The Frank-Starling mechanism is not mediated by changes in rate of cross-bridge detachment

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Almost a century ago, Frank and Starling described the effect of changes in ventricular volume on cardiac contractile function (25). The Frank-Starling relationship has been further characterized by experiments on muscle preparations, where it has been shown to be a fundamental property of myocardium, termed the force-length relationship (1, 8, 28). Further studies have shown that the force-length relationship is well preserved in skinned muscle preparations and therefore appears to operate mainly at the level of the sarcomere, manifesting as apparent changes in myofilament Ca$^{2+}$ sensitivity, with changes in sarcomere length (13). The mechanisms underlying these length-dependent changes in Ca$^{2+}$ sensitivity are not known. McDonald and Moss (21) found that osmotic compression of single cardiac myocytes eliminated the reduction in Ca$^{2+}$ sensitivity associated with a reduction in sarcomere length and proposed that the force-length relationship of cardiac muscle may be mediated in part on the basis of changes in myofilament lattice spacing. In a separate study, Zhao and Kawai (30) found that the rate of ATP hydrolysis in skeletal muscle was decreased with osmotic compression and was associated with an increase in myofilament economy, compatible with a reduction in the rate of cross-bridge detachment. The combined findings of these studies, therefore, suggest that changes in sarcomere length may modulate force development via changes in cross-bridge cycle kinetics. Specifically, they suggest that the rate of cross-bridge attachment is reduced at longer sarcomere lengths.

A reduction in the rate of cross-bridge detachment can be predicted to result in a reduction in the rate of ATP consumption for a given level of steady-state isometric force development. This can be illustrated using a simple two-state cross-bridge model (3), where N is the number of available cross bridges, X is the mean force per cross bridge, A is the fraction in the force-generating state, and D is the fraction in the non-force-generating state. In this case the steady-state force development (F) is predicted by the following equation

$$F = N \cdot A \cdot X$$

(1)

Furthermore, if $g$ is the rate of cross-bridge detachment and $f$ is the rate of attachment, then the overall absolute rate of cross-bridge turnover is predicted from the following equation

$$\text{Crossbridge turnover (cycles/s)} = g \cdot A \cdot N$$

(2)

All current cross-bridge models incorporate the concept that one molecule of ATP is consumed for a single, completed cross-bridge cycle. Therefore, from Eqs. 1 and 2

$$\text{Rate of ATP consumption} = g \cdot A \cdot N \cdot \frac{g}{X \cdot A \cdot N} = \frac{g}{X}$$

(3)

Equation 3 predicts that the slope of the ATP consumption-force relationship is a measurement of the rate of cross-bridge detachment. Implicit in this model are the assumptions that average force per cross bridge is constant, that a single molecule of ATP is consumed for a completed cross-bridge cycle, and that force development is proportional to the number of cross bridges in the force-generating state. These assumptions are common to most current cross-bridge models.

Therefore, we hypothesized that increases in sarcomere length in cardiac muscle would result in an increase in myofilament economy due to a reduction in the rate of cross-bridge detachment. If true, this should manifest as a reduction in the rate of ATP consumption for a given level of force generation at longer sarcomere lengths; i.e., the slope of the force-adenosinetriphosphatase (ATPase) relationship should be length dependent over the range of Ca$^{2+}$ activation that is associated with a steep force-length relationship. We simultaneously measured isometric force development and the rate of ATP hydrolysis in skinned rat cardiac trabeculae over a...
range of Ca$^{2+}$ activation and at three sarcomere lengths. We found that changes in sarcomere length did not alter the slope of the force-ATPase relationship, and concluded that the mechanisms that underlie the Frank-Starling relationship in cardiac muscle do not involve changes in the kinetics of the apparent detachment step in the cross-bridge cycle.

