ATP consumption rate per cross bridge depends on myosin heavy chain isoform

Young-Soo Han, Paige C. Geiger, Mark J. Cody, Rebecca L. Macken, and Gary C. Sieck
Departments of Anesthesiology and Physiology and Biophysics, Mayo Medical School, Rochester, Minnesota 55905

Submitted 9 July 2002; accepted in final form 11 February 2003

Han, Young-Soo, Paige C. Geiger, Mark J. Cody, Rebecca L. Macken, and Gary C. Sieck. ATP consumption rate per cross bridge depends on myosin heavy chain isoform. J Appl Physiol 94: 2188–2196, 2003.—In the present study, we tested the hypothesis that intrinsic differences in ATP consumption rate per cross bridge exist across rat diaphragm muscle (Diam) fibers expressing different myosin heavy chain (MHC) isoforms. During maximum Ca$^{2+}$ activation (pCa 4.0) of single, Triton X-permeabilized Diam fibers, isometric ATP consumption rate was determined by using an NADH-linked fluorometric technique. The MHC concentration in single Diam fibers was determined by densitometric analysis of SDS-PAGE gels and comparison to a standard curve of known MHC concentrations. Isometric ATP consumption rate varied across Diam fibers expressing different MHC isoforms, being highest in fibers expressing MHC2x (1.14 ± 0.08 nmol·mm$^{-3}$·s$^{-1}$) and/or MHC2B (1.33 ± 0.08 nmol·mm$^{-3}$·s$^{-1}$), followed by fibers expressing MHC2A (0.77 ± 0.11 nmol·mm$^{-3}$·s$^{-1}$) and MHCslow (0.46 ± 0.03 nmol·mm$^{-3}$·s$^{-1}$). These differences in ATP consumption rate also persisted when it was normalized for MHC concentration in single Diam fibers. Normalized ATP consumption rate for MHC concentration varied across Diam fibers expressing different MHC isoforms, being highest in fibers expressing MHC2x (2.02 ± 0.19 s$^{-1}$) and/or MHC2B (2.64 ± 0.15 s$^{-1}$), followed by fibers expressing MHC2A (1.57 ± 0.16 s$^{-1}$) and MHCslow (0.77 ± 0.05 s$^{-1}$). On the basis of these results, we conclude that there are intrinsic differences in ATP consumption rate per cross bridge in Diam fibers expressing MHC isoforms. ATP consumption rate = bng$_{app}$α$_{a}$ (1)

Skeletal muscle fibers; energetics; tension cost

Cross-bridge cycling in skeletal muscle fibers depends on the hydrolysis of ATP at the myosin heavy chain (MHC), and ATP consumption rate during cross-bridge cycling is a major determinant of the mechanical performance of skeletal muscle fibers (9, 10, 20, 25, 39). The chemomechanical transduction of the cross-bridge cycle is essentially an enzymatic reaction involving the consumption of one ATP molecule per cycle. Huxley’s original model described cross bridges cycling between two functional states: a force-generating state, in which cross bridges are strongly attached to actin, and a non-force-generating state, in which cross bridges are detached from actin (27, 28). Two apparent rate constants describe the transitions between these two functional states, one for strong cross-bridge attachment (f$_{app}$) and the second for cross-bridge detachment (g$_{app}$). On the basis of this two-state model of cross-bridge cycling, Brenner and colleague (6–8) proposed an analytical framework for chemomechanical transduction in which ATP consumption rate during cross-bridge cycling is described by the following equation.

**Address for reprint requests and other correspondence:** G. C. Sieck, Dept. of Physiology & Biophysics, 4-184 W. Joseph, Mayo Medical School, 200 First St. SW, Rochester, MN 55905 (E-mail: sieck.gary@mayo.edu)
METHODS

Studies were performed on adult male Sprague-Dawley rats (body wt ∼300 g). The Institutional Animal Care and Use Committee of the Mayo Clinic approved all procedures.

