Increased cross-bridge cycling rate in stunned myocardium

Wei Dong Gao, Tieying Dai, and Daniel Nyhan

Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted 13 May 2005; accepted in final form 13 September 2005

Gao, Wei Dong, Tieying Dai, and Daniel Nyhan. Increased cross-bridge cycling rate in stunned myocardium. Am J Physiol Heart Circ Physiol 290: H886–H893, 2006. First published September 19, 2005; doi:10.1152/ajpheart.00493.2005.—Decreased Ca2+ responsiveness of the myofilaments underlies myocardial stunning. Given that cross-bridge cycling is a major determinant of myofilament behavior, we quantified cross-bridge cycling rate in stunned myocardium. After stabilization, rat hearts were subjected to 20 min of no-flow global ischemia and 30 min of reperfusion at 37°C. Control hearts were perfused continuously at 37°C for 60 min. Trabeculae were dissected and chemically skinned with 1% Triton X-100. The muscles were then activated with solutions of varied Ca2+ concentration ([Ca2+]w). Force-[Ca2+]w relations, rate of force redevelopment after release (ktr), muscle stiffness (kM), and myofilament ATP consumption were determined. Maximal Ca2+-activated force (Fmax) was depressed in stunned myocardium (49 ± 5 vs. 82 ± 5 mM/m2, P < 0.01). Western immunoblotting showed degradation of troponin I in stunned myocardium. The ktr at Fmax was significantly increased in stunned muscles (19.82 ± 2.74 vs. 13.19 ± 0.96 s⁻¹, 22°C, P < 0.01; 7.49 ± 0.52 vs. 5.81 ± 0.54 s⁻¹, 10°C, P < 0.05). The ratio of ktr measured at 100 Hz over that at 1 Hz, during Fmax, is lower in stunned muscles (8.22 ± 1.56 vs. 12.94 ± 0.71, P < 0.05). In comparison with ktr at rigor, ktr at Fmax is significantly lower in the stunned group (78.82 ± 6.11 vs. 93.27 ± 3.03%, P < 0.05). Myofilament ATP consumption at Fmax did not change in stunned muscles (5,901 ± 952 vs. 5,596 ± 972 pmol·µL⁻¹·min⁻¹, P = 0.49). These results show that cross-bridge cycling is increased in stunned myocardium. Such increases are likely the result of increased transition rate from force-generating states to non-force-generating states. Thus stunned myocardium still maintains ATP consumption in spite of lower force development, rationalizing the long-standing paradox of decreased force but unchanged oxygen consumption in the postischemic heart. Cross-bridge cycling; contraction; myocardial stunning

Address for reprint requests and other correspondence: W. D. Gao, Dept. of Anesthesiology and Critical Care Medicine, Johns Hopkins Univ. School of Medicine, Tower 711, 600 N. Wolfe St., Baltimore, MD 21287 (e-mail: wga03@jhmi.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
redevelopment ($k_d$) to determine the rate of cross-bridge cycling. We found that $k_d$ increased significantly in stunned muscles. We also performed muscle stiffness ($k_m$) analysis by imposing sinusoidal length perturbations at 1 and 100 Hz and found that the ratio of $k_m$ at 100 Hz over 1 Hz was reduced in stunned muscles compared with controls. Also in stunned muscles, $k_m$ during maximal activation was lower than the stiffness during rigor, in which all cross bridges are expected to remain attached because of the absence of ATP. Myofilament ATP consumption was not different in stunned muscles compared with controls. These results show that there is an increased cross-bridge cycling rate in stunned myocardium. Stunned myocardium maintains ATP consumption in spite of decreased force development, likely because of an increased rate of cross-bridge turnover from force-generating states to non-force-generating states.

**MATERIALS AND METHODS**

**Rats.** Male LBN-F1 (~250–300 g body wt, n = 30) rats were used in these experiments. The care of the animals and the experiment protocol were approved by the Animal Care and Use Committee of The Johns Hopkins University.

