Differences in Load Dependence of Relaxation Between the Left and Right Ventricular Myocardium as a Function of Age in Rats

Joseph M. Capasso, Emily Puntillo, Giorgio Olivetti, and Piero Anversa

To determine whether the variation in the magnitude of work load sustained by the left and right ventricles during adulthood and senescence affects the load-dependent aspect of relaxation, posterior papillary muscles from the left and right ventricles of rats at 4, 10, and 20 months of age were studied under variably loaded conditions in vitro. Because of differences between the life spans of Fischer and Sprague-Dawley rats, the functional characteristics of relaxation were investigated to evaluate the possibility of a differential age-associated response in these two strains of animals. The kinetic performance of the diastolic phase of myocardial contraction was measured by assessing the relative time during which load bearing occurred in a series of afterloaded isotonic twitches. This measurement was expressed as the ratio of the duration of afterloaded isotonic shortening and relengthening to the time required for isometric force to decline to the same level during isometric relaxation. A ratio of less than unity identified a load-dependent state whereas a value greater than one reflected a load-independent condition. Results showed that the right myocardium was completely load independent whereas the left myocardium was fully load dependent at all physiological afterloads. Aging reduced the load independence of the right ventricle and the load dependence of the left ventricle in Fischer rats. In contrast, no aging effect on the properties of afterloaded isotonic relaxation was seen in Sprague-Dawley rats. In conclusion, distinct differences exist in the mechanical dynamics of inactivation between the left and right ventricular myocardium. Aging reduced these variations in Fischer rats but had no apparent influence in Sprague-Dawley animals up to 20 months after birth. (Circulation Research 1989;65:1499-1507)

Myocardial relaxation is a process whereby the myocardium returns to its precontraction tension and/or length after active force development and/or fiber shortening. The kinetic performance of the relaxation phase of the cardiac cycle is dependent on the interplay of the active removal of sarcoplasmic calcium ions, the crossbridge life cycle, and the prevailing loading conditions. The first two occurrences comprise the deactivation-dependent aspect of the relaxation process whereas the latter one reflects the load-dependent component of myocardial inactivation. However, the prevailing load is the dominant element in relaxation of muscle tissue in mammals when the capacity of the sarcoplasmic reticulum to uptake and release calcium is maintained.

Dissimilar load-bearing capacities between the left and right ventricles are present during early postnatal, adult, and aged life despite an identical stroke volume. This variation in physiological loading on the two pumps results in markedly different chamber geometry, hemodynamic performance, and mechanical characteristics. The decay rate of sarcoplasmic calcium is significantly lower in the right than in the left ventricle, and the calcium content of the myocardium is consistently greater in the right than in the left side of the heart. In addition, alterations in loading conditions can modulate calcium binding to the contractile proteins of myocytes with a direct relation existing between load on the individual cells and affinity of troponin C for calcium. In this regard, aging has been shown to affect the extent of load on a cellular basis in a distinct fashion in the two ventricles. Myocyte cell loss occurs as a function of age predominantly in the left ventricle and elevates systolic and diastolic stress on the remaining viable cells.

Therefore, the present study investigates whether load dependence of relaxation is affected by the
chronic differences in loading conditions that exist on the left and right ventricles throughout life. In addition, the possibility for a differential response of the myocardium of Fischer 344 and Sprague-Dawley rats was investigated since animals of the latter strain live longer and are characterized by a preservation of cardiac hemodynamics up to almost 2 years of age.

Materials and Methods

Male Sprague-Dawley rats (Harlan Sprague-Dawley) at 4, 10, and 20 months of age were used. Each group consisted of 8, 8, and 9 randomly bred animals, respectively. Identical groups of male Fischer rats (Harlan Sprague-Dawley) of the same age intervals were also used.

Animals were anesthetized with ether; the heart was rapidly excised and placed in oxygenated Tyrode’s solution containing 30 mM potassium to induce diastolic arrest. The left and right posterior papillary muscles were removed and suspended side by side in a muscle bath. The nontendinous end of each papillary muscle was inserted into a collet that was mounted to the end of a micrometer assembly that was used to adjust external muscle length. The tendinous end of the papillary muscle was tied to a steel wire with Ethicon braided silk. This was done to minimize muscle damage in the preparation. The wire was attached to the lever extension of a servo-controlled galvanometer. The location of the lever was measured by a variable capacitor positioned at the rear of the galvanometer’s moving iron core. Force at the tip of the lever was determined by scaling and amplifying the error signal produced in the position servo section of the circuitry during the contraction. These electronic feedback controls permitted operation in either afterloaded isotonic or isometric modes.