**MATERIALS AND METHODS**

Muscle preparation and experimental apparatus. All studies were conducted in accordance with institutional guidelines for the care and use of laboratory animals. We induced deep anesthesia in rats (Harlan LBN-F1, 225–250 g) by halothane inhalation. The hearts were then rapidly excised and immediately perfused with a cardioplegic, modified Krebs-Henseleit solution (see Solutions) as previously described (6). Under a binocular microscope, thin, unbranched trabecular muscles between the atrioventricular ring and right ventricular free wall were carefully excised. Muscle dimensions were determined via an ocular micrometer mounted in the dissecting microscope (resolution $\sim 10 \mu m$). On average the muscles were $1.44 \pm 0.36 \text{ mm}$ long, $0.95 \pm 0.19 \mu m$ thick, and $334 \pm 165 \mu m$ wide (means $\pm$ SD, measured at slack length). We incubated the trabeculae overnight in a relaxing solution containing 1% Triton X-100, which served to remove cell membranes and intracellular membrane-bound structures such as mitochondria and sarcoplasmic reticulum. Therefore, this procedure removed nonmyofilament ATPase, as well as sources of ATP generation, leaving the contractile myofilaments energetically isolated (12). Custom-made aluminum foil T clips were gently attached to the ends of the permeabilized muscles to serve as handles for mounting the preparation to the experimental apparatus (10). We mounted the muscles in a small bath (volume 60 $\mu l$) located on the stage of an inverted microscope (Nikon). The T clip on one end was hooked onto a servomotor (model 6350; Cambridge Technologies, Watertown, MA; $-250-250 \mu s$ 90% step response) that was used to control and adjust sarcomere length. The clip on the other end was attached to a modified semiconductor strain gauge (AE801; SenSonor; resonance frequency $\sim 2 \text{ kHz}$) for measurement of muscle force. The design of the bath was modified from Guth and Wojciechowski (12) and Stienen et al. (26) and is shown schematically in Fig. 1. The bath was designed to allow measurement of sarcomere length by laser diffraction and real-time measurement of ATP hydrolysis rate by enzyme-linked fluorescence (12). The sides of the chamber were Plexiglas, and the bottom was glass. The empty bath did not fluoresce under ultraviolet (UV) radiation. A small stirring rod traversed the length of the bath, parallel to the muscle and out of view of the microscope, and was driven by a small electric motor (Radio Shack). The bath was continuously stirred during an experiment to ensure rapid mixing so that enzyme reactions would not be limited by diffusion. Adequate stirring was confirmed by visual inspection of the time course of a step change in fluorescence after injection of a fluorescent indicator. A Hamilton syringe was fixed to a stand so that the tip of the needle entered one end of the bath. The plunger of the syringe was driven by a linear stepper motor under computer control using a custom computer program (LabVIEW, National Instruments). This enabled the precise injection of known amounts of reagents (such as NADH and ADP) into the bath, using a remote trigger. The bath was mounted on a copper base through which water was circulated for temperature control. The various solutions used to superfuse the muscles during an experiment were set in plastic cups on a copper plate, similarly temperature controlled. Temperatures of the bath and of the solutions were controlled using a heater-circulator (Fisher Scientific). A thermocouple thermometer (Digi-Sense, Cole-Palmer) was used to continuously monitor bath temperature, which averaged $23.8 \pm 0.66\text{°C }\pm$ SD over all experiments.

Measurement of sarcomere length. Sarcomere length was measured by laser diffraction as previously described (6). Briefly, a beam of laser light (632 nm) perpendicular to the longitudinal axis of the muscle was directed onto the center of the specimen. The resulting first-order diffraction band was projected onto a 512-element photodiode array (Reticon), which was scanned electronically every 0.5 ms. An analog circuit converted the intensity distribution of the diffraction band into a voltage proportional to median sarcomere length. Glass gratings of known spacing were used to calibrate the system. De Tombe and ter Keurs (6) found that errors due to muscle inhomogeneity and Bragg angle reflection artifacts are $<4\%$ using this approach. It was important to control sarcomere length both in the passive condition before activation and during force development to avoid the problem of internal shortening that could potentially impact on the force-ATPase relationship (26).

Measurement of ATP consumption. Because the mitochondria had been removed, it was necessary to add ATP to the solutions to fuel muscle contraction (see Solutions). In addition, we added the necessary enzymes to allow for the regeneration of ATP by the oxidation of NADH to NAD. Using the technique proposed by Guth and Wojciechowski (12), we then determined the rate of ATP consumption by using an enzyme-coupled system. Briefly, the ADP formed by the muscle was converted back to ATP by the following chemical reactions (12): 1) $\text{ATP} \rightarrow \text{ADP}$, 2) $\text{phosphoenolpyruvate} \rightarrow \text{ADP}$, $\text{pyruvate} \rightarrow \text{ATP}$, and 3) $\text{pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{lactate} + \text{NAD}^+$. Reaction 2 is catalyzed by the enzyme pyruvate kinase, whereas reaction 3 is catalyzed by lactate dehydrogenase. Both enzymes, as well as NADH and phosphoenolpyruvate, were added in ample amounts to the skinned fiber solutions to ensure a quick response time. The response time of the enzyme system has been estimated to be $\sim 20 \text{ ms}$ (11). The adequacy of the enzyme concentrations has previously been demonstrated (12); in addition, we performed preliminary experiments with 10- and 20-fold higher concentrations of enzymes and found no difference in rate of ATP consumption.