**Whole hearts.** The hearts were exposed via midsternotomy after the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg). 0.3–0.5 ml (300–500 U) heparin was injected in the left atrium, and the aorta was clamped. The heart was then rapidly excised, and the aorta was cannulated. The hearts were perfused retrogradely (~15 ml/min) with Krebs-Henseleit (K-H) solution equilibrated with 95% O2-5% CO2. The K-H solution is composed of (in mmol/l): 120 NaCl, 20 NaHCO3, 5 KCl, 1.2 MgCl2, 10 glucose, and 1.0 CaCl2, pH 7.35–7.45. The hearts were beating at 270–300 beats/min. Isovolumic left ventricular pressure was measured with an intracavitary balloon filled with water and connected to a pressure transducer. The volume of the balloon was adjusted to a diastolic pressure of ~10 mmHg, which was kept constant throughout the experiments. The hearts were placed in a water-jacketed container to maintain a constant temperature of 37°C. All hearts were perfused at constant flow (15 ml/min). An implantable temperature probe was attached to the myocardium for one mole of ATP to ADP. Because NADH fluoresces at 470 nm (emission) under ultraviolet radiation at 340 nm (excitation), decrease in fluorescence at 340 nm excitation in the solution reflects a reduction in NADH amount in the solution and thus the rate of ATP hydrolysis by the myofilaments (i.e., ATPase activity). Pyruvate kinase and lactate dehydrogenase, and their substrates (NADH and pyruvate), were added to both relaxing and activating solutions. To allow better diffusion into myofibrils, muscles were bathed in relaxing solution containing these substrates for a few minutes before the next activation. The concentrations of these substrates in both relaxing and activating solutions were as follows: 0.6 mmol/l NADH, 10 mmol/l phosphoenolpyruvate, 100 U/ml pyruvate kinase, and 5N a2ATP, 4.75 MgCl2, and 0.5 leupeptin, pH 7.2. Varied Ca2+ concentrations ([Ca2+]i) was calculated by a computer program that was based on the stability constants and the enthalpy values for the various reactions from Martell and Smith (21), except values for Mg2+-ATP and Ca2+-ATP reaction from Petit and Siddiqui (29). Skinning was considered to be complete when the preparation lost its pink color and sarcomeres were visualized. Diastolic sarcomere length was determined by direct visualization under ×100 magnification and was set at ~2.2 μm. Resting force was usually 5–10% of maximal activated force at this sarcomere length.

The steady state force-[Ca2+] relations were fit with a function of the Hill equation

\[ F = F_{\text{max}}[\text{Ca}^{2+}]^{n}/(C_{\text{Ca}_0} + [\text{Ca}^{2+}]^{n}) \]

where $F$ is the steady-state force at varied [Ca2+]i values, $F_{\text{max}}$ is the maximal Ca2+-activated force, $C_{\text{Ca}_0}$ is the Ca2+ required for 50% of maximal activation, and $n$ is the Hill coefficient.

**Western immunoblotting of troponin I.** In separate experiments, frozen (~80°C) tissue samples (100–200 mg) from control and stunned hearts were ground in liquid nitrogen and decanted into preweighed ice-cold Eppendorf tubes with lysis buffer (6.0 mol/l urea, 2.0 mol/l thiourea, 4% CHAPS, and 40 mmol/l Tris, pH 8.8). The mixture (10% wt/vol) was homogenized by sonication and centrifuged at 12,000 rpm for 40 min at 4°C. The supernatant was collected. Protein contents were determined with the Bio-Rad Protein Assay Kit.

Myofilament proteins separated by SDS-PAGE were transferred to a nitrocellulose membrane. After being blotted, the membrane was incubated with anti-Tnl (31-35; epitope 175–189 fragment, dilution 1:30,000; Spectral Diagnostic) for 1 h at room temperature with gentle agitation. After being washed, the membrane was probed with anti-mouse IgG (1:30,000) conjugated to horseradish peroxidase (HRP). HRP activity was visualized with Super Signal Chemiluminescent Substrate(Pierce) for 5 min, and the membranes were exposed to X-ray film (KODAK BioMax XAR Film) for 1–120 s to detect the blot.

