Ejection has both positive and negative effects on left ventricular isovolumic relaxation

DAVID S. BERGER,1 KATHERINE VLASICA,1 CHRISTOPHER M. QUICK,2 KIMBERLY A. ROBINSON,1 AND SANJEEV G. SHROFF1

Ejection has both positive and negative effects on left ventricular isovolumic relaxation. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2696–H2707, 1997.—In isovolumically beating hearts, the speed of left ventricular (LV) relaxation is uniquely determined by peak active stress (σmax). In contrast, such a succinct description of relaxation is lacking for the ejection beats, although ejection is generally thought to hasten relaxation. We set out to determine how ejection modifies the relaxation-σmax relationship obtained in the isovolumically beating hearts. Experiments were performed on five isolated rabbit hearts subjected to various loading conditions. Instantaneous LV pressure and volume were recorded and converted to active stress, from which isovolumic relaxation time (Trelax) was defined as the time for stress to fall from 75 to 25% of σmax (isosomatic beats) or its end-ejection value (ejection beats). Steady-state and transient isovolumic beat and steady-state ejection beat data were used to develop a multiple regression model. This model identified stress, current beat ejection, and previous beat ejection history as independent predictor variables of Trelax, and fit the data well in all hearts (r² > 0.98). Furthermore, this model could predict relaxation in transient ejection beats (r² = 0.80 for all hearts). Whereas the coefficient for the current beat ejection was negative (i.e., negative effect or hastening relaxation), the ejection history coefficient was positive (i.e., positive effect or slowing relaxation). The sum of these two coefficients was negative, corresponding to the commonly observed net negative effect of ejection on relaxation. The expected positive inotropic effect of ejection was also observed. The dissipations of both positive inotropic and relaxation effects were slow, suggesting a nonmechanical underlying mechanism(s). We postulate that these two effects are linked and caused by ejection-mediated changes in myofilament Ca²⁺ sensitivity.

isolated heart; ventricular function; lusitropy; load and relaxation

IF CONTRACTING CARDIAC MUSCLE is allowed to shorten, the shortening process itself affects the contractile properties and performance of the muscle. For example, ejection has been shown to have variable effects on the inotropic state: augmentation or depression, depending on the amount of ejection (7, 22, 37). Recent experimental work has shown that these shortening-mediated phenomena exist at the cardiac muscle level (11). Moreover, theoretical modeling supports the notion that these shortening-mediated phenomena may originate from the cross-bridge properties (28). Given that contraction and relaxation are governed by many of the same physical processes (e.g., activation, cross-bridge kinetics, activation-cross bridge interaction), it is likely that the ejection-mediated effects on relaxation are also variable.

Although many experiments have been performed to elucidate the determinants of relaxation speed, a quantitative description that unifies data from various ejection conditions is still lacking. The amount of ejection as well as the timings of the onset and end of ejection all seem to affect the speed of isovolumic relaxation (19, 20, 42). This dependence of relaxation speed on ejection has also been treated in terms of arterial system load (3, 27). A common observation has been that increasing ejection hastens relaxation (6, 14, 20). Recently, Tobias et al. (39) clarified the picture considerably for nonejecting conditions, finding that relaxation in isovolumically beating hearts is uniquely determined by the developed (active) stress such that relaxation is prolonged with increasing stress. Janssen and Hunter (25) have reported similar observations using an isolated cardiac muscle preparation. Thus it seems reasonable that any attempt to understand relaxation in normally ejecting hearts should start from and extend Tobias’ observation.

Figure 1 contains an example of data from previous experiments (4) and shows left ventricular pressure (Pp) for steady-state ejection followed by isovolumic Pp. Data corresponding to three levels of peripheral resistance, resulting in a wide range of stroke volumes (SV), are shown. Clearly shown in Fig. 1A is that post-ejection peak isovolumic pressure increases with increasing SV (ejection-mediated positive inotropy). Figure 1B shows normalized active isovolumic Pp from the same data. With the data in this form, it is clear that increased SV, either directly or indirectly through its effects on increasing peak isovolumic pressure, or both, also leads to longer relaxation times in the subsequent isovolumic beats. It is this observation that led us to the hypothesis that ejection can also slow relaxation (i.e., ejection has a positive effect on relaxation). Thus we designed a new set of experiments, using isolated rabbit hearts, to test the hypothesis that ejection can have both negative and positive effects on relaxation. An attempt to quantify these phenomena in terms of the amount of ejection and load on the heart is presented. Finally, we discuss the possible mechanisms underlying the experimental observations, including the positive effect of ejection on relaxation, the new finding of the present study.

METHODS

All protocols were reviewed and approved by The University of Chicago Institutional Animal Care and Use Committee and conform with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.
EJECTION AND ISOVOLUMIC RELAXATION

Experimental Preparation and Isolated Heart Setup

Experiments were performed on hearts isolated from adult male rabbits (New Zealand White) weighing 2.0–3.0 kg. Rabbits were preanesthetized with 5.0 mg/kg xylazine (Ben Venue Laboratory, Bedford, OH) and 0.01 mg/kg glycopyrrolate (Robinul-V; Elkins-Sinn, Cherry Hill, NJ) and, after 10 min, anesthetized with 30–50 mg/kg ketamine (Kedalar; Parke-Davis, Morris Plains, NJ) and 1.0 mg/kg acepromazine (Fermenta Animal Health, Kansas City, MO). Tracheotomy was performed after anesthesia, and rabbits were artificially ventilated (Harvard Ventilator, model 683; Harvard Apparatus, South Natick, MA) with room air at a respiratory rate of 43 breaths/min and a tidal volume of 25–30 ml. After a median sternotomy and ligation of great vessels, a metal cannula connected to the perfusion system was inserted into the brachiocephalic artery and immediately flushed with heparinized saline (3.0 ml, 1,000 U/ml). Retrograde perfusion of the coronary arteries was then begun at a constant perfusion pressure of 80 mmHg and temperature of 37°C. The heart was perfused with oxygenated modified Krebs-Henseleit solution (36), which was not recirculated. Connective tissue was cut away and the heart removed from the chest while being constantly perfused. Therefore, at no time was coronary circulation interrupted.