The muscles were continuously perfused with Tyrode’s solution of the following composition (mM): Na+ 151.3, Ca2+ 2.4, K+ 4.0, Mg2+ 0.5, Cl− 147.3, H2PO4 12.0, and dextrose 5.5. This solution was maintained at 30°C and gassed with 95% O2-5% CO2, which yielded a pH of 7.2. Preparations were stimulated at 0.1 Hz by rectangular depolarizing pulses 10 msec in duration and twice diastolic threshold in intensity. All contractions were made from an initial muscle length that was associated with maximum developed isometric force (Lmax). Lmax was determined from the actively developed and passive stress-strain relations after an equilibrium period of 120 minutes during which the muscle contracted isometrically with a resting stress of approximately 9.8 mN/mm². The stress-strain curve was generated by reducing muscle length in 0.1-mm steps from Lmax to approximately 90% of Lmax while recording resting and developed tensions. Step changes were made in a reproducible sequence to minimize the effects of hysteresis. Isometric contractions for measurement were determined after 10 identical isometric contractions to minimize the effects of prior loading history.

The approach to the characterization of load dependency in cardiac muscle has been described by Brutsaert, Sys, and coworkers. Since differences in isotropic state may play a role in the comparison of load dependency of muscles removed from the left and right ventricle and from animals of different ages and strains, the methodology of Sys et al was used in which the relation between the relative load (isotonic load/isometric load [L/L]) and the ratio of isotonic to isometric contraction duration was plotted (Figure 1). This relation was established at Lmax for a series of afterloaded contractions by progressively increasing the load on the muscle from preload to the isometric level by varying the current passed through the servomotor. Isometric contractions for measurement were determined after 10 identical isometric beats to preclude the effects of prior history of loading on contractile activity.

Figure 1 depicts the method for measurement of the various parameters of load and time involved in the generation of a load-dependency profile. The ratio of the time necessary for the initiation of the isometric relaxation phase of an isotonic afterloaded twitch to the time required for an isometric twitch to decline to the same load point is defined as the contraction duration ratio (T/Tm). This ratio was generated for each of a series of isotonic contractions in which the afterload was progressively increased from preload to the isometric level. These individual ratios were then plotted as a function of Lmax and a curve obtained to describe the degree of load dependency and independency of the relaxation phase of contraction. Values of T/Tm greater than unity reflect a load-independent state; a ratio of less than one characterizes a load-dependent condition.
TABLE 1. General Characteristics and Dimensional Properties of Left and Right Posterior Papillary Muscles From Sprague-Dawley Rats at 4, 10, and 20 Months of Age

<table>
<thead>
<tr>
<th></th>
<th>4-Month (n=8)</th>
<th>10-Month (n=8)</th>
<th>20-Month (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>419±7.20</td>
<td>474±13.90</td>
<td>527±10.37</td>
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<tr>
<td>p</td>
<td>0.007</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV (mg)</td>
<td>806±24</td>
<td>924±28</td>
<td>1.007±42</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV (mg)</td>
<td>213±8</td>
<td>236±14</td>
<td>297±17</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>0.01</td>
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<td>p</td>
<td>0.001</td>
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</tbody>
</table>

Values are mean±SE. BW, body weight; LV, left ventricular heart weight; RV, right ventricular heart weight; ML, length of posterior papillary muscle; MW, width of posterior papillary muscle; XS, cross-sectional area of posterior papillary muscle; NS, not significant.

The parameters of load and length were displayed as a function of time on a multichannel storage oscilloscope, and photographs were taken with an oscilloscopic camera for permanent record. At the completion of each experiment, the muscle length and muscle diameter at Lmax were measured with a reticle in the eyepiece of a dissecting microscope set at a magnification of ×30. The diameter of each papillary muscle was determined by averaging five equally spaced measurements of muscle diameter. This value was then used to compute the mean cross-sectional area of the muscle preparation. Force values were divided by papillary muscle cross-sectional area to obtain tension (force/mm²).