**Mechanical measurement of cross-bridge kinetics.** The quick release/restretch protocol was used to measure $k_d$. The skinned muscle was mounted between a force transducer and a motor arm via aluminum foil T-clips, the arms of which were folded and crimped directly on both ends of the muscle. The muscle length was decreased by a rapid release step (20% of muscle length) to drop force to zero in 1 ms, held for 25 ms, and then stretched back to the original length. The redeveloped force was recorded. These experiments were performed at both 10 and 22°C. The redeveloped force was fitted with the following single exponential equation

\[ F_t = F_{\text{max}}(1 - e^{-k_t}) \]

where $F_t$ is the level of force redevelopment at any time (t) after release and $k$ is $k_d$.

**Measurement of km.** The $k_m$ was measured using small (~<0.5% of muscle length) sinusoidal oscillations at 1 and 100 Hz. The ratio of force perturbations over muscle length changes will be used to indicate $k_m$.

**Measurement of myofilament ATP consumption.** Isometric ATPase activity was measured using the following coupled enzyme system (15), which was later adopted by Wannenburg et al. (37) in cardiac trabeculae (37):

1. pyruvate kinase converts: ADP + phosphoenolpyruvate → ATP + pyruvate;
2. lactate dehydrogenase converts: pyruvate + NADH + H+ → NAD+ + lactate.

In this coupled system, ATP is added to fuel contraction. ADP thus produced is converted back to ATP by the oxidation of NADH to NAD. Also in this system, one mole of NADH is converted to NAD for one mole of ATP to ADP. Because NADH fluoresces at 470 nm (emission) under ultraviolet radiation at 340 nm (excitation), decreases in fluorescence at 340 nm excitation in the solution reflects a reduction in NADH amount in the solution and thus the rate of ATP hydrolysis by the myofilaments (i.e., ATPase activity). Pyruvate kinase and lactate dehydrogenase, and their substrates (NADH and phosphoenolpyruvate), were added to both relaxing and activating solutions. To allow better diffusion into myofibrils, muscles were bathed in relaxing solution containing these substrates for a few minutes before the next activation. The concentrations of these substrates in both relaxing and activating solutions were as follows: 0.6 mmol/l NADH, 10 mmol/l phosphoenolpyruvate, 100 U/ml pyruvate kinase, and 5N a2ATP, 4.75 MgCl2, and 0.5 leupeptin, pH 7.2. Varied Ca2+ concentrations ([Ca2+]i) were achieved by mixing the activating solution and relaxing solution in various ratios. [Ca2+] was calculated by a computer program that was based on the stability constants and the enthalpy values for the various reactions from Martell and Smith (21), except values for Mg2+-ATP and Ca2+-ATP reaction from Petit and Siddiqui (29). Skinning was considered to be complete when the preparation lost its pink color and sarcomeres were visualized. Diastolic sarcomere length was determined by direct visualization under ×100 magnification and was set at ~2.2 μm. Resting force was usually 5–10% of maximal activated force at this sarcomere length.
kinase, 12 U/ml lactate dehydrogenase, 0.2 mMol/l ADP, and 100 