From the above reactions, it is apparent that one mole of NADH is converted to NAD for every mole of ATP converted to ADP. NADH, but not NAD, fluoresces at 470 nm under UV
radiation of 340–380 nm (12). Thus, by measuring fluorescence decay at 380 nm, we determined the rate of ATP consumption by the muscle. The signal was calibrated by injection of a known amount (1–2 nmol) of ADP into the solution during each activation. The ADP injection resulted in a rapid step reduction in fluorescence, and the magnitude of this step was used to calculate the rate of ATP consumption from the rate of fluorescent decay. In addition, the ADP injection served to confirm that the chemical response time and the bath stirring were adequate.

Fluorescence measurement. Figure 2 schematically depicts the optical arrangement for the laser sarcomere length measurements and the fluorescence measurements. All experiments were conducted in a dark room, and the sarcomere length system was interrupted with a shutter mechanism during fluorescence measurements. UV light (75-W lamp; Oregon, Stratford, CT) was passed through a 380-nm band-pass filter (band width 10 nm), chopped at 1,000 Hz (SR540 Chopper controller; Stanford Research Instruments, Stanford, CA), and transmitted to the microscope via liquid light guides (Oregon). The chopped UV light was projected on the muscle bath via a dichroic mirror (400 nm; Nikon) and a 20× UV-capable objective (Olympus). The resultant fluorescence signal, as well as other incident light collected through the microscope objective, was passed, via a 550-nm dichroic mirror, through a 480-nm, 20-nm band-pass filter to a photomultiplier tube (PMT) (R1527, Hamamatsu) with a voltage gradient of 900 V. The output of the PMT was input to a dual-phase lock-in amplifier that locked in on the fluorescence signal at the chopper frequency. By locking in on the chopping frequency, we were able to filter out non-UV-related light, and reduce noise more effectively than with a band-pass filter alone.

![Fig. 2. Schematic diagram of laser and fluorescent optical system.](image)

First-order diffraction band of a laser beam that was projected vertically onto muscle was passed through an inverted microscope and projected onto a photomultiplier for measurement of sarcomere length. Ultraviolet (UV) light (solid line) chopped at 1 kHz was directed through microscope and into bath by reflection off a 400-nm dichroic mirror. Resultant fluorescence (hatched line) was routed through microscope to a photomultiplier by reflection off a 550-nm dichroic mirror.

### Table 1. Solutions

<table>
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<th></th>
<th>MgCl₂</th>
<th>Na₂ATP</th>
<th>EGTA</th>
<th>HDTA</th>
<th>Ca-EGTA</th>
<th>KProp</th>
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<td>Relaxing</td>
<td>8.37</td>
<td>5.80</td>
<td>0.0</td>
<td>19.5</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>Preactivating</td>
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<td>5.80</td>
<td>0.5</td>
<td>19.5</td>
<td>43.6</td>
<td></td>
</tr>
<tr>
<td>Activating</td>
<td>7.63</td>
<td>5.87</td>
<td>2.0</td>
<td>19.5</td>
<td>43.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are concentrations in mmol/l. Ca-ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (Ca-EGTA) was made by dissolving equimolar amounts of CaCO₃ and EGTA. In addition, all solutions contained 0.6 mmol/l NADH, 100 mmol/l N,N,N,N'-tetrathiocyanatetramine (TMT), 0.012 mmol/l manganese dehydrogenase (870 U/mg), 10 µM oligomycin, 0.2 mmol/l ATP, and 100 µM leupeptin. Ionic strength was set at 200 mmol/l by potassium propionate (KProp); pH = 7.1; 24°C. Free Mg²⁺ and MgATP concentrations were calculated at 1 and 5 mmol/l, respectively. To achieve a range of free Ca²⁺ concentrations, activating and relaxing solutions were appropriately mixed assuming an apparent stability constant of the Ca-EGTA complex of 10⁶. HDTA, 1,6-diaminohexane-N,N,N',N'-tetraacetic acid.

