Load as an acute determinant of end-diastolic pressure-volume relation

ADELINO F. LEITE-MOREIRA AND JORGE CORREIA-PINTO
Department of Physiology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

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Abstract
Leite-Moreira, Adelino F., and Jorge Correia-Pinto. Load as an acute determinant of end-diastolic pressure-volume relation. Am J Physiol Heart Circ Physiol 280: H51–H59, 2001.—Afterload-induced changes in myocardial relaxation are a mechanism for diastolic dysfunction when afterload is elevated beyond certain limits. The present study investigated the effects of acute afterload and preload changes on the position of the end-diastolic (ED) pressure-volume (P-V) relation. Beat-to-beat afterload elevations were induced in seven open-chest rabbits by gradually occluding the ascending aorta to increase peak left ventricular pressure (LVP) from baseline to isovolumetric level. Afterload elevations were performed at three ED LVP: 2.0 ± 0.2 (low), 5.7 ± 0.2 (mid), and 9.6 ± 0.6 (high) mmHg. Preload was altered with caval occlusions and/or intravenous dextran. Afterload elevations induced an upward shift of the diastolic P-V relation, which became more important as afterload and/or preload increased. For instance, maximal afterload elevations shifted this relation upward 2.2 ± 0.5, 5.1 ± 0.8, and 12.1 ± 1.7 mmHg at low, mid, and high preload, respectively. These effects were partially due to changes in relaxation rate and time available to relax. In conclusion, load is an acute determinant of the ED P-V relation, which, therefore, does not provide a load-independent assessment of diastolic function.

Keywords: afterload; diastole; heart failure; myocardial relaxation; preload; load

The position of the end-diastolic (ED) pressure-volume (P-V) relation reflects left ventricular (LV) compliance and distensibility and, therefore, filling conditions during diastole (33). Classically, it was considered that this curvilinear relationship between LV diastolic pressure and volume could be modified only by chronic factors that affect either myocardial elasticity or LV geometry (11). In the seventies, this concept was challenged when several studies revealed that the ED P-V relation could be acutely and transiently shifted upward during “demand-induced” ischemia (2, 3, 6, 21, 22, 26). In the process of explaining these clinical observations, it was shown that various factors could affect the ED P-V relationship acutely (1, 8, 20, 27). Amongst these factors, decreased extent of myocardial relaxation with residual cross-bridge activation had been largely associated with decreased myocardial distensibility and compliance and, therefore, with diastolic dysfunction and failure (33). On the contrary, until very recently, it was considered that changes in the relaxation rate could never shift the entire diastolic P-V relationship because none of the changes in relaxation rate would be large enough to affect LV pressure (LVP) at ED (33). Changes in relaxation rate could, however, affect the atrioventricular pressure gradient and, therefore, LV filling rate during early diastole (33). Circumstantial evidence indicated that load-dependent slowing of relaxation could contribute to increased ED LVP, but this crucial issue still was controversial and not experimentally demonstrated (7). We recently tested the hypothesis that selective heavy afterload elevations, such as induced by aortic clamping, would increase ED LVP and result in diastolic dysfunction (13). These studies revealed that afterload elevations had a biphasic effect on relaxation rate (13–16) and diastolic LVP (13). Whereas small elevations accelerated LV relaxation and did not affect ED LVP, greater elevations markedly slowed LV relaxation rate, increased ED LVP, and resulted in diastolic dysfunction, confirming our hypothesis and challenging the prevailing view that relaxation rate could never affect ED LVP (13). There still remains, however, to be investigated the effects of acute afterload and preload changes on the position of the ED P-V relation. This was, therefore, the main goal of the present study.