μmol/l leupeptin. To ensure adequate mixing of all substrates, 

the volume of the bath was limited to 100 μL and the solution was stirred 

by air bubbles from a microneedle placed at one end of the bath. The 

small disturbance in the solution allows adequate mixing but does not 

interfere with fluorescent measurement. Calibration was performed 

separately, using ADP (10 mMol/l) that was manually injected in the 

bath containing the coupled enzyme system. The step drop in fluo-

rescence (in mV) after ADP injections was measured and was later 

used to calculate ATPase conversion (decrease in mV was used as the 

denominator). In most of our experiments, ATPase measurements 

were performed only during maximal activations. We therefore in-

jected ADP in the same maximal activating solution after removing 

the muscle and calculated ATPase activity based on the drop for that 

muscle. We also injected ADP in the bath containing the coupled 

enzyme system during rest. We pooled the data of the injections and 

determined that the step drop of fluorescence after 10 mMol/l ADP was 

6.5 ± 0.3 (SD) mV in <20 s (n = 7). Thus, in our system, there were 

only small variations in the step drops after ADP injection across 

experiments. The decreases of fluorescence at 340 nm were monitored 

during activations, and the rate of NADH decrease (and thus the rate 

of ATP consumption) was obtained. ATP consumption was normal-

ized to muscle volume and expressed as picomoles per microliters per 

second. We also calibrated NADH fluorescence over an NADH 

determination range of 0–400 nmol/l, a range over which our 

experiments were performed.

Statistics. Student’s t-test and one-way ANOVA was used for 

statistical analysis of the data (32, 38). A value of P < 0.05 was 

considered to indicate significant differences between groups. Unless 

otherwise indicated, pooled data are expressed as means ± SE.

RESULTS

Force-[Ca²⁺] relations in skinned stunned muscles. Figure 

1 shows the pooled data from all muscles studied. In Fig. IA the 

data are shown in absolute values, and in Fig. IB the data 

are normalized to their maximal values. These results are 

consistent with our previous findings in stunned muscles (11). 

Fₘₐₓ decreased significantly in stunned muscles (49 ± 5 vs. 

82 ± 5 mN/mm², P < 0.01). Ca₅₀ increased in stunned muscles 

(1.68 ± 0.14 vs. 1.22 ± 0.14 μMol/l in control 

muscles, P = 0.04), with no changes in the Hill coefficient n 

(2.5 ± 0.41 in stunned muscles vs. 2.1 ± 0.19 in control 

muscles, P = 0.5). Thus stunned muscles showed decreased 

Ca²⁺ responsiveness.

Western immunoblots of TnI. The role of TnI degradation in 

the stunned myocardium is controversial. To determine if TnI 

degradation occurred in our experiments, we performed Western 

immunoblot analysis of troponin I in tissues from hearts that 

underwent 20 min global ischemia followed by 30 min 

reperfusion. Figure 2 reveals a degradation band of TnI in 

stunned myocardium. Brief ischemia (20 min) alone did not 

cause TnI degradation. Similar results were obtained in three 

stunned hearts. These results are consistent with our previous 

studies (12), confirming that TnI is indeed partially and selec-

tively degraded in stunned myocardium.

Changes in kᵣ after stunning in skinned muscles. One of the 

major changes in stunned myocardium is the depression of 

Fₘₐₓ (see Fig. 1A). We thus investigated whether altered 

kinetic behavior of cross bridges affected force development 

during maximal activation. Brenner (3, 4) showed that the 

turnover kinetics of the cross bridges is important in regulating 

force development. When the turnover rate of the attached 

cross-bridges changes, the force-Ca relationship changes. We 

performed quick-step releases during Fₘₐₓ to assess changes in 

kᵣ to probe the behavior of the cross bridges in stunned 

myocardium. Figure 3A shows the typical traces of force 

redevelopment after a quick-release step of 25 ms duration. 

After normalization, it is clear that the rate of force recovery 

is faster in stunned trabecula than control (Fig. 3, A and B, insets). 

These force redevelopment traces were fitted with a single 

exponential equation, and kᵣ values were determined. Figure 

3C shows the pooled data of kᵣ from both control and stunned 

muscles. The kᵣ increased significantly in stunned muscles 

compared with control muscles (19.82 ± 2.74 vs. 13.19 ± 0.96 

s⁻¹, P < 0.02, temperature 22°C). We repeated these 

experiments at 10°C to verify these changes (Fig. 3, B and D). 

Despite slowing of kᵣ as temperature drops, it remains 

accelerated in stunned muscles (7.49 ± 0.52 vs. 5.81 ± 0.54 s⁻¹, 

P < 0.05). We also performed quick-release experiments at
two submaximal \([\text{Ca}^2+]\) (Table 1). The \(k_{tr}\) was not statistically different between control and stunned groups at these submaximal activations.