Tissue preparation and single fiber dissection. Animals were anesthetized by intramuscular injection of ketamine (60 mg/kg) and xylazine (2.5 mg/kg), and the right side of the Diaₘₐₜ was excised. Muscle fiber bundles were then stretched (~20% of relaxed length approximating optimal length, Lₒ), pinned on cork, and placed for 24 h in a relaxing solution consisting of 100.0 mM KCl, 1.0 mM MgCl₂, 4.0 mM Na₂ATP, 5.0 mM EGTA, and 10 mM imidazole at a pH of 7.0 at 5°C. The fiber bundles were then stored in relaxing solution containing 50% glycerol (vol/vol) for up to 3 wk. Before measurements of ATP consumption rate, the fibers were transferred from the relaxing solution to a relaxing solution (pCa 9.0).

Measurement of ATP consumption rate and maximum iso- metric force. Isometric force and ATP consumption rate were measured concurrently in a Guth Scientific Instruments muscle research system (21, 29) as previously described (23, 32, 37, 38). Briefly, permeabilized fibers, ~3 mm in length, were mounted between force and length transducers in a quartz cuvette that was perfused with solutions containing free ionized Ca²⁺ concentrations of either 1 nM (pCa 9.0) or 100 nM (pCa 4.0) maintained at 15°C. Muscle fiber length was adjusted to obtain an average sarcomere length of 2.5 µm as determined from calibrated video images of the fiber.

An NADH-linked fluorometric technique (22, 37, 38) was used to measure isometric ATP consumption rate of skinned fibers. In this method, the ATP hydrolyzed by actomyosin ATPase is regenerated by the biochemical reaction of ADP and phospho(enol)-pyruvate, which is catalyzed by the enzyme pyruvate kinase. This reaction is coupled to the reduction of pyruvate to lactate, which is catalyzed by lactate dehydrogenase, and the associated oxidation of NADH to NAD⁺. For each mole of ATP regenerated by these coupled reactions, 1 mol of NADH is oxidized to NAD⁺. Important in the quantification of ATP consumption is the fact that NADH is fluorescent (fluoresces at 470 nm when excited at 340 nm), whereas NAD⁺ is nonfluorescent. Thus the rate of extinction of NADH fluorescence is proportional to the rate of ATP consumption. NADH fluorescence was excited at 340 nm by use of a mercury lamp and an interposed band-pass filter. Emitted fluorescence was measured at 470 nm by using a photomultiplier tube equipped with a cutoff filter. The ATP solutions consisted of relaxing (pCa 9.0) and activating (pCa 4.0) solutions, both containing 5 mM phospho(enol)-pyruvate, 0.2 mM reduced B-nicotinamide adenine dinucleotide (NADH), 100 U/ml pyruvate kinase, 140 U/ml lactate dehydrogenase, and 0.2 mM PⅠ⁺PⅡ⁺-diadenosine-5’-pentaphosphate (A₂₉P₃). The computer program described by Fabiato and Fabiato (12) with stability constants listed by Godt and Lindley (18) was used to determine the activating and relaxing solutions. The solutions contained the following (in mM): 7.0 EGTA, 1.0 free Mg²⁺, 5.0 MgATP, and 70.0 imidazole, with a total ionic strength of 150 mM.

In this NADH-linked fluorometric technique, for each mole of ADP produced by the hydrolysis of ATP, 1 mol of NADH is converted to NAD⁺. The system was calibrated for known concentrations of NADH ranging from 0 to 400 µM. To measure ATP consumption rate, perfusion through the cuvette was stopped for 15 s, and the amount of ATP consumed by the actomyosin ATPase reaction was determined by measuring the rate of extinction of the NADH fluorescence signal (Fig. 1). Thereafter, flow through the cuvette was resumed for 1 s to replenish the enzymatic substrates before being stopped again. Such cycling was continued throughout the period of fiber activation.