Solutions. In every experiment, the muscle was dissected while the heart was perfused with a low-Ca²⁺ Krebs-Henseleit solution containing (in mmol/l) 140.5 NaCl, 5.0 KCl, 127.5 Cl⁻, 1.2 MgCl₂, 2.0 H₂PO₄, 1.2 SO₄²⁻, 19 HCO₃⁻, 10.0 glucose, and 0.1 CaCl₂. In addition, a Ca²⁺-desensitizing agent, 2,3-butanedione monoxime (20 mmol/l) was added to minimize damage to the ends of the trabeculae during dissection (22). The trabecular muscles were then bathed overnight in a relaxing solution to which 1% Triton X-100 was added to dissolve lipid membranes. After this “skinning period,” the muscles were bathed in a physiological solution that simulated intracellular conditions. Ca²⁺ in this solution was highly buffered so that Ca²⁺ concentration ([Ca²⁺]) could be strictly controlled. Three types of solutions were used: relaxing solution, preactivating solution, and activating solution. The compositions of these solutions are shown in Table 1. The solute concentrations were determined using an iterative computer program as described by Fabiato and Fabiato (7) using dissociation constants of Godt and Lindsey (9). We mixed various fractions of relaxing and activating solutions to obtain a variety of [Ca²⁺] in activating solutions. Experimental protocols. Muscles were activated or relaxed by exchanging the superfusate. Various levels of Ca²⁺ activation were obtained by mixing different proportions of activating and relaxing solutions. Muscles were allowed a minimum of 4 min in relaxing and preactivating solutions in between activations. An injection of 1 mmol ADP into the bath during each contraction was used to calibrate the rate of ATP consumption (Fig. 3). Muscle length was manually adjusted to maintain sarcomere length at the preactivation level during contraction. Data were collected when force development reached steady state.

We conducted two groups of experiments. The first group was a series of control experiments to determine whether the inevitable deterioration of force development during an experiment could affect myofilament efficiency directly and to test the effectiveness of the skinning process for the removal of sarcoplasmic Ca²⁺-ATPase. We wanted to be sure that we were indeed measuring only myofibrillar ATPase activity and needed to confirm the absence of other significant sources of Ca²⁺-sensitive ATPase. Therefore, in five muscles we conducted the following control experiments. At a constant sarcomere length, each muscle was activated at a minimum of five different levels of activation. Force development and ATP consumption were measured during each activation to determine a baseline force-ATPase relationship (run 1). This
series was then repeated in the same muscle at the same sarcomere length and the same levels of activation (run 2). Finally, we once again repeated the same series, this time with the addition of 10 µM cyclopiazonic acid (CPA), which is a potent inhibitor of Ca²⁺ATPase (CPA run) (18, 20). This protocol enabled us to test the effect of preparation deterioration during the passive state was also measured at each sarcomere length and the same levels of activation (ranging from 2.0 to 2.2 µm) at each sarcomere length, we stimulated contractions at a minimum of four different levels of Ca²⁺ activation (ranging from pCa 5.7 to 4.3), for a minimum of 12 activations. During each activation, steady-state force development and the rate of ATP consumption were measured. The rate of ATP consumption during the passive state was also measured at each sarcomere length. The order in which sarcomere length and the level of Ca²⁺ activation was changed was randomized between experiments.

Data analysis. The rate of ATP hydrolysis was calculated from linear regression of the slope of the fluorescent decay of NADH during each measurement period. This was measured in volts per second and was divided by the voltage step resulting from the injection of 10³ pmol ADP for conversion to picomoles per second. ATP consumption was normalized to muscle volume, and force generation was normalized to cross-sectional area. The economy of force development was assessed by determination of the slope of the force-ATPase relationship. Note that whereas the relationship between NADH concentration and fluorescence is nonlinear and saturates at high NADH concentrations, the range over which our experiments were conducted is almost linear, and correcting for the nonlinearity did not affect our findings.

![Diagram](https://example.com/diagram.png)

**Fig. 3. Measurement of rate of ATP hydrolysis.** Time course of changes in isometric force development (top), NADH fluorescence (middle), and sarcomere length (bottom) during a muscle contraction from a representative experiment are shown. Note that sarcomere length was monitored and adjusted to maintain 2.0 µm during rise in force development. When force transient reached steady state, a shutter mechanism shielded the laser and allowed UV light (380 nm) to fall on bath, and fluorescence was recorded. An injection of ADP (1 nmol) into bath served to calibrate signal and confirm adequate stirring and response time of enzyme cascade. Rate of ATP hydrolysis was determined from linear regression of fluorescent decay (see text for details).