Because \(k_{tr}\) is the sum of \(f_{app}\) (the rate constant for cross bridges from a non-force-generating state to a force-generating state) and \(g_{app}\) (the rate constant from a force-generating state to a non-force-generating state), we derived \(f_{app}\) and \(g_{app}\) based on \(k_{tr}\) measured at maximal \([\text{Ca}^2+]\) and \(k_m\) at rigor and during \(F_{\text{max}}\) (see below). Table 2 shows the derived \(f_{app}\) and \(g_{app}\) in control and stunned muscles. Thus the rate for cross bridges from a force-generating state to a non-force-generating state is increased in stunned muscles.

\(k_m\) in stunned myocardium. In this study, we also determined \(k_m\) by use of sinusoidal muscle length perturbations at frequencies of 1 and 100 Hz. In an active muscle, \(k_m\), when measured at 100 Hz of length perturbations, increases as more cross bridges are attached, whereas \(k_m\) at 1 Hz remains nearly the same compared with a nonactivated muscle (19, 36). We have also shown previously that measurement of the \(k_m\) ratio (100 over 1 Hz) is a sensitive method to establish whether active cross-bridge cycling is present. In each individual muscle during \(F_{\text{max}}\), \(k_m\) was first measured at 1 Hz and then at 100 Hz, and the ratio of \(k_m\) at 100 Hz over 1 Hz was determined (Fig. 4). The ratio of \(k_m\) at 100 over 1 Hz is greater in control muscles compared with stunned muscles, indicating a higher number of cross bridges during \(F_{\text{max}}\) in control muscles.

Rigor defines a state when cross bridges are unable to detach when ATP is absent. During rigor, all cross bridges are “locked” and \(k_m\) is expected to be maximal. To determine

![Western immunoblot of troponin I (Tnl) of tissue sample from control (ctr), stunned (stun), and 20-min ischemic (isch) hearts. Each lane was loaded with 10 \(\mu\)g sample protein. Note that an additional [degradated (degrad)] band of Tnl is seen in the stunned sample but not in control and 20-minute ischemic samples. MW, mol wt.](image)

![Fig. 2. Western immunoblot of troponin I (Tnl) of tissue sample from control (ctr), stunned (stun), and 20-min ischemic (isch) hearts. Each lane was loaded with 10 \(\mu\)g sample protein. Note that an additional [degradated (degrad)] band of Tnl is seen in the stunned sample but not in control and 20-minute ischemic samples. MW, mol wt.](image)

![Fig. 3. Force redevelopment after a step release during maximal Ca\(^{2+}\) activation. A: representative traces of force redevelopment after a step release during maximal activation in a control and a stunned muscle at 22°C. Inset compares the time courses of the two traces after normalization to each maximal value. The stunned muscle has faster recovery time to the prerelease level. The traces were fitted by a single exponential equation, and the rate constants of force redevelopment \(k_{tr}\) were obtained in each muscle. [Ca\(^{2+}\)] = 24 \(\mu\)mol/l. C: summery of \(k_{tr}\) from control and stunned muscles at a temperature of 22°C. B: representative traces of force redevelopment after a step release during maximal activation in a control and a stunned muscle at 10°C. D: summery of \(k_{tr}\) from control and stunned muscles at a temperature of 10°C. Please note that, although decreased at 10°C, \(k_{tr}\) remains accelerated in stunned muscles. Data are means \(\pm\) SE; \(n = 8–10\) in each group. *\(P < 0.01\) and **\(P < 0.03\) vs. control.](image)