Muscle fibers were imaged in the cuvette by using a calibrated monocular microscope (×10 objective), and fiber length and width were measured. Subsequently, a ×40 objective (Olympus LWD CD Plan 40, 0.55 NA) was used to measure the number of sarcomeres in series as well as the xy (width) and xz (depth) dimensions of the fibers. In a previous study (16), the xy- and xz-plane measurements obtained by using this inverted microscope system were directly compared with measurements obtained by using a confocal microscope (Olympus Fluoview). As expected, there were no differences in xy-axis measurements; however, xz-axis measurements made by use of the inverted system were ~20% shorter than the xz diameter measured by using the confocal system. On the basis of these differences, a correction factor for xz-axis distortion was obtained by the method of Cohen et al. (5). Measurements made by use of the inverted system were compared with measurements obtained by use of a confocal microscope (Olympus Fluoview). As expected, there were no differences in xy-axis measurements; however, xz-axis measurements made by use of the inverted system were ~20% shorter than the xz diameter measured by using the confocal system. On the basis of these differences, a correction factor for xz-axis distortion was obtained by the method of Cohen et al. (5).

Baseline force and ATP consumption rate measurements were obtained while fibers were perfused with a pCa 9.0 solution. The perfusate was then switched to a pCa 4.0 solution to maximally activate the fibers. After maximal activation, the fiber was again perfused with a pCa 9.0 solution to verify that force and ATP consumption rate returned to baseline levels (Fig. 1). Maximum specific force (Fmax N/cm²) was calculated by dividing the maximum isometric force by the corrected fiber CSA (see above). In a subset of muscle fibers, resting and activated stiffness were determined by using sinusoidal length oscillations (0.2% Lₒ) at 2 kHz, normalized for fiber CSA. The ratio of fiber stiffness during maximal activation in a rigor solution (pCa 4.0 without ATP) vs. activation in a normal pCa 4.0 (with ATP) solution was used to determine the αₛ (Fig. 2). The gₛₚₑₑ was calculated by using Eq. 1 on the basis of the measured parameters of b, n, αₛₑₑ, and ATP consumption rate. The value n was derived by multiplying MHC concentration (µg/µl) by half-sarcomere fiber volume (µl). The unit of MHC concentration (µg/µl) was converted to µmol/l by dividing MHC concentration by the molecular weight of the specific MHC isoform (~220 kDa) (31) and then the unit of mole in n was replaced by Avogadro’s number (Table 1).

Measurement of fiber MHC concentration. The methods used to determine MHC concentration in single Diaₘₐₜ fibers have been previously described (14–17). Single dissected Diaₘₐₜ fibers were placed in 25 µl of SDS sample buffer containing 62.5 mM Tris-HCl, 2% (wt/vol) SDS, 10% (vol/vol) glycerol, 5% 2-mercaptoethanol, and 0.001% (wt/vol) bromophenol blue at a pH of 6.8. The fiber samples were denatured by boiling for 2 min. Gradient gels were prepared using a modified procedure by Sugiuira and Murakami (41). The stacking gel contained a 3.5% acrylamide concentration (pH 6.8), and the separating gel contained 5–8% acrylamide (pH 8.8) with 25% glycerol (8 × 10 cm, 0.75 mm thick; Hoefer SE250). To compare migration patterns of the MHC isoforms, control samples of Diaₘₐₜ bundles in a 1:200 dilution of SDS sample buffer (~9.0 ng/µl MHC concentration determined by the Bradford method (4)) were run on the gels. Sample volumes of 10 µl were loaded per lane. The gels were silver stained according to the procedure described by Oakley et al. (30).