**RESULTS**

ATP hydrolysis. Figure 3 shows the raw data collected during a single contraction in a representative experiment. The muscle was activated by exchanging the bath solution for activating solution. Force developed rapidly (Fig. 3, top tracing), then reached a plateau. During this time, the laser was directed onto the muscle and sarcomere length was determined (Fig. 3, middle tracing). If internal shortening occurred, the muscle was stretched to maintain sarcomere length at the passive level. In many cases (as in that in Fig. 3), internal shortening was negligible and little adjustment was necessary. If internal shortening was observed, sarcomere length was checked again before the muscle was relaxed to ensure that sarcomere length was indeed clamped during each activation. Once force reached steady state, the laser was shielded and UV light was projected on the muscle for measurement of fluorescent decay. Fluorescence decayed linearly over time during the activation (Fig. 3, middle tracing), confirming steady-state conditions. Halfway through...
the data collection 1 nmol ADP was injected into the bath to calibrate the signal. This resulted in a rapid step in fluorescence and then recovery of the same rate of linear decay. Linear regression analysis of the slope of fluorescent decay before and after the ADP step was performed. This slope represented the rate of ATP hydrolysis by the myofilaments during that activation. These data show that we were successful in simultaneously measuring force and the rate of ATP hydrolysis in a cardiac trabeculum while controlling sarcomere length during the activation. Control of sarcomere length was important in this study because internal shortening per se may result in changes in the force-ATPase relationship that would confound our analysis.

Preparation stability. The results from five experiments testing the effect of deterioration in force generation by the muscle on the force-ATPase relationship are shown in Fig. 4. The relationship between force and ATP consumption from two consecutive data runs are shown, as well as a third data run performed with the addition of CPA to the solutions. On average maximum force deteriorated by 27 ± 18% (i.e., 5-6% per activation) from the first run to the second run. The force-ATPase relationships, however, remained linear, and the slopes of the force-ATPase relationships were not significantly affected by time-dependent deterioration (P = 0.64). This suggests that the time- and/or activation-dependent decay commonly seen in this kind of preparation is due to loss of contractile units rather than to changes in cross-bridge kinetics. Therefore, the preparation was adequate to compare the effect of various interventions on cross-bridge kinetics. Similarly, the slope of the force-ATPase relationships were not altered by the addition of CPA to the superfusate (P = 0.38). This indicated that the skinning procedure adequately removed nonmyofilament Ca\(^{2+}\)-dependent ATPase and, therefore, the slope of the force-ATPase relationship represented myofilament efficiency and was not contaminated with ATP consumption by remnants of sarcoplasmic reticula.

Effect of sarcomere length. The effect of sarcomere length on the force-Ca\(^{2+}\) relationships in eight experiments is shown in Fig. 5. The maximal force developed was, on average, 90 mN/mm\(^2\). The force-Ca\(^{2+}\) relationship was consistently shifted to lower [Ca\(^{2+}\)] at higher sarcomere lengths, with a resultant decrease in the EC\(_{50}\) from 4.41 ± 0.57 µM at 2.0 µm to 3.39 ± 0.24 µM at 2.1 µm and to 2.32 ± 0.32 µM at 2.2 µm (P < 0.01). This is consistent with an increase in myofilament Ca\(^{2+}\) affinity at higher sarcomere lengths and is in agreement with the findings of other investigators (21, 27, 29). These data show that the Frank-Starling relationship was preserved in our preparation over the range activation levels and sarcomere lengths used and further confirms the validity of our preparation for investigating possible mechanisms for the Frank-Starling relationship.