METHODS


Experimental Preparation

Male New Zealand White rabbits (Oryctolagus cuniculus, 3.0 ± 0.1 kg) were premedicated with ketamine hydrochloride (50 mg/kg im) and xylazine hydrochloride (5 mg/kg im). An auricular vein was cannulated, and a prewarmed solution containing 20 meq KCl and 40 meq NaHCO₃ in 500 ml of 0.9% NaCl was administered at a rate of 8 ml·kg⁻¹·h⁻¹ to compensate for perioperative fluid losses. A tracheostomy was performed, and mechanical ventilation was initiated (Harvard Small Animal Ventilator, model 683), delivering oxygen-enriched air. Respiratory rate and tidal volume were...
adjusted to keep arterial blood gases and pH within physiological limits. Anesthesia was maintained with ketamine hydrochloride (33 ml·kg⁻¹·h⁻¹ im), pentobarbital sodium (12.5 mg/kg iv before opening the chest and then 2.5 mg/kg iv as needed), and vecuronium bromide (0.5 mg/h iv) (13). A 20-gauge catheter was inserted in the right femoral artery and connected to a pressure transducer to monitor heart rate and arterial pressure and to obtain samples for blood gas analysis. The heart was exposed by a median sternotomy, and the pericardium was widely opened. One silk suture was placed around the ascending aorta, and another suture was placed around the inferior vena cava. Each one was then passed through a plastic tube to perform transient aortic and caval occlusions during the experimental protocol. A 3-F high-fidelity micromanometer (MTC P3FC, Dräger Medical Electronics) was inserted through an apical puncture wound into the LV cavity, positioned at the midventricular level, and secured in place with a purse-string suture to measure LVP. The manometer was calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. The zero was set at the level of the right atrium. LV dimensions were measured with miniaturized ultrasonic dimension gauges using a sonomicrometer amplifier (Triton Electronics, San Diego, CA); one pair of crystals (3-mm diameter) was sutured in place onto the LV anterior and posterior epicardial surfaces to measure LV external anterior-posterior diameter. A third crystal (1-mm diameter) was tunneled at a 30–45° angle into the subendocardium facing the LV anterior epicardial crystal. The anterior epicardial crystal and subendocardial crystal were combined to measure wall thickness. A limb electrocardiogram (DII) was recorded throughout. At the end of the experiment, the animal was killed with an overdose of anesthetics, and the pericardium was widely opened. One silk suture was placed around the inferior vena cava. Each one was then passed through a plastic tube to perform transient aortic and caval occlusions during the experimental protocol. A 3-F high-fidelity micromanometer (MTC P3FC, Dräger Medical Electronics) was inserted through an apical puncture wound into the LV cavity, positioned at the midventricular level, and secured in place with a purse-string suture to measure LVP. The manometer was calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. The zero was set at the level of the right atrium. LV dimensions were measured with miniaturized ultrasonic dimension gauges using a sonomicrometer amplifier (Triton Electronics, San Diego, CA); one pair of crystals (3-mm diameter) was sutured in place onto the LV anterior and posterior epicardial surfaces to measure LV external anterior-posterior diameter. A third crystal (1-mm diameter) was tunneled at a 30–45° angle into the subendocardium facing the LV anterior epicardial crystal. The anterior epicardial crystal and subendocardial crystal were combined to measure wall thickness. A limb electrocardiogram (DII) was recorded throughout. At the end of the experiment, the animal was killed with an overdose of anesthetics, and the position of the crystals and manometer were verified at necropsy.

**Experimental Protocol**

After complete instrumentation, we allowed the animal preparation to stabilize for 30 min before the beginning of the experimental protocol, which consisted of sudden increases in afterload (beat-to-beat interventions) at three different levels of preload. The animals were not paced, but heart rate did not vary during the experimental protocol (222 ± 11 beats/min).

**Afterload manipulation.** Multiple graded aortic occlusions were performed by abruptly narrowing or occluding the ascending aorta during the diastole separating two heartbeats. This was achieved by pushing the plastic tube against the aorta with one hand while pulling the silk suture with the other hand. The preceding beat is control, and the following beat is test heartbeat. The analyzed intervention, therefore, was a selective alteration of afterload without changes of preload or long-term load history. Systolic LVP of the first heartbeat after the intervention varied as a function of the strength of the tension on the aortic silk suture and, therefore, the extent of ascending aorta narrowing. The aortic clamp was quickly released to avoid neurohumoral reflex changes in cardiac function (12). The animal was stabilized for several beats before another intervention was performed. Multiple interventions, with variable degrees of aortic narrowing, were performed in a random manner.

**Preload manipulation.** Afterload interventions were performed at three different levels of preload: low (2.0 ± 0.2 mmHg), mid (5.7 ± 0.2 mmHg), and high (9.6 ± 0.6 mmHg). Preload was altered in a randomized manner, with caval occlusions and/or intravenous infusions of Dextran 40 diluted in saline at 10% (10–15 ml/kg over 5 min) to achieve the desired ED LVP. The animal was allowed to stabilize for 15 min at each preload level (17, 18) before proceeding with the afterload manipulations.