Identification of MHC isoforms by migration patterns was confirmed by Western blot analysis. MHC isoforms from rat...
Diam fiber bundles were separated on SDS-PAGE and transferred to nitrocellulose. After overnight transfer at 1 A, the nitrocellulose sheet was divided into five sections. One nitrocellulose segment was stained with colloidal gold to visualize protein bands and to ensure adequate protein transfer. The four additional segments were stained with one of the following mouse monoclonal or polyclonal antibodies: NCL (Novocastra, IgG), which reacts with MHCSlow; SC.71 (ATTC, IgG), which reacts with MHC 2A; BF-F3 (Schiaffino, IgM), which reacts with MHC 2B; and BF-35 (Schiaffino, IgG), which reacts with all but the MHC2X isoform. The specificity of these isoforms was previously determined (26, 36). Each nitrocellulose segment was stained with a biotinylated secondary antibody specific to IgG (NCL, SC.71, BF-35) or IgM (BF-F3).

Fig. 1. In a single Triton X-permeabilized rat diaphragm muscle (Dia, m) fiber, ATP consumption rate (NADH-linked fluorometry) and force were simultaneously measured during maximum isometric activation. The dimensions of the measured fiber are as follows: width (xy) = 75 μm, length = 3.1 mm, corrected cross-sectional area (CSA) = 3,534.3 μm². ATP consumption rate was normalized for fiber volume at rest and during maximum isometric contraction: 0.09 and 1.36 nmol-mm⁻³-s⁻¹, respectively. The value of isometric ATP consumption rate reported in this study was presented as difference between the values in maximum isometric contraction and at rest. Maximum isometric specific force: 11.3 N/cm². Myosin heavy chain (MHC) isoform of the fiber was identified as MHC2B/2X.

Table 1. Number of available cross bridges per half-sarcomere

<table>
<thead>
<tr>
<th>MHC Isoform</th>
<th>n (×10⁵)</th>
<th>Number of Fibers Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHCSlow</td>
<td>36.9 ± 3.7ᵃ</td>
<td>33</td>
</tr>
<tr>
<td>MHC2A</td>
<td>36.2 ± 4.1ᵃ</td>
<td>22</td>
</tr>
<tr>
<td>MHC2X</td>
<td>95.1 ± 8.6</td>
<td>26</td>
</tr>
<tr>
<td>MHC2B2X</td>
<td>108.2 ± 11.2</td>
<td>37</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of cross bridges. ᵃSignificantly different (P < 0.05) from fibers expressing myosin heavy chain (MHC)₂X and/or MHC₂B₂X.

Fig. 2. Stiffness measurements in a single Diaₘ fiber during maximal activation in rigor solution (pCa 4.0 without ATP), pCa 4.0 and pCa 9.0. The dimensions of the measured fiber are as follows: width (xy) = 77.2 μm, depth (xz) = 41.3 μm, length = 1.8 mm, corrected CSA = 2,497.6 μm². MHC isoform of the fiber was identified as MHC₂X.
and visualized with alkaline-phosphatase (Vectastain ABC-kit, Vector Labs).

To determine the MHC concentration of single Diam fibers, a standard curve was constructed by loading known concentrations of purified rabbit MHC [Sigma M-3889, protein concentrations verified with the Bradford method (4)] on a gel. The gels were silver stained, and a high-resolution scanner (Microtek ScanMaker 5, 600 dpi) was used for densitometric analysis. The brightness-area product (BAP) of each rabbit MHC sample was determined from the area and average brightness of each band after subtraction of local background. On the basis of the linear relationship between BAP and MHC concentration in these standard samples, the MHC concentration in single Diam fibers was determined (16) (Fig. 3).

Statistical analysis. One-way analysis of variance was performed to compare ATP consumption rate per myosin head, maximum specific force, fiber MHC content, and the fraction of cross bridges in the force-generating state across Diam fibers expressing different MHC isoforms. When appropriate, a Student’s t-test with Bonferroni correction was used to compare between fiber types. P < 0.05 was used to indicate statistical significance. Reproducibility of measurements of MHC concentration was assessed by analysis of the coefficient of variation across repeated BAP measurements.