The effect of sarcomere length on force development at two levels of Ca\(^{2+}\) activation is shown in Fig. 6. Note that the range of sarcomere lengths used in this study represents 50% of the working length in cardiac muscle (24) and resulted in an increase in force of 30–100% from the shortest to longest length at a given level of Ca\(^{2+}\) activation. If this force modulation were due to a
The effect of sarcomere length on the relationship of the rate of ATP consumption to force development is shown in Fig. 7. Figure 7A shows the data from a representative experiment, whereas Fig. 7B shows the pooled data. The raw data were subjected to multiple linear regression analysis (Eq. 7) to determine the relative effect of sarcomere length on the force-ATPase relationship. The fitted parameters are summarized in Table 2. The force-ATPase relationships at all sarcomere lengths, and the slopes of these relationships were not significantly different at different sarcomere lengths (P = 0.27). These data show that the rate of myofilament ATP consumption was determined solely by the level of isometric force generation and not by the [Ca\(^{2+}\)] or the sarcomere length. Therefore, it appears that both of these factors exert their effects on isometric force generation by modulation of the number of force-generating cross bridges, either via simple recruitment of cross bridges or changes in the attachment rate, but neither affect the rate-limiting step governing the apparent rate of cross-bridge detachment.

**DISCUSSION**

The slope of the force-ATPase relationship has been proposed as an index of the rate of cross-bridge detachment (3). Accordingly, any length-dependent change in the rate of cross-bridge detachment should manifest as a change in the slope of the force-ATPase relationship. Therefore, we simultaneously measured steady-state isometric force development and the rate of ATP consumption in skinned rat cardiac trabeculae at various levels of Ca\(^{2+}\) activation while rigorously controlling sarcomere length. From the data, we determined the relationship between force and the rate of ATP consumption at different sarcomere lengths.

The force-ATPase relationships at all sarcomere lengths were linear. This is in agreement with prior studies in skeletal (3, 23) and cardiac muscle (5, 18). With the assumption of a uniform development of force per cross bridge and stoichiometric coupling of cross-bridge turnover and ATP consumption, the linearity of the force-ATPase relationships suggests that changes in the level of Ca\(^{2+}\) activation per se do not affect the overall rate of cross-bridge detachment. This is supported by similar findings in cardiac muscle by de Tombe and Stienen (5).

Our findings of an average force-ATPase slope of 15.5 pmol·mN\(^{-1}\)·mm\(^{-1}\) and a maximal rate of ATP consumption of 1.5 nmol·s\(^{-1}\)·µl fiber vol\(^{-1}\) are both higher than previously reported for rat myocardium (5, 18). Assuming a myosin head concentration of 0.15 mmol/l (2), we deduced a maximum cycling rate of 10 s\(^{-1}\) per myosin head as opposed to ~3 s\(^{-1}\) from the prior studies (5, 18, 23). This is probably because superfusate temperatures in our experiments were ~4°C higher. An increase in the rate of ATP hydrolysis during isometric contraction would be expected to result in a decrease in economy due to a decrease in the average duration of cross-bridge force-generating states.

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**Table 2. Effect of sarcomere length on the force-ATPase relationship as shown by multiple linear regression analysis**

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<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
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<td>&lt;0.01</td>
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<tr>
<td>δ, pmol·µl(^{-1})·s(^{-1})·mN(^{-1})·mm(^{-1})</td>
<td>0.66</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Data from 8 trabeculae were analyzed by multiple linear regression (Eq. 6) to assess impact of sarcomere length on slope of force-ATPase relationship. The parameter represents average slope of force-ATPase relationship, whereas the parameter represents average effect of changes in sarcomere length from 2.0 to 2.2 µm on this slope. The parameter is average rate of ATP consumption in passive state, whereas the parameter represents the average impact on changes in sarcomere length on resting ATP consumption. Effect of changes in sarcomere length from 2.0 to 2.2 µm had no significant effect on slope or intercept of force-ATPase relationship.
We had expected to find that increases in sarcomere length would result in an increase in myofilament economy due to a reduction in the cross-bridge detachment rate, mediated by a decrease in actin-myosin lattice spacing. Evidence linking sarcomere length effects to lattice spacing was provided by McDonald and Moss (21), who showed that osmotic compression of isolated cardiac myocytes restored Ca$^{2+}$ sensitivity at short sarcomere lengths. In addition, Zhao and Kawai (30) found that osmotic compression was associated with a decrease in the rate of ATP hydrolysis and an increase in myofilament economy in skeletal muscle. Thus it appeared likely that increases in sarcomere length in cardiac muscle might, by the same mechanism, result in an improvement in myofilament economy. Our results, however, do not bear this out. We found no effect of changes in sarcomere length between 2.0 and 2.2 µm on the slope of the force-ATPase relationship. It should be noted that the degree of change in lattice spacing over the range of sarcomere lengths tested in our study may not have been sufficient to result in a significant change in cross-bridge cycle kinetics. For example, Zhao, Kawai, and co-workers (17, 30) found that the tension cost was cut in half at a dextran concentration of 8%, which corresponded approximately to a fiber width reduction of 20%. With the assumption of constant volume behavior, this degree of lattice spacing change would require a sarcomere length change of 156% from 1.8 to 2.9 µm, which is out of the physiological range for cardiac muscle. The same data would predict that a sarcomere length change from 2 to 2.2 µm as used in our study would have resulted in a fiber width change of only 5% and a reduction in the force-ATP relationship of ~20%. Therefore, although the range of sarcomere lengths tested in this study is relatively small, it is representative of the physiological range in cardiac muscle and covers a steep portion of the force length relationship at submaximal Ca$^{2+}$ activation (19). Indeed, the Frank-Starling relationship was well preserved in our skinned fibers, and this could not be accounted for by changes in the rate of cross-bridge detachment. Assuming no change in the rate of attachment, we would have predicted a decrease of at least 50% in the rate of detachment to account for the force changes between the shortest to longest sarcomere lengths in our studies. This would have been easily detectable with our system. On the basis of the variance of our slope estimates, we calculated a >80% power to detect a 20% slope change (P < 0.05) from our experiments. We feel that the lattice spacing changes in our study were smaller than those induced by osmotic compression in the previous studies. Although changes in lattice spacing may still underlie the mechanism of the Frank-Starling relationship, our data would suggest that this is not mediated by changes in the rate of cross-bridge detachment.