**Data Acquisition and Analysis**

Recordings were made with respiration suspended at end expiration. Parameters were converted on-line to digital data with a sampling frequency of 500 Hz. Some of the recorded parameters, along with the performed measurements, are illustrated in Fig. 1. An analog signal of the first derivative of LVP (dP/dt) was recorded with a cut-off filter set at 50 Hz. Peak rates of LVP rise (dP/dt max) and fall (dP/dt min) were measured. The internal diameter (ID) was calculated as

![Fig. 1. Representative example showing some of the recorded parameters and performed measurements (○). The time courses of left ventricular (LV) pressure (LVP; top), its first derivative (dP/dt; middle), and internal diameter (ID; bottom) of 3 consecutive heartbeats (two control and one isovolumetric) are shown. The ascending aorta was totally occluded during the diastole separating control and isovolumetric heartbeats. The vertical solid line represents end diastolic pressure (ED pre) at the beginning of the control beat, whereas the dashed line represents ED at the end of the control cycle (ED post). Note that ED post of the control beat corresponds to ED pre of the test beat. LVP max and LVP min, ED LVP at the beginning and end of the heartbeat, respectively; ID ED pre and ID ED post, ED inner diameter (ID) at the beginning and end of the heartbeat, respectively; LVP min, minimal LVP; LVP max, peak systolic LVP; dP/dt max and dP/dt min, peak rates of LVP rise and fall, respectively. The dotted line is placed at dP/dt min.](image-url)
Table 1. Effects of preload and afterload on parameters of LV contraction and relaxation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
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<tbody>
<tr>
<td><strong>Contraction</strong></td>
<td></td>
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<tr>
<td>LVPmax, mmHg</td>
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</tr>
<tr>
<td>Low</td>
<td>82.2 ± 1.7</td>
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<tr>
<td>Mid</td>
<td>88.9 ± 2.7a</td>
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<tr>
<td>High</td>
<td>91.9 ± 3.8abc</td>
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<tr>
<td>dP/dtmax, mmHg/s</td>
<td>3.041 ±240</td>
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<tr>
<td>Fractional shortening, %</td>
<td>26.3 ± 4.6</td>
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<tr>
<td>Low</td>
<td>28.2 ± 4.3a</td>
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<tr>
<td>Mid</td>
<td>29.3 ± 4.6ab</td>
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<tr>
<td>High</td>
<td></td>
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<tr>
<td>dP/dtmin, mmHg/s</td>
<td>−2,600 ± 61</td>
<td>−2,767 ± 72</td>
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<tr>
<td>Time to dP/dtmin, ms</td>
<td>147 ± 5</td>
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<tr>
<td>Time constant τ, ms</td>
<td>15.7 ± 1.1</td>
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<tr>
<td><strong>Relaxation</strong></td>
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<tr>
<td>LVPmax, mmHg</td>
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<tr>
<td>Low</td>
<td>2,610 ± 4</td>
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<tr>
<td>Mid</td>
<td>2,677 ± 10a</td>
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<tr>
<td>High</td>
<td>−3,545 ± 177a</td>
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<tr>
<td>dP/dtmin, mmHg/s</td>
<td>−1,991 ± 81a</td>
<td></td>
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<tr>
<td>Time to dP/dtmin, ms</td>
<td>157 ± 7a</td>
<td></td>
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<tr>
<td>Time constant τ, ms</td>
<td>17.0 ± 1.6a</td>
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<tr>
<td>LVPmax, mmHg</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low</td>
<td>5.5 ± 0.4</td>
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<tr>
<td>Mid</td>
<td>5.6 ± 1.1</td>
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<tr>
<td>High</td>
<td>5.4 ± 0.8</td>
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</table>

Results are presented as means ± SE; n = 7 rabbits. LVPmax, peak systolic left ventricular (LV) pressure (LVP); dP/dtmax and dP/dtmin, peak rates of LVP rise and fall, respectively. Significant differences between preload levels are the following: aP < 0.05 vs. low, bP < 0.05 vs. mid. Significant differences between afterload levels are the following: cP < 0.05 vs. control, dP < 0.05 vs. 70%, eP < 0.05 vs. 80%, and fP < 0.05 vs. 90%.