RESULTS

ATP consumption rate and maximum specific force. ATP consumption rate and maximum isometric force were simultaneously measured (Fig. 1) in a total of 65 rat Diam fibers. In addition, maximum isometric force was measured in another 41 Diam fibers in which fiber stiffness was also assessed. Because of technical constraints, fiber stiffness and ATP consumption rate could not be determined in the same fibers. Maximum force for each fiber was normalized for CSA to determine Fmax (Table 2). Fibers expressing MHC2X, either alone or together with MHC2B, exhibited the greatest specific force, followed by fibers expressing MHC2A and MHCSlow. Fibers expressing MHC2A generated greater specific force compared with fibers expressing MHCSlow.

Isometric ATP consumption rate varied across Diam fibers expressing different MHC isoforms (Fig. 4). Fibers expressing MHC2X either alone or together with MHC2B displayed the highest ATP consumption rate, followed by fibers expressing MHC2A and MHCSlow. The isometric ATP consumption rate of fibers expressing MHC2A was higher than that of fibers expressing MHCSlow. αfs. The ratio of fiber stiffness determined in a pCa 4.0 activating solution with or without ATP (rigor) provided an estimate of the αfs. The αfs was similar across all Diam fibers regardless of MHC isoform expression (Table 2).

$g_{app}$. The $g_{app}$ was derived from the simultaneous measurements of force and ATP consumption rate (see Fig. 3. A: MHC isoforms in the rat Diam identified by SDS-PAGE. B: graph representing the linear relationship between brightness-area product and MHC concentration. ▲, Known amounts of myosin loaded in 25-ng increments; ■, rat Diam single fibers loaded in 10-μl volumes and assayed for MHC content on the basis of the standard curve.
Similar to force and ATP consumption rates, $g_{\text{app}}$ was found to vary across Dia_m fibers expressing different MHC isoforms, being fastest in fibers expressing MHC_{2X} either alone or together with MHC_{2B}. The $g_{\text{app}}$ of fibers expressing MHC_{2A} and MHC_{Slow} was ~30% and 50% slower than that of fibers expressing MHC_{2X} and/or MHC_{2B} (Fig. 5). The $g_{\text{app}}$ of fibers expressing MHC_{2A} was also faster than that of fibers expressing MHC_{Slow}.

**Fiber MHC concentration.** Fiber MHC concentration did not vary across fibers expressing different MHC isoforms. (Fig. 6).

**ATP consumption rate per myosin head.** ATP consumption rate per myosin head, derived by dividing ATP consumption rate by fiber MHC concentration, varied across Dia_m fibers expressing different MHC isoforms. ATP consumption rate per myosin head was highest in fibers expressing MHC_{2X} alone or together with MHC_{2B}, followed by fibers expressing MHC_{2A} and MHC_{Slow} (Fig. 7). The ATP consumption rate per myosin head of fibers expressing MHC_{2A} was significantly higher than that of fibers expressing MHC_{Slow}.

**Isometric tension cost.** Isometric tension cost of rat Dia_m fibers was determined by the ratio of ATP consumption rate to the corresponding isometric force. Isometric tension cost varied across Dia_m fibers, being highest in fibers expressing MHC_{2X} alone or together with MHC_{2B} followed in the rank order by fibers expressing MHC_{2A} and MHC_{Slow} (Fig. 8). The isometric tension cost of fibers expressing MHC_{2A} was higher than that of fibers expressing MHC_{Slow}.

### DISCUSSION

The results of the present study supported the hypothesis that intrinsic differences in ATP consumption rate per cross bridge (myosin head) exist across rat Dia_m fibers expressing different MHC isoforms. As previously reported (1, 37, 38), isometric ATP consumption rate was found to vary across Dia_m fibers expressing different MHC isoforms, being slowest in fibers expressing MHC_{Slow} followed in rank order by fibers expressing MHC_{2A}, MHC_{2X}, and/or MHC_{2B}. The results of the present study indicate that fiber-type differences in isometric ATP consumption rate are not due to differences in fiber MHC concentration. Intrinsic differences in ATP consumption rate per cross bridge are entirely consistent with differences in cross-bridge cycling rate (37, 38) and maximum unloaded shortening velocity (1, 11, 37, 38).

