood gas and pH values guided the adjustment of respiratory rate and tidal volume. The rats were immobilized on a surgical board containing a rheostat-controlled heating element so that body temperature, monitored by a rectal thermistor probe (Yellow Springs Instruments, model 43TD), could be maintained in the physiological range. A laparotomy was performed, a polyethylene cannula was placed in the abdominal aorta through an arteriotomy made below the renal arterial level, and the abdominal wall was closed. This cannula was advanced to the thoracic aorta, heparinized, and connected directly to a Statham P23Db transducer to record systemic arterial pressure. A polyethylene cannula was placed in the left common jugular vein and advanced to the superior vena cava to administer fluids and anesthetic. A midsternal thoracotomy was performed and an electromagnetic flow probe (model DR8) was mounted above the superior vena cava to monitor right atrial pressure. The heart was excised, placed in oxygenated physiological buffer, and the small, anterolateral left ventricular papillary muscle was dissected free and mounted in a vertical, temperature-controlled muscle bath that contained a modified Krebs-Henseleit buffer solution gassed with 95% oxygen and 5% carbon dioxide at a temperature of 30°C. The concentrations of bath constituents in mM were: deoxyglucose, 16.6; NaCl, 118.5; KCl, 4.7; CaCl\(_2\), 2.5; MgSO\(_4\), 1.2; KH\(_2\)PO\(_4\), 1.2; and NaHCO\(_3\), 25.0. The holders used to clamp the specimens in place could be adjusted to permit calibrated length changes and contained bipolar field electrodes. The opposite end of the muscle was attached by an inelastic suture to an isometric force transducer (Statham, model UC2) mounted above the bath. Muscles were stimulated at 20 times per minute with a Grass stimulator (model SD9) using monophasic pulses of 10-msec duration that exceeded threshold voltage by 25%. Calibrated isometric tension and the rate of tension development, obtained with an active differentiator circuit, were monitored and photographically recorded (Electronics for Medicine, model DR8). The muscles were preloaded at 1 g and allowed to contract for a 1-hour equilibration period. The length corresponding to the peak of the length-active tension relationship (\( L_{\text{max}} \)) was then determined by progressively stretching the specimen from its unloaded (zero force) length to the point where active tension plateaued. The muscle length at \( L_{\text{max}} \) was measured with a telescope cathetometer, and the length was adjusted to 84% of \( L_{\text{max}} \). Length-tension curves were then performed by stretching the muscle from 84% of \( L_{\text{max}} \) to 106% of \( L_{\text{max}} \) in 2% increments. Bath gas tensions (\( P_O_2 \) and \( P_CO_2 \)) and pH were determined before and after the length-tension curves with a Radiometer blood gas analyzer.

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probe (Zapeda Instruments, type RA, 2.0-2.5 mm i.d.) placed around the ascending aorta. Pulsatile and mean aortic flow was monitored with a Zapeda flowmeter, model SWF4. The flow probes were calibrated on an excised segment of rat thoracic aorta by the timed collection of blood that perfused the segment at various rates from a gravity reservoir. A 21-gauge, stainless steel needle cannula was passed through the lateral chest wall and placed in the midportion of the LV chamber by direct puncture of the LV lateral wall. The cannula was connected directly to a Statham P23Db transducer, positioned outside the chest wall, and used to record LV pressures and the first derivative of pressure. Heart rate was recorded continuously from a cardiotachometer that was triggered by either the LV or systemic arterial pressure pulse. All hemodynamic variables were recorded on an Electronics for Medicine recorder (DR8).

After a 15-minute equilibration period, which included the adjustment of body temperature, arterial blood gases, and pH, resting hemodynamics (heart rate, mean systemic arterial pressure, LV systolic and end-diastolic pressures, maximal LV dp/dt, and mean aortic flow) were recorded. The hemodynamic variables required for calculating a ventricular function curve were recorded continuously during the constant rate infusion (2.25 ml/min) of saline through the venous cannula. This infusion was maintained until aortic flow and systemic pressure peaked. LV stroke work was calculated by the equation: SW(gM/beat) = (stroke volume) (mean aortic pressure) (1.36)/100. Ventricular function curves were constructed by plotting calculated values of cardiac output, stroke volume, and stroke work (both in absolute units and after adjustment for body weight) at LV end-diastolic pressures (LVEDP) of 2, 4, 6, 8, and 10 mm Hg. These values of LV filling pressure were achieved consistently during the performance of saline infusions. LV function (cardiac output) plateaued between 6 and 10 mm Hg LVEDP in all hearts from both control and PCU groups.