External diameter minus two times wall thickness. ED was set at the lower right corner of the LVP-ID loop. To distinguish between ED at the start and completion of the analyzed cardiac cycle, ED at the start was referred to as EDpre, whereas ED at the completion was referred to as EDpost. LVP was measured at the start of the cardiac cycle (LVPEdpost), at peak systole (LVPmax), at its minimum value (LVPmin), and at the end of the cardiac cycle (LVPEdend). The ID was measured at the start of the cardiac cycle (IDEdpost), at its minimum value (IDmin), and LVP Edpost at the end of the cardiac cycle (IDEdend). Fractional shortening was calculated as the following (in percent): (IDEdpost − IDmin)/IDEdpost. Afterload levels were presented as relative load, previously defined as LVPmax/peak isovolumetric LVP (measure in percent) (7). Time intervals were measured from EDpre to dP/dtmin and from dP/dtmin to the next ED (EDpost). Pressure fall was evaluated with dP/dtmin and the time constant (τ). For calculating τ, the portion of the LVP tracing between dP/dtmin and a pressure equal to LVPEdend was selected. The curve was then fitted to a monoexponential model with a nonzero asymptote, given by the following equation

\[ P(t) = P_0 e^{-\tau t} + P_x \]  

(1)

where the infinite pressure (P0) is a nonzero asymptote (in mmHg), P0 is an amplitude constant (in mmHg), τ is time (in ms), and P0 is the time constant of the exponent (in ms). The correlation coefficient \( r^2 \) yielded values >0.99. According to this formula, relaxation will be 97% complete after a time interval of 3.5 times τ (in ms), starting at dP/dtmin (13, 38).

Statistical Analysis

Group data are presented as mean values ± SE. Several data sets, combining the three preload and five afterload levels, failed in the Kolmogorov-Smirnov test for normality. The nonparametric Friedman repeated measures analysis of variance on ranks was therefore selected. When treatments were significantly different, the Student-Newman-Keuls test was selected to perform pairwise multiple comparisons. Statistical significance was set at P < 0.05.

RESULTS

Effects of Load on Systolic Function

The LVPmax of the control beat increased from 82.2 ± 1.7 to 88.9 ± 2.7 and 91.9 ± 3.9 mmHg when preload was elevated from the low to the mid and high levels, respectively (Table 1 and Fig. 2). These systolic LVP differences between preload levels are the following: aP < 0.05 vs. low, bP < 0.05 vs. mid. Significant differences between afterload levels are the following: cP < 0.05 vs. control, dP < 0.05 vs. 70%, eP < 0.05 vs. 80%, and fP < 0.05 vs. 90%.

LOAD AND END-DIASTOLIC P-V RELATION

H53
Fig. 2. Effects of selective afterload elevations on left ventricular pressure (LVP) time courses (in mmHg) at 3 different preload levels (low, mid, and high). Five superposed heartbeats with increasing afterloads are displayed: control (beat 1), 70% (beat 2), 80% (beat 3), 90% (beat 4), and 100% (beat 5), isovolumetric. Peak systolic LVP increased progressively with preload elevation at each relative load. LVPEDpost increased with preload elevation but was not affected by afterload, whereas LVPEDpre increased both with preload and with larger afterloads. Effects of afterload on LVPEDpost became more important as preload increased.

control heartbeat, so that LVPEDpost (Table 2) and dP/dtmax (Table 1) remained unaffected by these interventions.

Effects of Load on LV Relaxation and Diastolic Function

Effects of load on LVPmin, LVPEDpre, and LVPEDpost are illustrated in Figs. 2, 3, and 4 and reported in Table 2, where the corresponding LV dimensions are given as well. All these LV pressures and dimensions increased progressively with preload elevation but showed distinct responses to afterload elevations. In fact, whereas LVPEDpost, IDEDpre, and IDEDpost were not affected, LVPEDpre, LVPmin, and ID at LVPmin increased with afterload elevations. Because IDEDpost did not change with afterload, indicating that in the first diastole after an elevation of afterload there was no significant filling beyond IDEDpost, the difference between ED LVP at the end and beginning of the cardiac cycle (LVPEDpost − LVPEDpre) therefore reflected the magnitude of the shift upward of the ED LVP-ID relation and the occurrence of diastolic dysfunction at each preload level. In Fig. 4, mean ED LVP were plotted as a function of the corresponding LV internal diameter at all preload and afterload levels. The extrapolated end-diastolic pressure-internal diameter (ED P-ID) relations were also represented for each afterload level. Similar relations were obtained for the two lowest afterload levels (control and 70%). For the 80, 90, and 100% afterload levels, an upward shift of the ED P-ID relations were, however, observed. This shift was