### Table 1. \(k_{tr}\) and myofilament ATPase activity in control and stunned muscles at submaximal activations

<table>
<thead>
<tr>
<th>[Ca(^{2+})](\mu)mol/l</th>
<th>(k_{tr}, \text{s}^{-1})(\text{mol}^{-1}\text{l}^{-1})</th>
<th>ATPase, pmol(\text{s}^{-1}\text{mol}^{-1})</th>
<th>Control</th>
<th>Stunned</th>
<th>Control</th>
<th>Stunned</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.67</td>
<td>4.47±0.46</td>
<td>811±215</td>
<td>4.43±0.46</td>
<td>811±215</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.68</td>
<td>10.45±0.76</td>
<td>2,051±365</td>
<td>10.76±0.76</td>
<td>2,298±468</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means \(\pm\) SE; \(n = 4–6\) rat hearts in each group. [Ca\(^{2+}\)], Ca\(^{2+}\) concentration; \(k_{tr}\), rate of force redevelopment after release. Temperature = 22 °C.
whether a decreased capability of forming cross bridges is responsible for the decreased $F_{\text{max}}$, we induced rigor in stunned muscles by exposing the muscles to ATP-free activating solutions containing maximal $\text{Ca}^{2+}/\text{Ca}_{\text{ATP}}$, and $k_m$ was measured at 100 Hz. There is no change in $k_m$ at rigor in stunned muscles (4,799.81 ± 544.83 vs. 5,385.58 ± 887.58 mN·mm⁻²·μm⁻¹, $P = 0.49$). This indicates that the total number of cross bridges available for attachment is not different in stunned myocardium compared with control. However, the maximal active $k_m$ during $F_{\text{max}}$ decreased over 20% when comparing with $k_m$ at rigor in stunned muscles (Fig. 5). In control muscles, $k_m$ during $F_{\text{max}}$ decreased ~7% in comparison with the $k_m$ at rigor.

Myofilament ATP consumption in stunned myocardium. To complement the above findings, we assessed myofilament actomyosin-ATPase activity in separate groups of skinned muscles (Fig. 6). These measurements would add the analysis of cross bridge in stunned myocardium. Because the major change of $\text{Ca}^{2+}$ responsiveness in stunned myocardium is depression of force development during maximal activations, we focused our measurement of myofilament ATP consumption at $F_{\text{max}}$. Figure 7 shows the summary of the data. In spite of significant decreases in $F_{\text{max}}$, myofilament ATP consumption was not reduced in stunned muscles. We also measured ATP consumption at two different submaximal activations, and there was no difference between control and stunned groups.

<table>
<thead>
<tr>
<th>Table 2. $f_{\text{app}}$ and $g_{\text{app}}$ in control and stunned muscles during maximal $\text{Ca}^{2+}$ activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{\text{app}}$</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Stunned</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 7$–9 rat hearts in each group. $f_{\text{app}}$, the rate constant for cross bridges from a non-force-generating state to a force-generating state; $g_{\text{app}}$, the rate constant from a force-generating state to a non-force-generating state. Temperature = 22 °C. *$P > 0.02$ vs. control.
ATPase activity at rest was not different between control and stunned groups (results not shown).

**DISCUSSIONS**

This study in stunned myocardium reveals increased $k_{tr}$, decreased $k_m$ during maximal activations with no changes in $k_m$ at rigor, and no changes in myofilament ATP consumption despite reduced maximal force development. These findings suggest that cross-bridge cycling rate is increased in stunned myocardium. The increased cross-bridge cycling results in decreased $F_{max}$ and maintained myofilament ATP consumption.

**Cross-bridge cycling in stunned myocardium.** We have previously modeled the cross-bridge behavior of stunned myocardium using a cross-bridge model with experimentally constrained rate constants (10). Using measured intracellular $[\text{Ca}^{2+}]$ transients as inputs, we predicted that decreased force production could be the result of decreased cross-bridge attachment rates and increased cross-bridge detachment rates. In this study, we assessed cross-bridge cycling directly using a quick-step release method to determine $k_{tr}$.