Statistical Analysis

Results are presented as mean±SE computed from the average measurements obtained from each rat. Comparisons between the left and right ventricles in each group of rats at each age were performed by a paired Student’s t test. Statistical significance in multiple comparisons among independent groups of data was determined by the Scheffe method. Values of p<0.05 were considered to be significant.

Results

General Characteristics

Body weights of Sprague-Dawley rats revealed a consistent increase from 4 to 10 months and from 10 to 20 months, while Fischer rats demonstrated an augmentation from 4 to 10 months only (Tables 1 and 2). In comparing Sprague-Dawley with Fischer rats, the latter exhibited a significantly lower body weight at every age interval examined.

The weight of the left ventricle in Sprague-Dawley animals tended to increase from 4 to 10 months and from 10 to 20 months, but it reached statistical significance only over the entire time interval, from 4 to 20 months. Left ventricular heart weight in Fischer rats increased from 4 to 10 months with no further change at 20 months. Although no difference in right ventricular weight was found in Fischer rats, a significant increase in this parameter was observed in Sprague-Dawley rats as a function of age. Moreover, hearts from Sprague-Dawley animals were consistently heavier than those from age-matched Fischer rats (Tables 1 and 2).

Tables 1 and 2 present the length, diameter, and cross-sectional area of posterior papillary muscles removed from the left and right ventricles of Sprague-Dawley and Fischer rats at 4, 10, and 20 months after birth. The left muscle was significantly longer and wider than the right at each age group. With the exception of differences in right muscle length at 4 months and cross-sectional area of left and right muscles at 4 and 10 months, no other statistically significant differences between the two strains of rats were noted.

Load-Dependency Versus Load-Independency Profile

Figure 2 depicts representative force tracing from left and right posterior papillary muscles for a series of afterloaded isotonic contractions up to and including the isometric load. A significant prolongation of the time to the onset of the iso-
metric relaxation phase for each afterloaded isotonic contraction was observed in papillary muscles removed from the right ventricle. No differences in resting and developed isometric forces were seen between right and left muscles at any age when these parameters were expressed per unit area of muscle tissue. Comparable results were obtained when the amount of myocardial shortening was expressed as a percent of initial muscle length (data not shown). Therefore, all muscles were analyzed at identical absolute loads and at the same degree of muscle shortening.

Figure 3 illustrates the load dependency profile of papillary muscles from Sprague-Dawley rats. The right and left muscles from 4-, 10-, and 20-month-old animals demonstrated a progressive elevation in $T/T_M$ as a function of $L/L_M$. These data indicated that the myocardium became more load

| Table 2. General Characteristics and Dimensional Properties of Left and Right Posterior Papillary Muscles From Fischer Rats at 4, 10, and 20 Months of Age |
|---------------------------------|-----------------|-----------------|-----------------|-------|
|                                 | 4-Month (n=8)   | 10-Month (n=8)  | 20-Month (n=9)  |
| BW (g)                          | 350±6.2*        | 420±9.8*        | 418±22.0*       |
| $p$                             | 0.01            | NS              |                 |
| LV (mg)                         | 539±16*         | 637±18*         | 679±17*         |
| $p$                             | 0.003           | NS              |                 |
| RV (mg)                         | 135±6*          | 155±6*          | 148±11*         |
| $p$                             | NS              | NS              |                 |
| ML (mm)                         | 5.98±0.22       | 2.33±0.23*      | 6.00±0.20       |
| $p$                             | 0.0003          | 0.0001          | 0.0001          |
| MW (mm)                         | 1.01±0.09       | 0.66±0.08       | 1.28±0.11       |
| $p$                             | 0.0164          | 0.011           | 0.0132          |
| XS (mm²)                        | 0.80±0.14*      | 0.34±0.14       | 1.29±0.15       |
| $p$                             | 0.0221          | 0.011           | 0.021           |

Values are mean±SE. BW, body weight; LV, left ventricular heart weight; RV, right ventricular heart weight; ML, length of posterior papillary muscle; MW, width of posterior papillary muscle; XS, cross-sectional area of posterior papillary muscle; NS, not significant.

*Significant difference (p<0.05) from similarly aged Sprague-Dawley rats.
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**FIGURE 3.** Graphs showing contraction duration ratio ($T_T/T_M$) at increasing relative loads ($L/L_M$) for posterior papillary muscles removed from the left and right ventricles of 4- (panel A), 10- (panel B), and 20-month-old Sprague-Dawley rats. *Significantly different ($p<0.05$) from left ventricular values.