A thin latex balloon, secured at the end of a piston-cylinder device, was positioned in the left ventricle via the mitral orifice. A thread tied to the end of the balloon was passed through the apex of the left ventricle to secure the balloon in the chamber. A purse string tied around the mitral orifice secured the heart to the piston-cylinder device attached to a linear motor. The piston position was sensed by a linear voltage displacement transformer. All hearts were paced using unipolar electrodes attached to the apex of the left ventricle. More comprehensive details of the isolated heart setup can be found elsewhere (4, 36).

Experimental Protocols

In this study, each heart was subjected to three protocols: 1) single-beat Frank-Starling (SBFS) (9), 2) steady-state ejection (SSEJ), and 3) transient ejection (TREJ). The heart rate was 120 beats/min for all protocols and all hearts. Data were recorded at a sampling rate of 1,000 Hz. The specifics of the protocols were as follows.

SBFS. In the SBFS protocol (Fig. 2A), the heart was allowed to beat isovolumically at a constant reference volume \( V_{\text{ref}} \) until it reached steady state, at which time the volume \( V_v \) was changed over a short period of time in late diastole. After several cardiac cycles occurred at this perturbed volume, \( V_v \) was changed back to \( V_{\text{ref}} \). The \( P_v \) and \( V_v \) from two cardiac cycles were sampled: one steady-state cycle at \( V_{\text{ref}} \) and the other as the first beat after the volume change (the beat that will be analyzed). A full SBFS protocol consisted of 10–12 equispaced volume changes centered around \( V_{\text{ref}} \). If an arrhythmia occurred during the data collection, for example, a mechanically induced premature contraction, the \( P_v \)–\( V_v \) pair was omitted from the analysis.

SSEJ. In the SSEJ protocol, the real-time artificial arterial loading system controlled the instantaneous \( V_v \) (4). With reference to Fig. 2B, the following order of events was executed: 1) the heart beat isovolumically at \( V_{\text{ref}} \) until steady state; 2) the heart was allowed to eject into the artificial load that mimics the arterial system (4); 3) on reaching steady-state ejection (~45 s), \( P_v \) and \( V_v \) were sampled, and the steady-state ejection-volume time course \( \{V_v(t)\} \) was also stored for use in the next protocol; 4) ejection was halted and the next 10 isovolumic beats were sampled; and 5) thereafter, every third isovolumic beat was sampled for a total of 10 more beats. Thus, over a period of 20 s, a total of 20 postejec tion isovolumic beats were sampled. These data were acquired for a range of \( SV \) as described in Data Collection.

TREJ. The TREJ protocol was similar to the SSEJ protocol, except that ejection was imposed using direct volume control. This protocol consisted of the following two steps (Fig. 2C): 1) the heart beat isovolumically at \( V_{\text{ref}} \) until steady state, and 2) one transient ejection was then achieved by imposing \( V_v(t) \) obtained from the SSEJ protocol. \( P_v \) and \( V_v \) from this beat were sampled.

Data Collection

First, an SBFS protocol was performed and analyzed (see Data Analysis) to identify the \( V_v \) that resulted in maximum active \( P_v \). The \( V_v \) was adjusted to be 80% of this volume and served as the first \( V_{\text{ref}} \). The heart was then subjected to the
following experimentation sequence (Fig. 3). First, one SBFS protocol was performed. Next, one SSEJ protocol was executed for a given value of peripheral resistance ($R_s$). This was followed immediately by the TREJ protocol, using $V_{ej}(t)$ from the previous SSEJ. This SSEJ-TREJ pair was repeated four more times using a different $R_s$ each time (4). After this series of ejection protocols, another SBFS protocol was performed. Finally, this entire series of ejection protocols sandwiched by SBFS protocols was repeated two more times at different $V_{ref}$ (0.2 ml above and below the first $V_{ref}$).

The range of $R_s$ used in the ejection protocols was large and, together with different $V_{ref}$, resulted in a range of ejection fractions at similar SV. An entire series of ejection protocols (5 SSEJ-TREJ pairs) for a given $V_{ref}$ took ~20 min to execute. The second SBFS protocol was performed so we could be sure that the condition of the heart did not degrade during this time. Thus the total experimental duration (3 $V_{ref}$ values) was ~1 h. A total of five hearts were used.

Data Analysis

The passive pressure-volume relationship was obtained by relating left ventricular end-diastolic pressure ($P_{ed}$) to end-diastolic volume ($V_{ed}$) using data from the SBFS protocol. $V_{ed}$ and $P_{ed}$ were taken as the averages of $V_v$ and $P_v$ over a 10-ms period just before contraction. The following equation was fit to this $P_{ed}$-$V_{ed}$ relationship, with $a$, $b$, and $V_0$ as passive pressure-volume parameters (Fig. 4)

$$P_{ed} = a[e^{b(V_{ed}-V_0)} - 1]$$

(1)

Left ventricular active pressure ($P_{act}$) was then calculated for both ejection and isovolumic beats by subtracting passive pressure ($P_{pass}$) from measured $P_v$

$$P_{act} = P_v - P_{pass} = P_v - [\alpha + P_{ed}]e^{b(V_{ed}-V_0)} - a$$

(2)

For isovolumic beats, $V_v = V_{ed}$, and Eq. 2 reduces to a simple difference between $P_v$ and $P_{ed}$.

Fig. 4. LV active (○) and end-diastolic (●) pressure-volume relationships derived from an SBFS protocol. End-diastolic pressure-volume ($P_{ed}$-$V_{ed}$) relationship was fit to Eq. 1 as displayed (where $\alpha$, $\beta$, and $V_0$ are constants) and used later to calculate LV active pressure ($P_{act}$) from measured $P_v$ (Eq. 2).
Left ventricular active wall stress (\(\sigma\)) was estimated using a thick-walled spherical model

\[
\sigma = \frac{P_{\text{act}}}{1 + \frac{M_v}{\rho V_v} \frac{2}{3}} - 1
\]

where \(M_v\) and \(\rho\) are left ventricular muscle mass and density, respectively (13).