Table 2. Effects of preload and afterload changes on diastolic parameters

<table>
<thead>
<tr>
<th>LVPEDpost, mmHg</th>
<th>Control</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Mid</td>
<td>5.7 ± 0.2</td>
<td>6.0 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>High</td>
<td>9.6 ± 0.6</td>
<td>9.7 ± 0.7</td>
<td>9.4 ± 0.6</td>
<td>10.5 ± 0.8</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>IDEDpost, mm</td>
<td>12.0 ± 1.1</td>
<td>11.9 ± 1.1</td>
<td>12.0 ± 1.1</td>
<td>12.2 ± 1.0</td>
<td>11.8 ± 1.1</td>
</tr>
<tr>
<td>Low</td>
<td>13.8 ± 0.9</td>
<td>13.9 ± 0.9</td>
<td>13.9 ± 0.9</td>
<td>13.9 ± 0.9</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>Mid</td>
<td>15.0 ± 0.7</td>
<td>15.1 ± 0.7</td>
<td>15.1 ± 0.7</td>
<td>15.1 ± 0.7</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td>LVPEDpost, mmHg</td>
<td>1.9 ± 1.0</td>
<td>2.0 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>3.3 ± 0.6</td>
<td>4.4 ± 0.8</td>
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<tr>
<td>Low</td>
<td>5.7 ± 0.2</td>
<td>5.9 ± 0.3</td>
<td>7.0 ± 0.3</td>
<td>9.0 ± 0.8</td>
<td>12.2 ± 0.8</td>
</tr>
<tr>
<td>Mid</td>
<td>9.7 ± 0.8</td>
<td>9.6 ± 0.7</td>
<td>10.6 ± 0.9</td>
<td>14.9 ± 0.9</td>
<td>22.3 ± 1.7</td>
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<tr>
<td>IDEDpost, mm</td>
<td>11.9 ± 1.1</td>
<td>12.0 ± 1.0</td>
<td>12.2 ± 1.1</td>
<td>12.2 ± 1.0</td>
<td>12.1 ± 1.0</td>
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<tr>
<td>Low</td>
<td>13.8 ± 0.9</td>
<td>13.8 ± 0.9</td>
<td>14.0 ± 0.9</td>
<td>14.1 ± 0.9</td>
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<tr>
<td>Mid</td>
<td>15.1 ± 0.6</td>
<td>15.0 ± 0.7</td>
<td>15.1 ± 0.7</td>
<td>15.2 ± 0.7</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>LVPmin, mmHg</td>
<td>1.0 ± 0.4</td>
<td>0.1 ± 0.5</td>
<td>0.6 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>2.5 ± 0.6</td>
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<tr>
<td>Low</td>
<td>1.6 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>6.1 ± 0.9</td>
<td>10.0 ± 1.1</td>
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<tr>
<td>Mid</td>
<td>3.7 ± 1.2</td>
<td>5.2 ± 1.1</td>
<td>6.9 ± 1.2</td>
<td>9.7 ± 1.6</td>
<td>21.1 ± 4.2</td>
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<tr>
<td>ID at LVPmin, mm</td>
<td>10.1 ± 1.3</td>
<td>10.0 ± 1.1</td>
<td>10.4 ± 1.1</td>
<td>11.1 ± 1.2</td>
<td>11.0 ± 1.4</td>
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<tr>
<td>Low</td>
<td>11.5 ± 1.2</td>
<td>11.7 ± 1.1</td>
<td>12.9 ± 1.1</td>
<td>13.1 ± 1.1</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>Mid</td>
<td>13.5 ± 1.6</td>
<td>11.7 ± 1.7</td>
<td>12.8 ± 1.5</td>
<td>13.8 ± 1.4</td>
<td>15.1 ± 0.7</td>
</tr>
</tbody>
</table>

Results are presented as means ± SE; n = 7 rabbits. LVPEDpre, and LVPEDpost, end-diastolic (ED) LVP at the beginning and at the end of the heart beat, respectively; ID at LVPmin, ED internal diameters (ID) at the beginning and the end of the heart beat, respectively; LVPmin, minimal LVP. Significant differences between preload levels are the following: *P < 0.05 vs. low and bP < 0.05 vs. mid. Significant differences between afterload levels are the following: *P < 0.05 vs. control, *P < 0.05 vs. 70%, *P < 0.05 vs. 80%, and *P < 0.05 vs. 90%.
mild for the 80% but significantly increased when afterload was elevated to 90 and 100%. In addition, please note that, at each afterload level, the upward shift also increased when preload was elevated.