Histology and Chemistry

Body weight was obtained immediately before each rat was killed. After excision of a papillary muscle or the performance of in situ hemodynamics, the heart was dissected, trimmed of fat and vascular connections, and weighed in the wet (blotted) condition. A small sample was taken from the LV apex, weighed wet, and oven dried at 90°C for 24 hours before reweighing to permit calculation of dry-to-wet weight ratios. A section of the LV free wall, midway from base to apex, was removed from 18 PCU and 18 control rats used for papillary muscle studies. These sections were fixed in buffered 10% formalin for 48 hours and then stored in refrigerated sucrose buffer. Microsections were cut from paraffin-embedded samples and stained with Harris-Lilie hemotoxylin and aqueous eosin for subsequent histological examination by a pathologist.

The left ventricles of 25 PCU and 17 control rats used for hemodynamic studies were dissected from the remaining chambers and valvular tissue and frozen at minus 4°C for future biochemical analysis. Individual ventricles were thawed, homogenized in ice cold distilled water (20% homogenate), and homogenate was divided for analysis. RNA was estimated by the orcinol method (Schneider, 1957) and DNA by the diphenylamine method (Richards, 1974). Hydroxyproline was determined by a modification of the method of Kivirikko et al. (1967), and multiplied by 7.46 to obtain collagen values. The remaining ventricles were paired and homogenized with cold Weber-Edsall solution. Actomyosin content then was estimated according to the method of Benson et al. (1955).

Hematocrits and serum chemistries (total protein, albumin, calcium, magnesium, phosphorus, potassium, and sodium) were performed on caval blood drawn from PCU and control rats used for papillary muscle studies.

Results

Body Weight and Heart Weight

Table 1 presents body weight and heart weight data from both the starved and PCU rats. One week of food deprivation was not associated with death or overt evidence of illness in the starvation group of rats. They lost 22% of their initial body weight during the 7 days of starvation, whereas their ad libitum fed controls gained 4% in body weight. On the other hand, 12 of the 55 rats fed 10 g of chow daily for 6 weeks died during the 4th to 6th weeks of the protocol. All surviving rats demonstrated a considerable degree of generalized weakness at the end of the 6th week. The average body weight of the PCU rats decreased by 44% (432 ± 16 SE to 242 ± 10 g, P < 0.001). None of the chronic PCU control rats died, and the body weight in this group increased by 27% (402 ± 17 to 512 ± 15 g, P < 0.001) over the 6-week period. The final body weight of PCU rats was approximately 48% of the final body weight of the control rats. Gross dissection of the PCU rats revealed a complete absence of adipose tissue.

Cardiac atrophy was detectable in the acutely starved rats, whose average heart weight was 85% of the heart weight in control rats, and was marked in the chronic PCU rats in which heart weight was 56% of control (0.674 ± 0.017 vs. 1.211 ± 0.032 g, P < 0.001). When heart weight was expressed as a percentage of total body weight (Table 1), the values were higher significantly in both starved and PCU rats, suggesting a degree of cardiac sparing in undernutrition. The relative sparing of cardiac mass in the undernourished rats cannot be explained by myocardial edema, since there were no significant
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Table 1: Heart Weight and Body Weight Data for Undernourished and Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body wt (g)</th>
<th>Terminal body wt (g)</th>
<th>% Control body wt</th>
<th>Heart wt (g)</th>
<th>% Control heart wt</th>
<th>Heart wt (g/100 g BW)</th>
<th>Dry/wet heart weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starvation (n = 9)</td>
<td>405 ± 20</td>
<td>316 ± 16*</td>
<td>80</td>
<td>0.983 ± 0.025*</td>
<td>85</td>
<td>0.282 ± 0.010</td>
<td>0.235 ± 0.004</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>379 ± 11</td>
<td>393 ± 11</td>
<td>100</td>
<td>1.036 ± 0.026</td>
<td>100</td>
<td>0.264 ± 0.051</td>
<td>0.231 ± 0.006</td>
</tr>
<tr>
<td>PCU (n = 28)</td>
<td>417 ± 21</td>
<td>242 ± 10†</td>
<td>58</td>
<td>0.674 ± 0.017</td>
<td>56</td>
<td>0.294 ± 0.006†</td>
<td>0.241 ± 0.005</td>
</tr>
<tr>
<td>Control (n = 29)</td>
<td>402 ± 17</td>
<td>512 ± 15</td>
<td>130</td>
<td>1.211 ± 0.032</td>
<td>130</td>
<td>0.237 ± 0.004</td>
<td>0.228 ± 0.006</td>
</tr>
</tbody>
</table>

Starvation = 7 days without food; PCU = 6 weeks of PCU. Results are expressed as mean ± SE.
*P < 0.005, †P < 0.001, experimental vs. control determined by unpaired Student’s t-test.