Isovolumic relaxation time (\(T_r\)) was characterized by \(T_r = T_{25} - T_{75}\). For isovolumic beats, \(T_{25}\) and \(T_{75}\) are the times at which stress falls to 75 and 25% of its peak active stress (\(\sigma_{\text{max}}\)), respectively (Fig. 5) (39). For ejection beats, \(T_{25}\) was determined in a manner similar to that for the isovolumic beats, except that \(T_{25}\) and \(T_{75}\) refer to end-ejection stress (\(\sigma_{\text{ee}}\)) rather than \(\sigma_{\text{max}}\) (see Fig. 5B). The rationale for using \(\sigma_{\text{ee}}\) as a reference will be discussed later.

As described by Tobias et al. (39), the left ventricular relaxation process for the isovolumic beats was characterized by relating \(T_r\) to \(\sigma_{\text{max}}\). By extension, the relaxation process for the ejection beats was characterized by relating \(T_r\) to \(\sigma_{\text{ee}}\). To determine the effects of ejection on relaxation, several determinants of ejection values, respectively.

Multiple regression analysis was used to evaluate the determinants of \(T_r\). The regression model was developed in a stepwise manner to develop a unified analytical framework that describes isovolumic relaxation. We will show this development in detail for one heart, the results of which are representative. For the remaining four hearts, results are presented for the final model only.

Relaxation in Isovolumic Beats

Figure 6A shows the \(T_r\)-\(\sigma_{\text{max}}\) relationships derived from six SBFS protocols (i.e., two SBFS protocols at three \(V_{\text{ref}}\) values). To facilitate quantitative analyses, absolute \(T_r\) and \(\sigma_{\text{max}}\) values were converted to \(\Delta T_r\) and \(\Delta \sigma\), respectively (Fig. 6B)

\[
\Delta T_r = T_r - T_{25, V_{\text{ref}}} \quad \text{and} \quad \Delta \sigma = \sigma_{\text{max}, V_{\text{ref}}} - \sigma_{\text{max}, V_{\text{ref}}}
\]

where \(T_{25, V_{\text{ref}}}\) and \(\sigma_{\text{max}, V_{\text{ref}}}\) are average values for the steady-state isovolumic beats at \(V_{\text{ref}}\) (solid symbols in Fig. 6A). With the use of this data set, the regression model describing the relationship between \(\Delta T_r\) and \(\Delta \sigma\) (Fig. 6B) took the following form. (Note that the intercept is 0 by definition.)

\[
\Delta T_r = a_1 \Delta \sigma + a_2 \Delta \sigma^2
\]

where \(a_1\) and \(a_2\) are regression coefficients. Equation 4 fit the data well \((r^2 = 0.99)\), indicating that, as found by Tobias et al. (39), relaxation in isovolumic beats is determined by \(\sigma_{\text{max}}\). Table 1 contains the coefficient values, their errors, and the coefficient of determination for this and subsequent regression analyses. For the SBFS data, the coefficient for the linear term, \(a_1\), was roughly six times more important than that for the quadratic term, \(a_2\) (inference based on standardized regression coefficients), yet both were statistically sig-

![Fig. 5. LV \(P_v\) (top) and \(V_v\) (bottom) for a steady-state ejection beat followed by a postejection isovolumic beat (A). Data were analyzed as follows. First, \(P_{\text{act}}\) was calculated using Eq. 2. Next, with assumption of a thick-walled spherical model, \(P_{\text{act}}\) and \(V_v\) were used to calculate active wall stress (B). From active stress, isovolumic relaxation time (\(T_r\)) was calculated for isovolumic beats as difference between times for active stress to fall to 75 (\(T_{25}\)) and 25% (\(T_{75}\)) of its maximum value. For ejection beats, \(T_r\) was similarly defined, except that reference stress was end-ejection stress. \(T_{\text{max}}\) and \(T_{\text{ee}}\) times for active stress to reach its maximum and end-ejection values, respectively.](http://ajpheart.physiology.org/)
significant. This isovolumic ΔT$_r$-Δσ relationship will now be used as a nomogram, against which we will compare the ΔT$_r$-Δσ relationships from the ejection protocols.

Relaxation in Steady-State Ejection Beats

Figure 7 contains ejection beat data from the SSEJ protocol along with the SBFS data presented in Fig. 6B. For ejection beats, ΔT$_r$ and Δσ were calculated from the absolute T$_r$, and σ$_{ee}$ (end-ejection stress)

$$\Delta T_r = T_r - T_r(V_v = V_{ref})$$
$$\Delta \sigma = \sigma_{ee} - \sigma_{max}|V_v = V_{ref}$$

where, as above, $T_r(V_v = V_{ref})$ and $\sigma_{max}|V_v = V_{ref}$ are the values for the steady-state isovolumic beat. All ejection beat ΔT$_r$-Δσ points lie below the nomogram. As SV decreases, the ejection beat ΔT$_r$-Δσ relationship moves toward the reference isovolumic ΔT$_r$-Δσ point. To characterize this combined isovolumic and ejection beat data set, the regression equation (Eq. 4) was modified to include a term for ejection

$$\Delta T_r = a_1\Delta \sigma + a_2\Delta \sigma^2 + a_3SF$$  \hspace{1cm} (5)

where $a_3$ is the coefficient relating SF to ΔT$_r$. For the SBFS beats, SF = 0. Equation 5 described this data set well ($r^2 = 0.99$) and coefficients $a_1$ and $a_2$ did not change from the previous regression (Table 1), indicating no interaction between the independent variables, Δσ and SF. Note the negative value of $a_3$ in Table 1, which indicates a negative effect of ejection on relaxation.

We analyzed alternative independent variables to quantify ejection (e.g., SV, EF, SL) in place of SF. Although the model fits were all good ($r^2 > 0.94$), SF appeared to be slightly superior: residual sum of squares was lowest with SF in four of five hearts. Moreover, using more than one independent variable for ejection did not improve the model-based fits.

Relaxation in Postejection Isovolumic Beats

In Fig. 8, the first postejection isovolumic beat ΔT$_r$-Δσ relationships are included along with the data from Fig. 7. Once again, ΔT$_r$ and Δσ for these postejection isovolumic beats were calculated by subtracting $T_r(V_v = V_{ref})$ and $\sigma_{max}|V_v = V_{ref}$ of the steady-state isovolumic beat. Note that, similar to the ΔT$_r$-Δσ data from ejection beats, as SV decreases, the post-ejection, isovolumic ΔT$_r$-Δσ points move toward the nomogram reference point. Unlike the ejection beat ΔT$_r$-Δσ points, all post-ejection ΔT$_r$-Δσ points lie above the nomogram, indicating a positive effect of ejection on relaxation (i.e., ejection slows relaxation).