*Equation 1* provides a conceptual framework to better understand the chemomechanical transduction of the cross-bridge cycle in skeletal muscle fibers. From *Eq. 1* it can be seen that ATP consumption rate in single muscle fibers is dependent on a number of factors, including the total number of cross bridges (the product of b, the number of half sarcomeres within a muscle fiber, and n, the number of available cross bridges), the number of half sarcomeres within a muscle fiber, and n, the number of available cross bridges.
bridges per half-sarcomere), the rate constant for cross-bridge detachment \( (g_{\text{app}}) \), and the fraction of strongly bound cross bridges \( (\alpha_{fs}) \) (5, 29, 37). Consistent with previous studies (13, 16), we found that \( \alpha_{fs} \) during maximum \( \text{Ca}^{2+} \) activation was comparable across \( \text{Dia}_m \) fibers expressing different MHC isoforms (\( \sim 77\% \)). This result is in good agreement with those reported by Goldman and Simmons (\( \sim 75\% \); Ref. 19) and by Higuchi et al. (80%; Ref. 24). Previously, we found that cross-bridge cycling rate varied across \( \text{Dia}_m \) fibers expressing different MHC isoforms, being fastest in fibers expressing MHC2X and/or MHC2B and slowest in fibers expressing the MHC\text{Slow} (37, 38). Thus the estimates of \( g_{\text{app}} \) reported in the present study, on the basis of force and ATP consumption rate measurements, were consistent with these previous observations.

ATP consumption rate per myosin head. The estimates of isometric ATP consumption rate per myosin head in single \( \text{Dia}_m \) fibers provided by the present study are comparable to the value (2.3 ATPs hydrolyzed per second per myosin head) reported by Kerrick et al. (29) for rabbit adductor magnus muscle fibers. However, Kerrick and colleagues did not address whether differences in ATP consumption rate per myosin head existed across different MHC isoforms. Differences in ATP consumption rate per myosin head across \( \text{Dia}_m \) fibers expressing different MHC isoforms reflect a difference in the rate of ATP consumption per cross bridge. To the best of our knowledge, this is the first report of isoform-specific differences in ATP consumption rate per myosin head.

Isometric ATP consumption rate. In the present study, isometric ATP consumption rate differed across \( \text{Dia}_m \) fibers expressing different MHC isoforms, being highest in fibers expressing MHC2X alone or together with MHC2B, followed by fibers expressing MHC2A and MHC\text{Slow} (Fig. 4). The ATP consumption rate across rat \( \text{Dia}_m \) fibers expressing different MHC isoforms had the same rank order as the maximum shortening velocity.
results reported by Bottinelli et al. (1) and Sieck et al. (37). On average, the resting ATP consumption rate across Diam fibers expressing different MHC isoforms was found to be $0.09 \pm 0.008 \text{nmol-mm}^{-3} \text{s}^{-1}$. This relatively low resting ATP consumption rate represented a weak binding state in cross-bridge cycling. These results are generally consistent with the previous studies in rats (1, 37, 38). However, the values of ATP consumption rate reported by Bottinelli et al. for rat Diam fibers expressing different MHC isoforms were substantially lower than those found in the present study. Bottinelli et al. reported the ATP consumption rate and $F_{\text{max}}$ as $0.045-0.230 \text{nmol-mm}^{-3} \text{s}^{-1}$ and $6.8-11.4 \text{N/cm}^2$, respectively, across all fibers expressing different MHC isoforms. In particular, the discrepancy of ATP consumption rate between the two studies may relate to significant differences in measurement of muscle fiber CSA and in the methods used to measure ATP consumption rate. ATP consumption rate normalized for the CSA could significantly contribute to the reported values. Temperature difference (12 vs. 15°C) and different preparations (rat hindlimb muscle and rat diaphragm muscle) in the two studies might also contribute to the discrepancy. In contrast to the present study, Bottinelli et al. measured NADH concentration by absorbency rather than fluorometry.