Differences in dry to wet heart weight ratios in any of the groups.

Histology and Chemistry

Histological examination by light microscopy of LV sections from 18 chronic PCU rats also failed to demonstrate interstitial edema. There were no pathological alternations in the left ventricles of these undernourished rats aside from what appeared to be a modest decrease in myofiber size.

The levels of RNA and actomyosin per unit of LV wet weight did not differ between PCU and control hearts (Table 2). RNA was decreased by 5% and actomyosin was decreased by 9% in PCU left ventricles. DNA and collagen concentrations were increased significantly in the LV muscle of PCU hearts, and the RNA/DNA ratio in these hearts was depressed. DNA was increased by 46%, RNA/DNA ratio decreased by 31%, and collagen increased by 123% in PCU left ventricles.

Serum protein levels were depressed in six chronic PCU rats compared to 13 control rats. Total protein was 4.50 ± 0.26 g/dl in PCU vs. 5.79 ± 0.16 g/dl in control (P < 0.001). Serum albumin was 2.98 ± 0.17 g/dl in PCU vs. 4.42 ± 0.15 g/dl in control (P < 0.001). A mild depression of hematocrit was noted when 13 PCU rats (37.9 ± 0.9%) were compared with 22 controls (43.8 ± 0.9%, P < 0.001).

Papillary Muscle Mechanics

The resting length-tension relationships for starved and PCU rats and their respective ad libitum fed controls are illustrated in Figure 1. These curves, plotting mean resting tension in LV papillary muscles against resting muscle length expressed as a percent of Lmax, fail to show any difference in myocardial compliance among the various groups of rats. Active length-tension relationships (peak developed tension vs. resting muscle length) are presented in Figure 2. Since the age-matched control rats were quite obese after 6 weeks of ad libitum feeding, isometric mechanics also were studied in papillary muscles from a weight-matched control of young (2 months) rats (body weight 265 ± 7 g). In addition, papillary muscles whose total cross-sectional area (0.61 ± 0.27 mm²) was equivalent to that of PCU muscles (0.63 ± 0.05 mm²) were selected from the control groups, and their length-tension data was plotted as a third control curve. The length-tension curves from all three of these control groups were virtually identical, whereas papillary muscles from the chronic PCU rats manifest significantly augmented contractility. The active length-tension curve of papillary muscles from the starved rats was also augmented and fell midway between the tension values from PCU muscles and the various controls. Active length-tension curves using maximal dT/dt instead of peak developed tension also demonstrated augmented contractility in the muscles from PCU and starved rats.

Table 2: Chemical Analyses of Left Ventricles from Chronically Undernourished and Control Rats

<table>
<thead>
<tr>
<th>Nucleic Acids/Acmyosin</th>
<th>PCU (mean ± SE)</th>
<th>Control (mean ± SE)</th>
<th>P value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (µg/mg)</td>
<td>11 1.82 ± 0.10</td>
<td>15 1.25 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RNA (µg/mg)</td>
<td>11 2.25 ± 0.16</td>
<td>15 2.36 ± 0.28</td>
<td>NS</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>11 1.33 ± 0.14</td>
<td>15 1.94 ± 0.18</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Actomyosin (µg/mg)</td>
<td>11 20.49 ± 2.12</td>
<td>15 22.57 ± 2.62</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen (µg/mg)</td>
<td>11 70.61 ± 4.54</td>
<td>12 31.72 ± 2.44</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PCU = 6-week PCU rats, n = number of LV samples assayed, except for actomyosin where n = number of paired LV samples assayed. Results are expressed as micrograms per milligram of left ventricle wet weight. There was no difference in dry-to-wet weight ratios between the PCU and control tissues. NS = not significant.
FIGURE 1 Mean resting tension of LV papillary muscles (grams per mm$^2$ of muscle cross-sectional area) from starved rats $\bullet \cdots \bullet$ ($n=8$) and their controls $\circ \cdots \circ$ ($n=6$), and from chronic PCU rats $\circ \bullet$ ($n=16$) and their controls $\circ \circ$ ($n=18$) plotted against muscle length over the range from 84 to 102% of $L_{\text{max}}$. The tension values did not differ significantly between any of the curves when tested at 100% $L_{\text{max}}$.