Dynamics of Positive Effect of Ejection on Relaxation

The $T_r$ and $\sigma_{max}$ values for the isovolumic beats after steady-state ejection gradually returned to steady state (Fig. 9). Excess $T_r$ was defined as the difference between the measured $T_r$ and the $T_r$ seen in steady-state isovolumic beats at the same $\sigma_{max}$, the latter being obtained from the nomogram (Eq. 4). The temporal recovery patterns of normalized excess $T_r$ ($T_{re}$. by definition $T_{re} = 1$ for the first beat) for these post-ejection isovolumic beats are shown.

**Table 1.** Parameter values, errors, and coefficients of determination for each model used in stepwise regression analysis in one heart.

<table>
<thead>
<tr>
<th>Model</th>
<th>Data Used</th>
<th>$r^2$</th>
<th>$a_1$, s/mmHg</th>
<th>$a_2$, %</th>
<th>$a_3$, s/mmHg</th>
<th>$a_4$, %</th>
<th>Net Negative</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta T_r = a_1 \Delta \sigma + a_2 \Delta \sigma^2$</td>
<td>SBFS</td>
<td>0.99</td>
<td>4.97 $\times$ 10$^{-4}$</td>
<td>1.2</td>
<td>3.57 $\times$ 10$^{-6}$</td>
<td>5.0</td>
<td>$a_5$, s</td>
<td>$a_6$, %</td>
<td>$b_1$, s</td>
</tr>
<tr>
<td>$\Delta T_r = a_1 \Delta \sigma + a_2 \Delta \sigma^2 + a_3SF$</td>
<td>SBFS</td>
<td>0.98</td>
<td>4.98 $\times$ 10$^{-4}$</td>
<td>1.2</td>
<td>3.56 $\times$ 10$^{-6}$</td>
<td>4.8</td>
<td>$a_5$, s</td>
<td>$a_6$, %</td>
<td>$b_1$, s</td>
</tr>
<tr>
<td>$\Delta T_{re} = a_1 \Delta \sigma + a_2 \Delta \sigma^2 + b_3SF + b_4SS$</td>
<td>SSEJ, ejection</td>
<td>0.99</td>
<td>4.98 $\times$ 10$^{-4}$</td>
<td>1.4</td>
<td>3.57 $\times$ 10$^{-6}$</td>
<td>5.6</td>
<td>$a_5$, s</td>
<td>$a_6$, %</td>
<td>$b_1$, s</td>
</tr>
</tbody>
</table>

$r^2$, Coefficient of determination; $a_1$, $a_2$, and $a_3$, model parameters; $a_0$, $a_1$, $a_2$, and $a_4$, SE of parameters; $T_r$, isovolumic relaxation time; σ, active stress; SF, shortening fraction; $b_3$, $b_4$, dummy variables; SBFS, single-beat Frank–Starling protocol; SSEJ, steady-state ejection protocol. Subscripts n and SS refer to current and steady-state beats, respectively. Δ denotes the difference from the value at the reference, steady-state isovolumic condition.
in Fig. 10. From these data, it appeared that the elimination of $T_{r,ex}$ followed a monoexponential function and that the relative speed of recovery is independent of the amount of steady-state ejection. Thus, to quantify the rate of $T_{r,ex}$ recovery, the data in Fig. 10 were fit to the following equation

$$T_{r,ex} = e^{-N_b t_{tb}}$$

where $N_b$ is the beat number and $t_{tb}$ is the beat constant.

Equation 6 fit this data well ($r^2 = 0.92$) with $t_{tb} = 4.14$ beats. The recovery was relatively large for all hearts (Table 2), ranging from 4.14 to 5.80 beats (or $\approx 2–3$ s).

To describe quantitatively the dynamics of the positive effect of ejection, we assumed the following.

1) Because the positive effect dissipates slowly, its onset is also slow. In other words, both the onset and dissipation of the positive effect depend on the history of ejection. 2) Like dissipation, the onset time course follows a first-order process. 3) The steady-state value of the positive effect ($\Pi_1$) is proportional to the amount of ejection, i.e., SF. Thus the positive effect for beat n ($\Pi_n$) can be written as

$$\Pi_n = \Pi_{n-1} e^{-t_{bd}} + b_d (1 - e^{-t_{bd}}) SF_n$$

where $t_{bd}$ and $t_{bo}$ are the dissipation and onset beat constants, respectively, and $\Pi_{n-1}$ is the value of the positive effect of the previous beat. The first term in Eq. 7 is the dissipation of the existing positive effect, and the second term represents the onset of a new positive effect.

Fig. 7. Steady-state ejection beat $\Delta T_{r,ex}$ data from SSEJ protocols. $\Delta T_{r,ex}$ nomogram (SBFS protocol) from Fig. 6B is superimposed to facilitate comparison. Note that all ejection beat $\Delta T_{r,ex}$ points fall below nomogram, and, as SV is reduced, ejection beat $\Delta T_{r,ex}$ point approaches reference $\Delta T_{r,ex}$ point (i.e., origin) indicated by dotted lines. Regression model in Eq. 5 described this data set well.

Fig. 8. $\Delta T_{r,ex}$ data from 1st post-ejection isovolumic beats. Data from Fig. 7 are superimposed to facilitate comparison. All $\Delta T_{r,ex}$ points from 1st post-ejection isovolumic beats fell above nomogram (SBFS protocol). Similar to steady-state ejection beats, post-ejection isovolumic $\Delta T_{r,ex}$ points approach reference $\Delta T_{r,ex}$ as SV becomes smaller. Full regression model in Eq. 10 fit this entire data set well.

Fig. 9. Time course of recovery of $\sigma_{max}$ and $T_r$ in isovolumic beats following steady-state ejection. Data are from 4 combinations of $V_{ed}$ and SV.