The effects of selective afterload elevations on the time available for the ventricle to relax and the relaxation rate at three different preload levels (low, mid, and high) were illustrated in Fig. 5 and reported in Tables 1 and 3. The time available to relax was computed as the time from dP/dt min to ED post. This time decreased both with preload and afterload elevations. Effects on relaxation rate were assessed with the fractional changes in the time constant $\tau$ ($\tau_{\text{test}}/\tau_{\text{control}}$).

From control to 70% relative load, the relaxation rate was accelerated ($\tau_{\text{test}}/\tau_{\text{control}} > 1$) similarly at all preload levels. Afterload elevations reaching or exceeding a relative load of 80% progressively decreased relaxation rate ($\tau_{\text{test}}/\tau_{\text{control}} < 1$). The amount of slowing increased from low to mid preload but did not change from mid to high preload. The transition from acceleration to deceleration occurred at similar relative load (73–76%) at all preload levels. From the analysis of Fig. 5, it is clear that the heartbeats with the highest preloads and afterloads, which are the ones that induce the biggest upward shifts of the ED P-ID relation, are also the ones with less time to relax and slower rates of relaxation. It remained, however, to evaluate to what extent the time available to relax and the relaxation rate could entirely explain diastolic dysfunction and the elevation of LVPEDpost.

In Fig. 6, the difference between ED LVP at the end and start of the cardiac cycle (LVPEDpost, LVPEDpre), which reflects the magnitude of the upward shift of the ED P-ID relationship (diastolic dysfunction), was plotted as a function of the difference between the available and predicted time for the ventricle to relax. Available time was considered the observed time from dP/dt min to ED post, whereas the predicted time was computed as 3.5 times $\tau$ (13, 38). When the difference between these times yields a negative value, this means that there was a time deficit, and the ventricle at that relaxation rate presumably did not have enough time to fully relax. When this happened, a significant diastolic dysfunction was observed. In Fig. 6, the symbols to the left of the dashed line do not fall, however, on the same line. Similar time deficits yield larger increases in LVPEDpost, when preload is higher, indicating that the time available to relax and the relaxation rate do not explain entirely the observed diastolic dysfunction and elevation of LVPEDpost.

**DISCUSSION**

The present study investigated the effects of acute afterload and preload changes on the position of the end-diastolic P-ID relation. Afterload elevations induced a progressive upward shift of this relation, which increased as preload was elevated.
Load and Systolic Function

Preload elevations resulted, as expected by the Frank-Starling mechanism, in an improvement of the indexes of systolic function. On the other side, when afterload was experimentally elevated, fractional shortening decreased, whereas ED pressures and dimensions at the beginning of the cardiac cycle (LVPEDpre and IDEDpre) and dP/dt max remained similar in control and test beats. This experimental situation corresponded to afterload mismatch (31) and represented abnormal systolic performance in the presence of normal contractility. We should, however, stress the fact that clamping the aorta to different degrees does not represent a physiological way to change the afterload, because we raise input resistance but cannot control the overall input impedance, which might affect the degree of afterload mismatch.

Load and LV relaxation

Increasing afterload had a biphasic effect on relaxation rate. LVP fall was accelerated up to a certain afterload level and slowed thereafter, as we described previously in detail (7, 8, 13–16). Briefly, small afterload elevations are imposed before the end of the calcium transient, allowing the cardiac muscle to respond by increasing the number of interacting cross-bridges. With higher afterload elevations, systolic LVP still increases after the end of the calcium transient when the number of interacting cross-bridges cannot increase anymore. This will elevate the systolic stress imposed on each individual interacting cross-bridge, resulting in less cross-bridge cycling and a slower course of myocardial relaxation (7, 8, 13–16). The transition from acceleration to deceleration occurs at a lower relative load in rabbits (13) than in dogs (15, 16).