Tension variables and contraction-relaxation intervals measured at $L_{\text{max}}$ are presented in Table 3. In addition to a greater level of contractility (peak developed tension and maximal $dT/dt$), the papillary muscles from the PCU hearts demonstrate a significant increase in the time to peak tension and in the recovery time.

In Situ Hearts

Baseline hemodynamic measurements in anesthetized chronic PCU rats and their controls are presented in Table 4. Initial body temperature was lower in PCU rats (mean 35.7 ± 0.3°C) than in control rats (36.9 ± 0.3°C) ($P < 0.01$). Chronic PCU rats demonstrated a significant depression in heart rate, aortic pressure, LV peak systolic pressure, $L_{\text{max}}$, $dP/dt$, cardiac output, and stroke volume. Their calculated total systemic resistance was increased when compared to controls. When cardiac output and stroke volume were calculated per gram of body weight, the resultant cardiac and stroke indices were actually higher in PCU than in control rats. Cardiac and stroke indices were usually higher in PCU than in control rats. LV function curves using stroke work as a function of LVEDP are shown in Figure 3. Whether absolute values of stroke volume, stroke work, or cardiac output were employed, the function curves for chronic PCU hearts were significantly depressed. However, when stroke volume index, stroke work index, and cardiac index based on body mass were substituted, no difference was noted in LV function of PCU and control hearts.

Discussion

Cachexia and Cardiac Atrophy

The chronic PCU protocol employed in the present study featured a marked reduction in consumption of a normal diet which resulted in a moderate reduction in dietary protein (approximate deficit of 33%) and a severe reduction in caloric content (approximate deficit of 65-75% in fat and energy content). A marasmic type of cachexia results from this form of chronic undernutrition and is the pattern most often encountered in affluent, developed countries where anorexia nervosa, cancer, and chronic digestive system disorders are the primary etiological factors. In contrast, the Kwashiorkor type of cachexia frequently seen in the children in impoverished, underdeveloped nations results from an

FIGURE 2 Mean peak developed isometric tension of LV papillary muscles (grams per mm$^2$ of muscle cross-sectional area) from starved rats $\bullet \cdots \bullet$ ($n=9$) and their controls $\circ \cdots \circ$ ($n=7$) and from chronic PCU rats $\bullet \bullet$ ($n=16$) and three groups of control rats fed ad libitum. Obese controls $\bigtriangleup \bigtriangleup$ ($n=17$); young, lean, normally nourished controls $\bigcirc \bigcirc$ ($n=6$); and controls with papillary cross-sectional area matched to PCU muscles (0.62 vs. 0.63 mm$^2$) $\square \square$ ($n=10$). The vertical bars at 100% $L_{\text{max}}$ represent standard errors of the mean for PCU, starved, obese PCU control and starvation control rats. $^* P < 0.001$ when PCU values at $L_{\text{max}}$ were compared with the obese control group using Student's t-test.
almost complete absence of dietary protein with a variable reduction in caloric intake. Marasmic cachexia is characterized by the absence of adipose tissue, hepatic atrophy, and only modest depression of serum protein levels without development of the edema or ascites that are characteristic of Kwashiorkor. Our PCU rats demonstrated marked cachexia, judging by total weight loss (44% of original body weight), a fatality rate of 22% after the 4th week of undernutrition, diffuse muscular weakness in the surviving rats, and the complete absence of edema or evidence of myocardial edema when cardiac dry to wet weight ratios and histological sections of the left ventricle were examined.