Fig. 10. Time course of recovery of normalized excess $T_r$ (see text) in a single heart for several combinations of $V_{ed}$ and SV. Data were fit to a monoexponential; beat constant ($t_b$) for this example was 4.14 (or $\approx 2$ s).
second term is the additional positive effect due to current beat ejection. In the following analyses, we assumed that \( \tau_{bp} = \tau_{bo} \) and that this common value is given by \( \tau_b \) (Eq. 6; Table 2). Thus the steady-state value of II is given by

\[
II_{ss} = b_3 SF_{ss}
\]

(8)

where the subscript ss denotes steady state. For the special case of steady-state isovolumic contractions (\( SF_n = SF_{n-1} = SF_{ss} = 0 \)), Eq. 7 reduces to \( II_n = II_{n-1} = 0 \).

Relaxation in Steady-State Ejection and Isovolumic Beats: A Unified Description

Given that there is a positive effect of ejection, the net negative value of coefficient \( a_3 \) in Eq. 5 indicates that both positive and negative effects exist and that the negative effect dominates. Assuming that the negative effect of ejection depends on the current ejection conditions and takes effect immediately, we can now modify Eq. 5 with Eq. 7 to incorporate both negative and positive effects of ejection

\[
\Delta T_{r,n} = a_1 \Delta \sigma_n + a_2 \Delta \sigma_n^2 + b_3 SF_n
\]

\[+ II_{n-1} \cdot e^{-\frac{t}{\tau_b}} + b_4(1 - e^{-\frac{t}{\tau_b}})SF_n \]

(9)

where the subscripts \( n \) and \( n-1 \) refer to the current beat and the immediately preceding beat, respectively. As in Eq. 5, the third term in Eq. 9 represents the negative effect of ejection. The positive effect is represented by the fourth and fifth terms.

Data from SBFS and SSEJ protocols consist of three special cases of Eq. 9: 1) for steady-state isovolumic beats (SBFS protocol), \( SF_n = 0 \) and \( II_{n-1} = 0 \); 2) for steady-state ejection beats (SSEJ protocol), \( SF_n = SF_{ss} \) and \( II_{n-1} = II_{ss} = b_3 SF_{ss} \); and 3) for the first isovolumic beat after steady-state ejection, \( SF_n = 0 \) and \( II_{n-1} = II_{ss} = b_3 SF_{ss} \). When considering these three cases only, the general description in Eq. 9 reduces to

\[
\Delta T_{r,n} = a_1 \Delta \sigma_n + a_2 \Delta \sigma_n^2 + b_3 D_3 SF_n + b_4 D_4 SF_{ss}
\]

(10)

where \( D_3 \) and \( D_4 \) are dummy variables given by

\[
D_3 = \begin{cases} 
0 & \text{steady-state isovolumic} \\
1 & \text{steady-state ejection} \\
0 & \text{1st postejection isovolumic}
\end{cases}
\]

\[
D_4 = \begin{cases} 
0 & \text{steady-state isovolumic} \\
1 & \text{steady-state ejection} \\
e^{-\frac{t}{\tau_b}} & \text{1st postejection isovolumic}
\end{cases}
\]

Comparing Eq. 10 to Eq. 5, we see that \( a_3 \) emerges as the sum of \( b_3 \) and \( b_4 \) for steady-state ejection. This final regression model described well the entire data set from protocols SSFS and SSEJ (\( r^2 = 0.99 \)). Again, coefficients \( a_1 \) and \( a_2 \) retained their values (Table 1). Coefficient \( b_4 \) had a negative value, indicating a negative effect on relaxation due to the current beat ejection. In contrast, coefficient \( b_3 \) had a positive value, indicating a positive effect on relaxation due to the steady-state (history of) ejection. Coefficient \( b_2 \) had a larger negative value compared with \( a_3 \) (\( -0.300 \) vs. \( -0.176 \)), whereas the sum of \( b_2 \) and \( b_4 \) was equal to \( a_2 \); thus regression Eqs. 5 and 10 yielded quantitatively consistent results for steady-state ejection beats, and the net effect of steady-state ejection on relaxation was negative. Lastly, the magnitude of the \( b_2 \)-to-\( b_3 \) ratio was 0.473, meaning that in this heart, the positive effect of ejection on relaxation was 47% as strong as the negative effect.

Once again, the use of any other independent variable to quantify ejection in place of \( SF \) yielded the same quantitative results. That is, whereas values of the coefficients that relate ejection to \( \Delta T_r \) (\( b_2 \) and \( b_4 \)) varied in absolute magnitude, the following was always true: \( b_2 < 0 \) and \( b_4 > 0 \), and the positive-to-negative ratios (i.e., \( b_2 \)/\( b_4 \)) were similar.

Table 2 contains coefficient values for the final regression model (Eq. 10) along with their errors and coefficients of determination for all hearts. In every case, the regression model fit the data well (\( r^2 \geq 0.98 \)) and all coefficients were statistically significant. Furthermore, \( b_4 \) was always negative and \( b_2 \) was always positive. The positive-to-negative coefficient ratio was also consistent between hearts, ranging from 44 to 54% (Table 2).

Prediction of Relaxation Times for Transient Ejection Beats

Because the TREJ protocol data were not used in the regression analyses, they provide an independent source with which to verify Eq. 9. Thus parameter values estimated from the regression analysis presented (Table 2) were used to predict \( \Delta T_r \) for transient ejection beats. The transient beat \( \Delta T_r - \Delta \sigma \) points are shown in Fig. 11, along with the data from Fig. 8, and generally fell below the steady-state ejection beat points at a given \( \sigma_{ee} \). For the first transient ejection beat after steady-state isovolumic contraction, \( SF_n = SF_{tr} \) and \( II_{n-1} = II_{ss} = 0 \), where the subscripts \( tr \) denotes the transient ejection.
The major finding of this study is that ejection has both a negative and a positive effect on the rate of fall of left ventricular pressure during the isovolumic relaxation phase and that the negative effect dominates. In this section we will discuss first the rationale for this conclusion, which is qualitative and does not depend on any of the models developed above. Next, we will discuss our attempt to quantify the phenomena on the basis of muscle load and shortening. Last, we will consider the possible underlying mechanisms.

**DISCUSSION**

Fig. 11. ΔT_{re},Δσ data for transient ejection beats (TREJ protocol). Data from Fig. 8 are superimposed to facilitate comparison.