Table 3. Evaluation of the completion of myocardial relaxation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time available to relax, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>112 ± 12</td>
<td>102 ± 10c</td>
<td>95 ± 9cd</td>
<td>89 ± 9de</td>
<td>83 ± 6ode</td>
</tr>
<tr>
<td>Mid</td>
<td>114 ± 8a</td>
<td>99 ± 6a</td>
<td>90 ± 5cd</td>
<td>80 ± 6de</td>
<td>73 ± 5ade</td>
</tr>
<tr>
<td>High</td>
<td>105 ± 8ab</td>
<td>96 ± 8b</td>
<td>80 ± 8abcd</td>
<td>70 ± 7abcde</td>
<td>65 ± 8abcde</td>
</tr>
<tr>
<td>Predicted completion of relaxation, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>55.1 ± 3.8</td>
<td>49.9 ± 3.3c</td>
<td>63.6 ± 3.0ed</td>
<td>81.8 ± 5.0gde</td>
<td>109.9 ± 5.2gdef</td>
</tr>
<tr>
<td>Mid</td>
<td>59.5 ± 5.7a</td>
<td>55.5 ± 5.9bc</td>
<td>61.8 ± 4.7d</td>
<td>100.4 ± 17.5gdec</td>
<td>144.5 ± 17.5gdef</td>
</tr>
<tr>
<td>High</td>
<td>60.3 ± 4.6a</td>
<td>54.4 ± 4.2c</td>
<td>65.8 ± 2.7d</td>
<td>99.9 ± 7.4gdec</td>
<td>149.6 ± 12.8gdef</td>
</tr>
<tr>
<td>Available time – predicted time for ventricle to relax, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>55.9 ± 8.2</td>
<td>52.3 ± 8.9</td>
<td>31.2 ± 9.3ed</td>
<td>7.0 ± 8.2gde</td>
<td>−27.2 ± 9.0gdef</td>
</tr>
<tr>
<td>Mid</td>
<td>54.8 ± 7.0</td>
<td>43.9 ± 4.5</td>
<td>27.3 ± 5.5ed</td>
<td>−20.7 ± 18.5gdec</td>
<td>−71.6 ± 18.0gdef</td>
</tr>
<tr>
<td>High</td>
<td>44.9 ± 7.0</td>
<td>41.6 ± 7.0</td>
<td>14.2 ± 7.6abcd</td>
<td>−29.7 ± 13.3gdec</td>
<td>−84.6 ± 12.8gdef</td>
</tr>
</tbody>
</table>

Results are presented as means ± SE; n = 7 rabbits. Significant differences between preload levels are the following: aP < 0.05 vs. low and bP < 0.05 vs. mid. Significant differences between afterload levels are the following: cP < 0.05 vs. control, dP < 0.05 vs. 70%, eP < 0.05 vs. 80%, and fP < 0.05 vs. 90%. 

Fig. 5. Group data displaying effects of selective afterload elevations on the time available for the ventricle to relax (time to relax; left) and on relaxation rate (t_{test}/t_{control}) where t is the time constant; right) at 3 different preload levels (low, mid, and high). The time to relax was computed as the time from dP/dt_min to ED_post. This time decreased with both preload and afterload elevations. Effects on relaxation rate were assessed with the fractional changes in the time constant t (t_{test}/t_{control}). From control to 70% relative load, the relaxation rate was accelerated (t_{test}/t_{control} < 1) similarly at all preload levels. Afterload elevations reaching or exceeding a relative load of 80% progressively decreased the relaxation rate (t_{test}/t_{control} > 1). The amount of slowing increased from low to mid preload but did not further increase from mid to high preload. The transition from acceleration to deceleration occurred at similar relative load (73–76%) at all preload levels. Statistical significance symbols were not represented for the sake of clarity of the figure.
and can be anticipated with caffeine treatment (14). Such changes were attributed to the calcium transients (7). In the present study, we showed that this transition occurred at a similar relative load (73–76%) at all preload levels and, therefore, was not affected by preload (Fig. 5). This is in accordance with the absence of preload effects on the duration of the calcium transient (25).

Afterload elevations exceeding the relative load of the transition (80, 90, and 100% interventions) progressively slowed the rate of LVP fall, with the amount of slowing increasing from low to mid preload but not changing from mid to high preload. Previous studies reported contradictory effects of preload on rate of LVP fall, with some authors describing a decrease in relaxation rate (5, 9, 29, 36) and others describing no effects (35, 39) in response to preload elevations. Some of these discrepancies were attributed to the concomitant changes in preload and afterload in response to the majority of interventions used to modify preload (e.g., volume infusion and caval occlusions). It was proposed that the observed effects were, therefore, due to the afterload changes and that selective changes in preload were devoid of effects on rate of LVP fall (39). The present study, having analyzed the effects of preload at similar afterloads, may contribute to clarify this issue and explain some of the discrepancies. In fact, preload slows LVP fall when it is elevated from low (2.0 ± 0.2 mmHg) to mid (5.7 ± 0.2 mmHg) levels but does not affect rate of LVP fall when it is elevated from mid to high (9.6 ± 0.6 mmHg) levels.