A relative degree of cardiac sparing is suggested in our chronic PCU rats by their increased heart weight-to-body weight index and the observation that PCU body weight was 48% of control, whereas heart weight was 57% of control (Table 1). Cardiac atrophy during undernutrition probably occurs as a result of the decreased cardiac work performed with cachexia, whereas sparing of the cardiac mass relative to the skeletal muscle and other organs could reflect the constant demand for cardiac work and the heart’s ability to maintain protein synthesis and work output from a wide variety of substrates (Bing et al., 1954; Rannels et al., 1974). The data for myocardial biochemistry (Table 2) indicate that RNA and actomyosin concentrations were unchanged by chronic PCU, although the total amounts of these compounds fell as heart size decreased. These findings occurred in the absence of histopathological changes in the left ventricle and are compatible with the maintenance of a stable rate of protein turnover to match cardiac work. This is in contrast to the increased RNA concentration that accompanies myocardial growth or hypertrophy (Nair et al., 1971). The observed increase in DNA and collagen concentration in PCU does not necessarily imply de novo synthesis of these components, but probably represents a relative increase related to a decrease in myofiber size, as well as an

<table>
<thead>
<tr>
<th>Group</th>
<th>Papillary muscle area (mm²)</th>
<th>Resting tension (g/mm²)</th>
<th>Peak developed tension (g/mm²)</th>
<th>Max dT/dt (g/sec per mm²)</th>
<th>TPT (msec)</th>
<th>1/2 RT (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starvation (n = 9)</td>
<td>0.84 ± 0.11</td>
<td>2.22 ± 0.32</td>
<td>3.73 ± 0.51</td>
<td>56.75 ± 7.90</td>
<td>119 ± 3</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>Controls (n = 8)</td>
<td>0.78 ± 0.10</td>
<td>1.78 ± 0.14</td>
<td>3.02 ± 0.36</td>
<td>49.94 ± 6.12</td>
<td>106 ± 3</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>PCU (n = 16)</td>
<td>0.63 ± 0.06</td>
<td>2.20 ± 0.20</td>
<td>4.84 ± 0.21</td>
<td>66.07 ± 2.35</td>
<td>137 ± 3</td>
<td>124 ± 5</td>
</tr>
<tr>
<td>Control 1 (n = 17)</td>
<td>0.89 ± 0.04</td>
<td>2.00 ± 0.14</td>
<td>3.24 ± 0.31</td>
<td>50.06 ± 4.35</td>
<td>121 ± 2</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Control 2 (n = 6)</td>
<td>0.86 ± 0.10</td>
<td>1.64 ± 0.22</td>
<td>3.18 ± 0.34</td>
<td>50.66 ± 4.97</td>
<td>109 ± 5</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>Control 3 (n = 10)</td>
<td>0.62 ± 0.03</td>
<td>2.18 ± 0.17</td>
<td>3.41 ± 0.44</td>
<td>51.62 ± 5.03</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Papillary muscle area = cross-sectional area; TPT = time onset of contraction to peak tension; 1/2 RT = time from peak tension to 60% peak tension; Starvation = 7-day total food deprivation rate; PCU = 5-week PCU rats; PCU control 1 = age-matched, ad libitum-fed rats; PCU Control 2 = young (2-month) body weight-matched controls; PCU Control 3 = papillary muscle data selected from controls 1 and 2 matched for muscle cross-sectional area. P values indicate significant t-test results when experimental group was compared with control group.
Cachexia and Cardiac Function

The present study demonstrates that normal myocardial function prevails in rats exposed to uncomplicated acute and chronic PCU. Passive length-tension curves from LV papillary muscles failed to suggest differences in myocardial stiffness between the hearts of acutely starved, chronically undernourished, and normally nourished rats. Active length-tension curves from isometrically contracting papillary muscles demonstrated that chronic PCU was associated with a significantly enhanced state of myocardial contractility. Three control groups of healthy, normally nourished male rats were included to permit the matching of age, total body mass, and total papillary muscle area with those present in the PCU group. The contractility results were unaffected by these parameters. A relationship between the degree and/or the duration of undernutrition and contractility is suggested by the qualitatively similar, but less marked, myocardial response seen in rats starved for 7 days.

The absence of interstitial edema, fibrosis, or myofiber damage and the presence of normal myocardial actomyosin and RNA concentrations suggest that normal myocardial contractility should be maintained in the hearts of our PCU rats. We have no explanation for the apparent enhancement of contractile state in these rats. The observation that myocardial contractility is preserved or even enhanced in chronic PCU, however, is not without precedent. Cohen et al. (1976) demonstrated that isometric peak tension and the time-to-peak tension were significantly increased in the LV myocardium of chronically undernourished rats compared to normally nourished or thiamine-deficient rats. Normal or enhanced LV function at rest and during exercise also has been demonstrated in cachectic human patients with cardiac atrophy (Heymsfield et al., 1978a; Gottdiener et al., 1978). The presence of normal or augmented myocardial contractility makes it unlikely that latent cardiac failure exists in PCU and supports the concept that the depressed levels of cardiac output and systemic arterial pressure in PCU animals and patients are the result of chronic cardiovascular loading conditions and not intrinsic myocardial dysfunction. It should be noted that if far advanced PCU were accompanied by myocardial edema, myocardial necrosis and fibrosis, or a significant depletion of myocardial inorganic components such as calcium, phosphorus, or magnesium, impairment of myocardial contractility might be an expected consequence.