In this case, the general description in Eq. 9 becomes

\[ ΔT_{re,n} = a_1Δσ_n + a_2Δσ_n^2 + b_3SF_n + b_4(1 - e^{-c_n})SF_n \]  

Using Eq. 11, we were able to predict measured ΔT_{re} for the TREJ protocol. Data from all five hearts are presented in Fig. 12 (r² = 0.80, slope = 1.03).

Interpretation of T_{re}−σ_{max} and T_{re}−σ_{ee} Relationships

In steady-state isovolumic contractions, relaxation time is determined by peak active stress (39). Thus the plot of T_{re} vs. σ_{max} for a wide range of stress (from SBFS protocol, for example) offers a unique picture of the relaxation state of the heart, that is, a nomogram. As such, knowing the value of T_{re} is not enough to determine whether or not an intervention alters isovolumic relaxation; T_{re} must be compared at the appropriate value of σ_{max}. For example, a given intervention might result in simultaneous changes in T_{re} and σ_{max} such that the change in T_{re} is completely explained by the change in σ_{max}. In this case, the postintervention T_{re}−σ_{max} point would lie somewhere on the nomogram and we would conclude, even though T_{re} changed, that the relaxation process was not affected by the intervention. In contrast, if a new T_{re}−σ_{max} point lay below or above the nomogram, we would conclude that the intervention had a negative or positive effect on isovolumic relaxation, respectively.

This argument applies only to isovolumic beats. When relaxation is compared between isovolumic and ejection beats, σ_{max} is not a good index for muscle load at end systole in the ejection beats. We chose instead end ejection as a landmark in the ejection beats for several reasons. First, we wanted to refer to some load on the cardiac muscle in the early phase of relaxation. In isovolumic beats, σ_{max} occurs at end systole and is the appropriate choice. In ejection beats, σ_{max} occurs long before end systole and this time is highly dependent on arterial system load. Furthermore, it is possible that if σ_{max} occurs early enough, the T_{75}-T_{25} interval could begin before end ejection; such T_{re} measurements would not be a proper index of isovolumic relaxation. Thus σ_{ee}, which occurs very near end systole, is the appropriate and practical choice. Second, if we consider the isovolumic contraction to be a special case of ejection (i.e., SV = 0), then the transition from ejection to isovolumic beats (i.e., SV → 0) will force end systole, σ_{max}, and σ_{ee} to occur at the same time (see Fig. 5B). Thus the analyses of isovolumic and ejection beats are consistent with each other. With these criteria in mind we may now interpret and compare the results from the different protocols.

Qualitative Analysis of Data from SBFS and SSEJ Protocols

The steady-state ejection beat ΔT_{re}−Δσ points all lie below the nomogram (Fig. 7), meaning that relaxation is hastened in ejection beats. This ejection-mediated hastening of relaxation has been observed before (6, 14, 20). That the ejection beat ΔT_{re}−Δσ points converge to the origin with decreasing SF is expected because progressive reduction in shortening must ultimately lead to steady-state isovolumic behavior.

Because of positive inotropic effect, the first postejection isovolumic beat would have increased σ_{max}. If ejection only hastens relaxation (i.e., negative effect), we would expect that the ΔT_{re}−Δσ point from the first postejection beat would lie to the right of the reference
\( \Delta T_r - \Delta \sigma \) either 1) on the nomogram or 2) below the nomogram. The first case would mean that any negative effect on relaxation is short-lived and is only present in the ejection beat. The second case would indicate that the negative effect on relaxation persists and will disappear gradually. We see from Fig. 8 that neither of these expectations are met; the first post-ejection isovolumic \( \Delta T_r - \Delta \sigma \) points all lie above the nomogram, which indicates a positive effect on relaxation. The amount of this positive effect is directly related to SF (Figs. 1 and 8) and dissipates relatively slowly as evidenced by the values of \( \sigma_{\max} \) (Table 2). At this time we can conclude that in addition to the negative effect on relaxation observed in steady-state ejection, a positive effect of ejection exists. It is logical to assume that these two competing effects are present simultaneously in the ejection beats (with the negative effect dominating). The positive effect is unmasked in the post-ejection isovolumic beats because the negative effect is fleeting. This positive effect of ejection on relaxation has not been observed until now, and we have attempted to incorporate this phenomena into a more complete quantitative understanding of relaxation in the ejecting heart.

Quantitative Analysis

The small standard errors of the coefficients (Tables 1 and 2) indicate that all coefficients are significant and identifiable using our data set. More importantly, the values of \( a_3 \) or \( b_3 \) are the same for each model, confirming that there is no interaction between the stress coefficients (\( a_1 \) and \( a_2 \)) and the ejection coefficients (\( a_3 \) or \( b_3 \) and \( b_4 \)). In other words, there is a unique relationship between active stress and isovolumic relaxation, and ejection modifies relaxation independent of this relationship. The consistency of coefficients \( a_3 \) and \( a_2 \) also suggests that our choice of \( \sigma_{\max} \) for the ejection beat data is appropriate.

One would also predict that if both positive and negative effects of ejection on relaxation exist, they should be present simultaneously in the steady-state ejection beats. In other words, regression coefficient \( a_3 \) from Eq. 5, the coefficient relating \( \Delta T_r \) to SF, should contain information on both the positive and negative effects. This is revealed to be true in the full regression model (Eq. 10), where the sum of coefficients \( b_3 \) and \( b_4 \) is equal to \( a_3 \). If only steady-state ejection beat data were presented, the positive effect could not be identified. That the magnitude of \( b_3 \) is greater than that of \( b_4 \) is also consistent with the net negative effect found in the steady-state ejection beats; the negative effect dominates. Because both \( b_3 \) and \( b_4 \) linearly relate the amount of shortening to the speed of relaxation, their ratio represents their relative influences on \( T_r \). The \( b_3 \)-to-\( b_4 \) ratio for the five hearts was \( 0.45 \pm 0.04 \) (mean \( \pm \) SD), which demonstrates that, although the negative effect always dominates during ejection, the positive effect is not insignificant. Given these quantitative results, we feel that we were able to identify definitively both the negative and positive effects of ejection on isovolumic relaxation.