Independent with increasing afterloads. Because values of $T_T/T_M$ greater than 1.0 were obtained in the right muscle at all loads, the onset of the isometric relaxation phase of each isotonic contraction was determined to be fully load independent at the different age intervals examined. Moreover, age did not effect this ratio in either the right or left papillary muscle.

An identical analysis performed in Fischer rats is shown in Figure 4. As above, the myocardium of both ventricles became more load independent with greater afterloads, and the contraction duration ratio of right muscle was consistently greater than one. However, in Fischer rats, aging resulted in a continuous decrease of the contraction duration ratio in the right muscle whereas a progressive elevation was measured in the left muscle. These changes indicated that the right muscle became more load dependent and the left muscle more load independent as a function of age. Such shifts in the load-dependency profiles were particularly apparent at lower relative loads (Figure 4).

A further analysis of the magnitude of the difference in the load-dependence properties of the ventricular myocardium between Sprague-Dawley and Fischer rats is shown in Figure 5. At each increasing $L/L_M$, the difference in $T_T/T_M$ between the right and left ventricles was computed by $[(T_T/T_M)_\text{right}-(T_T/T_M)_\text{left}] / (T_T/T_M)_\text{right} \cdot 100$ and compared in the two strains of animals. This was done to emphasize that in Fischer rats the differences between the left and right myocardium progressively decreased as a function of age but became greater in Sprague-Dawley rats. Thus, aging generated greater variations between the two strains.

When the curves for Fischer and Sprague-Dawley rats were compared separately (Figure 6), it was found that the decrease in the relative difference between left and right muscles in Fischer animals was statistically significant. In contrast, Sprague-Dawley rats failed to reveal such an age-dependent adaptation.

Results presented in Figures 3–6 also show that values for right muscles exhibit large deviation from the mean than those of left muscles. This phenomenon most likely reflects a greater biological variability for the right muscle although technical difficulties in the study of right myocardial preparations cannot be excluded.

**Discussion**

The results of the present study indicate that differences exist between the left and right myo-
FIGURE 4. Graphs showing contraction duration ratio (T/Tw) at increasing relative loads (L/Lw) for posterior papillary muscles removed from the left and right ventricles of 4- (panel A), 10- (panel B), and 20-month-old Fischer rats. *Significantly different (p<0.05) from left ventricular values.

cardium in terms of their load-dependency profiles. The magnitude of time required for the onset of myocardial relaxation in the right papillary muscle is not dependent on the prevailing load throughout the physiological range of afterloaded contractions. In contrast, the abbreviation of the isotonic phase of an afterloaded contraction in response to the current load is operative in the left myocardium. These properties have been found to be characteristic of the myocardium of both Fischer and Sprague-Dawley rats. Aging affects the ventricular myocardium in a distinct fashion in the two strains of rats. In Fischer animals, the magnitude of load independency of the right muscle decreases from 4 to 20 months whereas the corresponding left muscle becomes progressively less dependent on the extent of the isotonic afterload. On the other hand, right and left myocardium maintain their typical relaxation profiles with age in Sprague-Dawley rats.

The current observations strongly suggest that diastolic filling may be substantially different in the two ventricles since the load independency of relaxation in the right ventricle may interfere with the rapid filling phase of diastole. Moreover, a reduced capacity of the right side of the heart to accommodate a sudden elevation in absolute load may imply that the right ventricle possesses a limited supportive role when left ventricular dysfunction develops. Right ventricular end-diastolic pressure may rapidly increase, and pressure transmitted backward may rise in the venous system.

Differences in the timing parameters of relaxation between isometric and isotonic muscle contraction have been well documented and are assumed to reflect an increase in the rate of delivery of calcium to the cell cytoplasm. The degree to which myocardial relaxation is dependent on the prevailing load has been related to the ability of the sarcoplasmic reticulum to sequester calcium after the initiation of muscular contraction. In cardiac muscle, the relative time of onset of the isometric relaxation phase of an afterloaded isotonic contraction is directly related to the magnitude of force necessary to overcome the absolute load on the muscle. These responses have been obtained in vitro by use of heart tissue from various animal species and in vivo by use of a servo-controlled canine heart preparation.