With regard to the time available for the ventricle to relax (time from dP/dt_{min} to ED{post}), it decreased with preload and afterload elevations. Because the duration of the cardiac cycle (time interval from ED{pre} to ED{post}) did not significantly vary throughout the experiment, the decrease in the time from dP/dt_{min} to ED{post} is mainly due to the prolongation of the time from ED{pre} to dP/dt_{min}, which is induced by preload and afterload.

Load and Diastolic Function

Afterload elevations reaching or exceeding a relative load of 80% progressively shifted upward the ED P-ID relation. This upward shift increased when preload was elevated, indicating a simultaneous decrease in ventricular distensibility and compliance (10).

At each preload, the afterload-induced increase in LVP ED{post} could be explained by a decrease in relaxation rate and a shortening of the time available for the ventricle to relax (Figs. 5 and 6). In Fig. 6, relaxation rate, as estimated by the time constant τ, was used to predict the time to completion of relaxation, computed as 3.5 times τ. The increase in LVP ED{post} was then plotted against the difference between the available and necessary times to relax. A negative difference was interpreted as a time deficit, indicating that the ventricle presumably did not have enough time to relax completely. This interpretation adequately explained the effects of afterload at each preload level. However, as we pointed out when describing Fig. 6, similar time deficits yielded larger increases in LVP ED{post} at higher preloads, indicating that the time available to relax and the relaxation rate do not explain entirely the observed diastolic dysfunction and elevation of LVP ED{post}. Preload should, therefore, influence some other factor(s) affecting LV filling and diastolic function. One possible factor is an increase in the diastolic tone induced by preload elevation. Diastolic tone is a sustained partial contraction, by virtue of which the cardiac muscle resists stretch more than it would by virtue of the inherent elasticity alone or of its mere physical properties (4, 8). Diastolic tone was previously shown to be influenced by ischemia (2, 3, 6, 21, 22) and subjected to a paracrine modulation by nitric oxide (27). Preload increases myofilament sensitivity for calcium (25), which might facilitate residual cross-bridge activation during diastole and, therefore, increase diastolic tone. Another possibility relates with the viscoelastic properties of the myocardium. Viscous forces increase with increasing length and/or increasing velocity of lengthening (28). Neither length nor the velocity of lengthening were increased by afterload. On the contrary, preload obviously increased ED length and, therefore, viscous forces. Viscoelastic properties should, however, mainly influence diastolic pressures and the diastolic P-V relation during the rapid filling
phases and not at ED, where its effects were considered minor (28, 30, 33).

Other factors affecting diastolic function were not likely to have contributed to our findings. The pericardium is an important modulator of the diastolic P-V curve (33), but it was widely opened in the present study, and, therefore, it could not constrain the heart. In addition, ventricular cross-talk is modest when the pericardium is opened (34). Coronary arterial engagement may be altered by afterload, but its effect on diastolic pressures is modest (24, 32, 37). If present, a significant increase of coronary engorgement would increase diastolic wall thickness (13, 19), which was not observed.

In conclusion, in addition to demand-induced ischemia (2, 3, 6, 21, 22, 26) and neurohormonal or pharmocological agents (1, 27), which have been previously shown to acutely influence the ED P-V relation, the present study demonstrated that this relation is also subjected to an acute modulation by load (preload and afterload). The ED P-V relation, therefore, does not provide a load-independent assessment of diastolic function.

Limitations of Present Study

The use of the 3.5 times \( t \) rule to estimate the time to completeness of relaxation (13, 38) might raise some discussion, because relaxation rate is influenced by ventricular filling. The rule applies well to nonfilling beats, but time to completeness of relaxation may be prolonged up to 5.4 times \( t \) in filling beats (23). If we had used a value larger than 3.5, we would have ended up with even clearer evidence of incompleteness of relaxation, including the 80% interventions as well, which in fact induced a slight, but significant, increase of coronary engorgement would increase diastolic wall thickness (13, 19), which was not observed.

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REFERENCES