The final model (Eq. 9), developed to some extent on an ad hoc basis, yielded excellent descriptive fits to experimental data. However, the prediction of \( \Delta T_r \), in transient ejection beats, which were not used in the regression analysis, was quite good (\( r^2 = 0.80 \) for all hearts combined). This prediction of an independent data set further establishes the validity of the model.

Possible Sources of Error

Because of their obvious potential to affect data and subsequent interpretation, two sources of error are addressed. The first is coronary turgor, a condition whereby increases in coronary vascular volume can augment both systolic and diastolic \( P_v \) at a fixed chamber volume (24). In our experimental setup, coronary perfusion pressure (\( P_{\text{cor}} \)) was constant. Thus coronary turgor might be present in conditions with low systolic \( P_v \) (e.g., low volumes in the SBFS protocols, ejections with low values of peripheral resistance). To evaluate the effects of coronary turgor on left ventricular isovolumic relaxation, a SBFS protocol was performed on one heart at three levels of \( P_{\text{cor}} \) (65, 80, and 125 mmHg). Figure 13A shows the diastolic pressure-volume relationship. The effects of turgor are clearly

![Figure 13A](http://ajpheart.physiology.org/)

**Fig. 13.** A: LV (passive) \( P_{\text{cor}}-V_{\text{ed}} \) relationship obtained using SBFS protocol (see Fig. 2A) at 3 levels of coronary perfusion pressure (\( P_{\text{cor}} \)). As \( P_{\text{cor}} \) was raised, LV passive pressure increased, especially at higher volumes. B: relationship between LV isovolumic \( T_r \) and \( \sigma_{\max} \) was not affected by changes in \( P_{\text{cor}} \).
evident, because $P_{ed}$ is higher at a given volume with higher $P_{cor}$. Despite this increased pressure, the $T_r-\sigma_{max}$ relationships for the three conditions were superimposable, as shown in Fig. 13B. Thus it appears that turgor acts simply like an increase in preload; increases in stress are accompanied by increases in $T_r$ that are consistent with the nomogram.

A second potential source of error is our assumption that left ventricular shape is spherical for all volumes. Given that different geometries yield different stresses for any pressure-volume pair, this assumption can affect stress calculations in two ways. First, when volume changes, as during ejection or in SBFS protocol, the shape could change (17, 30). Second, even during isovolumic conditions, the shape of the ventricle is known to change. Shape changes during isovolumic relaxation will alter the time course of stress, affecting $T_r$ calculations. Thus we would like to be certain that our assumption of spherical chamber does not impact on the results so as to render the observed phenomena artifactual.

The positive effect of ejection on relaxation was deduced by comparing the $\sigma_{max}$-$T_r$ data for steady-state isovolumic and postejection isovolumic beats. Olsen et al. (30) have shown unique volume-shape relationships for diastole and systole, independent of loading conditions (i.e., different preloads and ejection patterns). Thus, because left ventricular volume was the same for the two isovolumic conditions, it is reasonable to conclude that they have the same chamber shape and shape change during relaxation. Consequently, more realistic assumptions regarding left ventricular geometry will not eliminate the positive effect.

For the net negative effect during ejection beats to be artifactual, stress would have to be overestimated by $\sim50\%$ (moving the $T_r-\sigma_{ee}$ point too far right) or $T_r$ would have to be underestimated by $\sim15\%$ (moving the $T_r-\sigma_{ee}$ point too far down), or some combination of both. Regarding the first possibility, due to ejection-mediated effects on inotropic state, an ejection beat with $\sigma_{ee}$ equal to $\sigma_{max}$ of an isovolumic beat can have a different $V_v$ throughout the isovolumic relaxation period (7, 37). However, the differences in $V_v$ in our data at common levels of stress are very small ($\leq5\%$). Therefore, errors in stress estimation due to volume-induced stress differences between isovolumic and ejection beats are expected to be insignificant. For the second possibility to have an impact, one would have to postulate that the left ventricular shape change during relaxation is significantly slower in the ejection beat compared with that in the isovolumic beat. Existing data (17, 30, 31), although not from experimental conditions precisely the same as ours (especially controlled $V_{ed}$ and heart rate), do not support this postulate. From these considerations we are confident that more realistic assumptions regarding the geometry of the left ventricle will not eliminate the net negative effect during ejection beats.

It is acknowledged that other sources of error may exist. For example, differences in behavior exist between blood-perfused and crystalloid-perfused hearts. However, these differences are mostly quantitative, and therefore the existence of the positive effect of ejection on relaxation, the new finding of this study, is not likely to be an artifact of the crystalloid perfusion.

Comparison With Previous Studies

Previous investigations have clearly established that the effects of ejection (shortening) on the speed of relaxation are determined by several factors, such as muscle length (initial or end ejection) (11, 14, 15, 34, 38, 41), amount of shortening (3, 6), shortening (loading) pattern (e.g., timing of start and end shortening) (3, 5, 6, 8, 19, 20, 27, 42), and systolic load (5, 8, 14, 18, 34, 38, 41). However, the common observation has been that relaxation becomes faster with increasing amount of ejection (shortening), consistent with our observation that ejection has a net negative effect on isovolumic relaxation, which is directly related to the amount of ejection. In contrast, the positive effect of ejection on relaxation has not been reported previously. Only by combining data from both ejection and isovolumic beats could the positive effect be observed. Specifically, by examining the first postejection isovolumic beat and comparing it to the nomogram, we were able to identify the positive effect. We could do this because stopping ejection effectively removes the immediate negative effect of ejection, i.e., the postejection isovolumic beats contain only the remnants of the positive effect.

Sys and Brutsaert (38), using cat papillary muscle, and de Tombe and Little (11), using rat trabeculae, related relaxation to muscle length and reported that relaxation time constant was directly proportional to end-systolic length in both isometric and shortening contractions. Given that the peak active stress in an isometric contraction is directly related to the end-systolic length over the physiological range, this observation is consistent with our data. However, de Tombe and Little (11) found that the relationship between relaxation time constant and end-systolic sarcomere length was the same for isometric and shortening contractions. This is inconsistent with our observations that the relaxation process is quite different between ejection and isovolumic beats at common end-systolic stress (which is very nearly the same as common end-systolic volume). Although we cannot definitively identify the reasons for this discrepancy, possibilities include species difference (rat vs. rabbit), specifics of the loading protocol (e.g., sarcomere length vs. ventricular volume control), and analysis of data (grouped vs. individual experiment).