Cachexia and Myocardial Function

Increased number of nuclei and normal connective tissue components per unit of cardiac mass. This is also in contrast to the unchanged concentration of DNA and increased RNA/DNA ratio present during myocardial hypertrophy, where observed increases in total DNA have been shown to be due to hyperplasia of nonmyocardial tissue elements (Nair et al., 1971; Neffgen and Korecky, 1972).

There was no evidence of cardiac abnormality by light microscopy in our undernourished rats. Although few in number, pathological studies of human and animal hearts in undernutrition tend to support this observation (Smythe et al., 1962; Wharton et al., 1969; Svoboda et al., 1966). The observations of Piza et al. (1971) in malnourished children and those of Chauhan et al. (1965) in young rhesus monkeys that were exposed to severe, chronic protein undernutrition stand in contrast to these reports. These authors found extensive evidence of interstitial edema, cellular atrophy and damage, and fibrosis in the hearts of their malnourished patients and subjects.
anesthetized PCU rats. Ventricular function curves constructed by plotting stroke volume, cardiac output, or stroke work against LV filling pressure suggested a decreased cardiac reserve capacity in PCU rats. These results correspond to previous reports that have described a hypodynamic circulatory state in humans and animals suffering from, or exposed to, chronic undernutrition. When our data from the hearts of PCU rats were reexamined by calculating indices of stroke volume, cardiac output, and stroke work based on nondiastatic body mass, the resultant values equaled or exceeded those from control hearts. Furthermore, ventricular function curves constructed with stroke volume index and stroke work index (Fig. 3) indicated that cardiac reserve was normal in the PCU heart. These data suggest that ventricular function is adjusted to meet altered metabolic requirements in the undernourished body. This hypothesis is supported by the work of Landsberg and Young (1978) who showed that decreased sympathoadrenal activity and depression of active triiodothyronine levels occur in the fasted rat. These neural and hormonal alterations depress metabolic rate and hence the demand for cardiac output. Several reports from studies of malnourished humans also have indicated that total oxygen consumption is depressed but that oxygen consumption indices are in the normal range (Montgomery, 1962; Heymsfield et al., 1978b; Keys et al., 1950). An adaptive response in cardiac dynamics during PCU, as opposed to myocardial depression and cardiac dysfunction, is also favored by the prompt increase in heart rate, systemic pressure, and cardiac output that occurs when metabolic rate and circulatory demand increase during nutritional repletion of the malnourished subject (Alleyne, 1966; Heymsfield et al., 1978a; Keys et al., 1950).

Myocardial contractility and ventricular function, as evaluated in our animal model of uncomplicated chronic PCU, are normal and suggest that the atrophied heart of PCU falls at one end of a cardiac function spectrum that represents the matching of heart size and performance to chronic hemodynamic loading conditions and systemic metabolic requirements. These results may be applicable to the marasmic form of human cachexia but probably cannot be extrapolated to all forms of undernutrition. The presence of complicating factors, such as significant depletion of certain inorganic ions or vitamins and severe protein deprivation (Kwashiorkor), may result in pathological changes in the myocardium, depressed contractility, and cardiac dysfunction.

Acknowledgments

We appreciate the technical assistance provided by Albert Landers, Susan Tower, and Marijana Clark. Dr. Charles Sewell interpreted the histological sections of left ventricular tissue. Charline Hutton and Juanta Maclin provided valuable secretarial assistance.

References

Heymsfield SB, Bethel RA, Anody JD, Gibbs DM, Felner JM, Nutter DO (1978a) Cardiac abnormalities in cachectic patients before and during nutritional repletion. Am Heart J 95: 584-594
The Biology of Human Starvation, vol I. Minneapolis, University of Minnesota Press, pp 607-634
Toro H, de la malnutrition protidigue. Med Trop 32: 505-518

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The effect of chronic protein-calorie undernutrition in the rat on myocardial function and cardiac function.
D O Nutter, T G Murray, S B Heymsfield and E O Fuller

_Circ Res._ 1979;45:144-152
doi: 10.1161/01.RES.45.1.144

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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