Based on the current cross-bridge model (2), $k_{tr}$ obtained from the quick-release experiments (under conditions of low $P_i$ and maximal $\text{Ca}^{2+}$ activation) is the sum of $f_{app}$ and $g_{app}$. Also, force development depends on $f_{app}$

$$F = N \times F_o \times \frac{f_{app}}{f_{app} + g_{app}}$$  

where $N$ indicates the number of cross bridges available, $F_o$ indicates the mean force produced by each strongly attached cross bridge, and $f_{app}/(f_{app} + g_{app})$ represents the occupancy of force-generating states (2, 5). We have shown in this study that $k_{tr}$ increased significantly in stunned muscles (Fig. 3). Either increased $f_{app}$ or $g_{app}$, or both, would cause the increase in $k_{tr}$. However, an increase in $f_{app}$ would result in higher force development. On the other hand, increases in $g_{app}$ would predict a reduced occupancy of force-generating states of cross bridges and thus lower force development (Table 2).

The $k_{tr}$ did not change significantly at the submaximal activations in stunned muscles tested in this study. However, this does not necessarily mean cross-bridge cycling was not affected. Recent investigations have suggested that cooperative activation of the thin filament because of strong-bound cross bridges plays an important role in force redevelopment (6, 7), especially at submaximal activations. Thus $k_{tr}$ at submaximal $\text{Ca}^{2+}$ is not only dependent on $\text{Ca}^{2+}$ (4, 5) but also on cooperativity (6). More detailed experiments at submaximal $\text{Ca}^{2+}$ will be needed to define cross-bridge behavior at submaximal activations in stunned myocardium.

Changes in $k_m$ found in this study also support that the occupancy of cross bridges in force-generating states is lower (Table 1). ATPase activity at rest was not different between control and stunned groups (results not shown).
at maximal activation in stunned myocardium. The $k_m$ at rigor reflects all available cross bridges that are formed and locked in the attached states. Thus $k_m$ at rigor represents maximal stiffness under the same experimental conditions for the muscle. We have found that $k_m$ during $F_{\text{max}}$ is not different from the $k_m$ at rigor in control muscles. This suggests that the total number of cross bridges available for attachment and contraction is not different in stunned and control myocardium. However, $k_m$ during $F_{\text{max}}$ is 23% less than the $k_m$ at rigor in stunned muscles (Fig. 5), suggesting $>20\%$ less attached cross bridges during $F_{\text{max}}$. This could be caused by either reduced availability of the cross bridges (i.e., $N$ in Eq. 3) or reduced occupancy of the cross bridges in force-generating states (i.e., the total number of cross bridges engaged in cycling is not reduced but, as a result of increased $g_{\text{app}}$, the number of cross bridges at any given time is reduced). The fact that the $k_m$ at rigor is not different between control and stunned muscles indicates that stunned myocardium is capable of recruiting the same total number of cross bridges (i.e., the same $N$) as control, negating that a reduction in the number of cross bridges available for force generation is responsible for lower force development at maximal activation. Therefore, increased $g_{\text{app}}$ results in less $F_{\text{max}}$ in stunned myocardium. Our results show that $k_m$ during $F_{\text{max}}$ is $\sim 93\%$ of rigor stiffness in control muscles. A similar high ratio of $k_m$ during $F_{\text{max}}$ at rigor has also been reported from other laboratories (30). One potential explanation for the high ratio is the formation of rigor-like cross bridges during $F_{\text{max}}$ (36). If this is true, we could have overestimated the $k_m$ during $F_{\text{max}}$ in stunned myocardium. This high ratio also limits our ability to conclude how much $f_{\text{app}}$ and $g_{\text{app}}$ have changed in stunned muscles based on Table 2. This study, however, cannot rule out the possibility that the force generated by each individual cross bridge is reduced in stunned myocardium (i.e., lower $F_0$ in Eq. 3), thus causing decreased $F_{\text{max}}$. Some recent studies have focused on the structural alterations within the cross-bridge complex in stunned myocardium and have suggested decreased strains in the cross bridges formed in the presence of truncated troponin I (8). Direct evidence of decreased force production by each individual cross bridge is still lacking. Moreover, $k_n$ would not necessarily be affected when $F_0$ is decreased.