Investigators also have focused on ejection pattern as determining relaxation rate. For example, Hori et al. (19, 20) found, in isolated dog hearts, that for constant SV and EF, delays in end-ejection time increased the speed of relaxation. In contrast, they found that changes in the begin-ejection time did not affect relaxation. Although we did not include these timing aspects of ejection in the analysis, they are unlikely to affect our conclusions for the following two reasons. First, reducing $R_+$ to increase SV (SSE) protocol resulted in a marked earlier begin-ejection time, with almost no
effect on end-ejection time (Fig. 1). This is so because reducing R$_s$ yields much lower end-diastolic aortic pressures (2). Second, although a given transient ejection beat had an identical ejection pattern to a steady-state ejection beat, the transient beat relaxed faster. However, it is possible that, had the protocols further uncoupled SV from begin- and end-ejection times, the end-ejection time would emerge as an independent determinant of isovolumic relaxation in ejection beats; such protocols are under development.

That ejection exerts a positive inotropic effect has been demonstrated by others, both at the muscle (1, 26, 32, 33) and the ventricular (7, 22, 37) level. These studies indicate that ejection-mediated changes in inotropic state are better described by the relative amount of ejection (e.g., EF) (7, 37). In contrast, different measures of ejection described the positive effect of ejection on relaxation equally well in the present study. Because $T_r$ is so strongly dependent on $\sigma_{\text{max}}$ (or $\sigma_{\text{iso}}$), it is possible that the ranges of $V_{\text{ef}}$ and $R_s$ used did not sufficiently uncouple absolute amounts of ejection (SV or SL) from relative amounts (EF or SF).

Potential Mechanisms for Effects of Ejection on Relaxation

Thus far we have described and quantified the negative and positive effects of ejection on relaxation in a phenomenological manner. The mechanisms that underlie these phenomena are of ultimate interest. With regard to the negative effect, the most commonly cited mechanism is the shortening-induced deactivation. Specifically, shortening causes loss of myofilament-bound Ca$^{2+}$, perhaps due to the decrease in the Ca$^{2+}$ affinity of troponin C (1, 21, 35). This would lead to hastening of relaxation provided that the change in troponin C affinity persists after the cessation of shortening. A second possibility is shortening-induced changes in the kinetic parameters of cross-bridge cycling, especially the increase in the rate of dissociation with increasing amount of shortening (23). Once again, if these changes in the kinetic parameters persist beyond end-ejection, hastening of relaxation would occur. Finally, it is theoretically possible that shortening causes changes in the rate of Ca$^{2+}$ sequestration by the sarcoplasmic reticulum; however, no clear experimental evidence exists to support this possibility. Irrespective of the underlying mechanism, our results indicate that the negative effect of ejection on relaxation is short-lived compared with the longer lasting positive effect.

With regard to the positive effect of ejection on relaxation, increased inotropic state combined with slower relaxation observed in the postejec- tion isovolumic beats is curious, because positive inotropic agents that mobilize intracellular Ca$^{2+}$ (e.g., $\beta$-agonists, sympathetic stimulant, phosphodiesterase inhibitors, digitals-like compounds) hasten relaxation (8, 14, 16, 29). Conversely, conditions that typically prolong relaxation are also negative inotropes (e.g., $\beta$-antagonists, hypocalcemia) (14, 43).

It was shown long ago (26, 32) that peak tension in isometrically contracting muscle strips immediately following shortening is larger than that of a steady-state isometric contraction at the same length. This phenomena demonstrates a dependence of contraction on the history of shortening. Series-coupled viscoelasticity was suggested as a possible mechanism (32). Although series-coupled viscoelasticity can, in principle, yield higher postejec- tion peak isovolumic pressures (any mechanism that increases the end-diastolic contractile element length will do this), the decay of postejec- tion $T_{\text{max}}, T_r$, and excess $T_r$ appears too slow to be explained by series- or parallel-coupled viscoelastic behavior (monoeponential time constants on the order of seconds, Figs. 9 and 10). For example, using isolated rat trabeculae at 25°C, de Tombe and ter Keurs (12) determined that the time constants for series and parallel viscoelasticity were ~6 ms and 100 ms, respectively. Similar findings for cat papillary muscle were reported by Chiu et al. (10).

Recent experimental studies have shown that the positive inotropic effect of ejection is a property of cardiac muscle, perhaps related to the effects of ejection on myofilament interaction with cytosolic free Ca$^{2+}$ (11). These experiment-based inferences are supported by the model-based study of Landesberg (28). It is tempting to postulate that the positive effect of ejection on relaxation also has its basis at the muscle level and is related to the positive inotropic effect. One possible mechanism could be ejection-induced increased Ca$^{2+}$ sensitivity. Tobias et al. (40) recently showed that the Ca$^{2+}$ sensitizer EMD-57033 acted to increase $T_r$ greater than would be expected due to increased $\sigma_{\text{max}}$ alone (similar to excess $T_r$ in our postejec- tion isovolumic beats). Furthermore, $\sigma_{\text{max}}$ and excess $T_r$ were augmented by similar amounts. We too find that both excess $T_r$ and $\sigma_{\text{max}}$ were augmented by 8–10% (depending on the amount of ejection) in the immediate postejec- tion isovolumic beats. Therefore, our results are consistent with the hypothesis that the positive effects of ejection on ventricular relaxation are mediated via increased myofilament Ca$^{2+}$ sensitivity. That postejec- tion excess $T_r$ and $\sigma_{\text{max}}$ decay at different rates (Figs. 9 and 10) could indicate that some intermediate mechanism links the two and that this intermediary has different dynamic relationships between inotropic state and relaxation. Given the mechanisms for the negative effect described here, ejection might have a dual effect on myofilament Ca$^{2+}$ sensitivity and the physical transducers for the positive and negative effects must be distinct. The specific molecular mechanisms linking the mechanical event of shortening to changes in Ca$^{2+}$ sensitivity remain unknown.

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Address for reprint requests: S. G. Shroff, The Univ. of Chicago Medical Center, 5841 S. Maryland Ave., MC-5084, Chicago, IL 60637. Received 20 May 1997; accepted in final form 5 September 1997.

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