The load dependency of relaxation may also occur during the course of an afterloaded isotonic contraction as a result of crossbridge detachment due to the increased rotational load placed on these
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FIGURE 5. Graphs showing the difference in the contraction duration ratio \( T_{T/LM} \) between right and left posterior papillary muscles at increasing relative loads \( \eta = \frac{L}{L_{m}} \): \( \left( \frac{T_{T/LM}}{L_{m}} \right)_{\text{right}} - \left( \frac{T_{T/LM}}{L_{m}} \right)_{\text{left}} \cdot 100 \). These values are for Fischer and Sprague-Dawley rats at 4 (panel A), 10 (panel B), and 20 (panel C) months after birth. *Significantly different (p<0.05) from values obtained from Sprague-Dawley rats of the same age.

actin-myosin connections.\(^5\) This, in turn, dictates the premature return of the filaments to their original precontraction orientation.\(^\text{19,23} \) Whether calcium movements per se or crossbridge interactions or both may be involved in the relaxation properties of the left myocardium, the right myocardium appears not to be controlled by the extent and duration of the loading condition on the ventricular tissue. Therefore, the difference in load sensitivity of relaxation between the left and right myocardium may be explained by a decreased removal of sarcoplasmic calcium during an isotonic contraction, by a decreased rate of crossbridge detachment during an isotonic contraction, by an increased rate of crossbridge detachment during an isometric twitch, or by a combination of these three distinct processes. The possibility that a higher crossbridge formation rate (i.e., a slower rate of detachment) may be associated with a lower sensitivity of relaxation to load has been shown in studies comparing rat and cat heart\(^\text{20} \) and ventricular tissue with atrial myocardium in rodents.\(^\text{24} \)

The age-related changes in the mechanical characteristics of relaxation may result from the combination of the detrimental effects of aging on the heart and the tendency of the reserve capacity of the myocardium to preserve normal cardiac hemodynamics. The left ventricle becomes less load dependent with age, and this alteration is consistent with its reduced ability to withstand an increase in loading conditions.\(^\text{10,15} \) On the other hand, the right ventricle loses part of its load independency, enhancing its tendency to accommodate a greater load as a function of age. These observations, however, were restricted to Fischer rats,\(^\text{10,15} \) and no aging-related events were detected in Sprague-Dawley rats. This latter strain has been shown to be capable of sustaining episodes of elevated work demands since their inotropic and chronotropic responses are maintained up to 19–21 months after birth.\(^\text{9,13} \) In addition, Fischer rats show a consistent decline in mechanical contractile behavior\(^\text{14,15} \) and coronary blood flow distribution and reserve as a function of age.\(^\text{25} \)

It should be pointed out, however, that the volume composition of the muscles may change with age-influencing mechanical characteristics. Although this represents a potential problem, the load-dependency profile was different in the left and right muscles of rats at 4 months of age, a time when it is
unlikely that the process of aging had begun. An additional consideration regarding the differential response of the left and right muscles involves the possibility of less diffusion of oxygen in the left muscle because of its larger diameter. However, hypoxia would shift the load-dependency profile upward and to the left making the load-dependent hypoxia would serve to minimize the difference in magnitude of the phenomena described here.

An assumption made in the present investigation is that the localized damage produced by clamping one muscle end is similar in all groups. Direct evaluation of sarcomere dynamics would solve this potential complexity inherent in the in vitro studies of muscle mechanics, but posterior papillary muscles are generally too large to be analyzed in this manner. It has been shown that the mechanisms of an undamaged region of the muscle may yield results different from those obtained in the entire muscle although the timing parameters are not qualitatively affected. In this regard, no noticeable differences were found between the information obtained from the entire muscle itself and its inner portion; this finding suggests that the observations made in the present work are not significantly influenced by the potential contribution of damage in the preparation.

In conclusion, differences exist in the relaxation properties of the two ventricles; these differences further support the concept that the right and left ventricular myocardium are functionally, structurally, and biochemically distinct and may react differently under pathological conditions and as a result of aging.

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References

16. Sys SU, Housmans PR, Van Oocken ER, Brutsaert DL: Mechanisms of hypoxia-induced decrease of load depen-
Load Dependence of Relaxation in Cat Papillary Muscle. Pflugers Arch 1984;401:368–373

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