The results from this study contrast those of McDonald et al. (22), who found decreased unloaded shortening velocity in a porcine model of regional stunning. The discrepancy can be the result of a couple of factors: 1) different models of stunning can cause different changes in myofilaments and thus differences in cross-bridge behavior. For example, in the regional stunning in pigs, no proteolysis of troponin I is seen (17); 2) differences in experimental protocol (e.g., loaded vs. unloaded shortening, slack-length vs. rapid-release stretch back, cell segments vs. intact fibers) could account for the different observations.

**Myofilament ATP consumption in stunned myocardium.** We have measured myofibrillar ATP consumption during maximal activations and found that the myofibrillar ATP consumption remained unchanged compared with controls, whereas maximal force development decreased significantly (Fig. 7). Our findings are consistent with a number of other studies. It has long been recognized that stunned myocardium consumes more oxygen in spite of decreased contraction (18, 26). The increased $\text{MVO}_2$ is independent of coronary flow and tissue oxygen extraction (31). A recent study has shown that oxygen consumption by processes involved in excitation-contraction coupling and by myofibrils increased significantly in stunned myocardium (33). Foster et al. (8) have provided more direct evidence that the myofibrillar ATPase activity was increased in stunned myocardium. Using COOH-terminal truncated cardiac troponin I (cTnI$_{1-192}$) to reconstruct the troponin complex, they found that there is a $>50\%$ increase in Ca$^{2+}$-activated S1 ATPase activity, whereas force development was $\sim 76\%$ compared with myofilaments composed of reconstructed full-length cTnI. The mechanism of maintained ATP utilization in spite of decreased force in stunned myocardium is not entirely clear. Increases in the rate of cross-bridge detachment, $g_{\text{app}}$, predict increased ATP usage since cross-bridge detachment occurs when ATP is bound to the actomyosin complex. However, at a molecular level, the nature of the increased cross-bridge detachment remains elusive. We did not calibrate ATPase activity during activations, and this is a limitation to our study. Given that accurate ATPase calibration may be altered by solution volume, light intensity, NADH signal, and basal ATPase activity, we could not completely rule out the contribution of these confounding variables contributing to quantitative changes in ATPase activity.

When viewed in the context of the three-states model of thin filament regulation of contraction (13, 24, 35), the increased ATP consumption in spite of decreased force is likely the result of destabilization of the strongly bound actomyosin intermediates of the cross-bridge cycle (i.e., during the open state). In the absence of Ca$^{2+}$, troponin stabilizes tropomyosin in a position that blocks myosin binding to actin (the blocked state). In the presence of Ca$^{2+}$, troponin shifts tropomyosin and allows myosin to bind actin (the closed state). In the presence of myosin binding, tropomyosin adopts a state that permits myosin to bind all exposed sites on actin and hydrolyzes ATP (the open state). It is conceivable that the states of tropomyosin are affected by the troponins. In stunned myocardium, the position of tropomyosin in the open state is likely altered such that the transition to other states is accelerated and the strongly bound cross bridges are not fully supported as a result of degradation of troponin I (12, 23, 34). Thus ATP consumption by the cross bridges would be maintained in spite of decreased force generation.

We conclude that cross-bridge cycling rate is increased in stunned myocardium. The increased cycling rate is caused by an increased rate of transition from force-generating states to non-force-generating states ($g_{\text{app}}$), resulting in lower force development. Myofilament ATP consumption remained unchanged in spite of decreased force development. These findings have important implications for our understanding and management of stunned myocardium clinically.

**ACKNOWLEDGMENTS**

We thank Eduardo Marbán for helpful discussions throughout the work and for critical reading of the manuscript. We also thank Dr. Pieter de Tombe for suggestions about the manuscript.

**GRANTS**

This study was supported by National Heart, Lung, and Blood Institute Grant HL-44065 (to W. D. Gao).
REFERENCES