Fiber MHC concentration. In the present study, we found that MHC concentration was comparable across Diam fibers expressing different MHC isoforms. Previously, we reported differences in MHC content per half-sarcomere (14, 16), which are largely attributed to fiber-type differences in CSA and to a lesser extent to differences in myofibrillar density and thick-thin filament lattice spacing (13). With no change in MHC concentration, larger diaphragm fibers have greater MHC content. Different fiber types in the Diam vary in size and, thus, MHC content. We normalized ATP consumption rate to

![Fig. 7. Differences in ATP consumption rate per myosin head across Diam fibers expressing different MHC isoforms. Values are means $\pm$ SE. *Significantly different ($P < 0.05$) from fibers expressing MHCslow. **Significantly different ($P < 0.05$) from fibers expressing MHC2A. ***Significantly different ($P < 0.05$) from fibers expressing MHC2X.](http://jap.physiology.org/)

![Fig. 8. Differences in isometric tension cost across Diam fibers expressing different MHC isoforms. Values are means $\pm$ SE. *Significantly different ($P < 0.05$) from fibers expressing MHCslow. **Significantly different ($P < 0.05$) from fibers expressing MHC2A. ***Significantly different ($P < 0.05$) from fibers expressing MHC2X.](http://jap.physiology.org/)
fiber MHC concentration. In contrast to the MHC content, MHC concentration was not significantly different across different diaphragm fibers.

**Isometric tension cost.** Fibers expressing MHC<sub>Slow</sub> had the lowest values of tension cost followed by fibers expressing MHC<sub>2A</sub> and fibers expressing MHC<sub>2X</sub> and/or MHC<sub>2B</sub> (Fig. 8). Dia<sub>m</sub> fibers expressing MHC<sub>2X</sub> and/or MHC<sub>2B</sub> generated greater force, but their ATP consumption rate per cross bridge was also disproportionately higher compared with fibers expressing MHC<sub>Slow</sub> and MHC<sub>2A</sub> isoforms, hence the higher tension cost. In other words, Dia<sub>m</sub> fibers expressing MHC<sub>Slow</sub> and MHC<sub>2A</sub> are the most energy efficient. These results generally agree with those reported by Bottinelli et al. (1). They reported significant differences in tension cost between all groups of fibers. The only exception was between I<sub>a</sub> MHC and I<sub>x</sub> MHC: ~2.8, ~2.6, ~1.9, ~1.5, and ~0.7 pmol ATP mN<sup>-1</sup> mm<sup>-1</sup> s<sup>-1</sup> in I<sub>b</sub> MHC, mixed, I<sub>x</sub> MHC, I<sub>a</sub> MHC, and 1 MHC, respectively. It should be noted that the tension cost of rat Dia<sub>m</sub> fibers was significantly higher than that reported for human vastus lateralis muscle fibers (23). These results are consistent with the general principle that the energetic costs of generating muscular force are higher in small animals (43).

This research was supported by grants from the National Heart, Lung, and Blood Institute (HL-34817 and HL-37680).

**REFERENCES**


7. Brenner B. The necessity of using two parameters to describe isotonic shortening velocity of muscle tissue: the effect of various interventions upon initial shortening velocity (vi) and curvature (b). *Basic Res Cardiol 81: 54–69, 1986.*


34. Reiser PJ, Moss RL, Giulian GG, and Greaser ML. Shortening velocity in single fibers from adult rabbit soleus muscles is