In a previous study, Kentish and Stienen (18) found that at short sarcomere lengths, the level of force generation in skinned cardiac muscle was reduced out of proportion to the rate of ATP hydrolysis, resulting in a reduction in myofilament efficiency. This result does not conflict with our study, because this effect was only apparent below 1.95 µm and was thus probably the result of restoring forces opposing contraction. At higher lengths, data in that study were only collected at maximal activation, where the Frank-Starling mechanism was not operative. We purposely avoided making measurements at short sarcomere lengths to avoid the confounding effects of restoring forces.

Hofmann and Moss (14) have shown that unloaded shortening velocity in skinned rat myocardium is reduced at lower levels of Ca$^{2+}$ activation, which would seem to conflict with our finding a linear force-ATP consumption relationship. Along the same lines, it has been shown in intact myocardium that the level of force development determines the relaxation rate (4, 15, 16). However, the conditions in these experiments were markedly different from the current study. We studied skinned myocardium in an isometric, high-strain state. In this situation the myofilaments are isolated from the complex activation process present in intact muscle, and there are no possible shortening-mediated effects on activation level. The determinants of the rate of relaxation after a twitch in intact muscle are still unknown. However, our data suggest that at steady state there is no direct effect of Ca$^{2+}$, force, and length on the rate of cross-bridge cycling at the myofilament level. In intact twitching muscle, mechanisms such as Ca$^{2+}$ binding kinetics, cooperative activation-deactivation of actin binding sites, or passive viscous loads may effect the rate of force decay.

The limitations of this study involve the use of skinned cardiac trabeculae. Skinned preparations may not exhibit constant volume behavior, which is expected in intact preparations. Therefore, it is possible that intact myocytes may derive some energetic benefit, not apparent in our preparations, at longer sarcomere lengths. However, the force-length relationship is preserved in skinned preparations, suggesting that either lattice spacing is similar, or that lattice spacing effects do not directly mediate the force-length relationship. Another potential source of error in our preparation is the fact that small portions of the muscle at either end of the preparation are covered by aluminum clips and do not contribute to force generation. However, Kentish and Stienen (18) have shown that the ATP consumed by these portions of the preparation is negligible. Although it would be preferable to simultaneously measure myofilament force and ATP consumption in a more “physiological” preparation, this is not currently possible.

We conclude that the rate of ATP consumption is tightly and linearly coupled to the level of isometric force development in cardiac myofilaments. Neither changes in Ca$^{2+}$ activation nor changes in sarcomere length over the physiological range resulted in a significant departure from linearity in the relationship between force development and the rate of ATP consumption in our experiments. Specifically, there was no increase in myofilament economy at longer sarcomere lengths. We conclude that neither sarcomere length nor the level of Ca$^{2+}$ activation exert their effects on force...
development via changes in the rate of cross-bridge detachment.

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