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


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. First published February 14, 2003; 10.1152/japplphysiol.00618.2002.—In the present study, we tested the hypothesis that intrinsic differences in ATP consumption rate per cross bridge exist across rat diaphragm muscle (Dia) fibers expressing different myosin heavy chain (MHC) isoforms. During maximum Ca\(^{2+}\) activation (pCa 4.0) of single, Triton X-permeabilized Dia fibers, isometric ATP consumption rate was determined by using an NADH-linked fluorometric technique. The MHC concentration in single Dia 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 Dia 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 Dia fibers. Normalized ATP consumption rate for MHC concentration varied across Dia 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 Dia fibers expressing MHC isoforms.

\[ \text{ATP consumption rate} = b \cdot n \cdot g_{\text{app}} \cdot \alpha \]  

where \(b\) is the number of half sarcomeres within a muscle fiber, \(n\) is the number of available cross bridges per half-sarcomere, and \(\alpha\) is the fraction of available cross bridges that are in a strongly bound state.

The rate of ATP consumption varies across fibers expressing different MHC isoforms in humans (2, 23, 40) and in rats (1, 11, 37, 38). These MHC isoform-dependent differences in ATP consumption rate generally correspond with differences in maximum shortening velocity across different fiber types reflecting differences in \(g_{\text{app}}\) (3, 11, 33–35, 37, 38, 42). In addition, recent studies indicate that MHC content per half sarcomere (reflecting \(n\), the number of available cross bridges) varies across muscle fibers expressing different MHC isoforms (14–17). Certainly, differences in fiber MHC content might affect ATP consumption rate independent of differences in ATP consumption rate per myosin head. In the rat diaphragm muscle (Dia) fibers, differences in force generated by fibers expressing different fast MHC isoforms (i.e., MHC2A, MHC2X, and MHC2B) disappear after normalization for MHC content per half-sarcomere, whereas force generated by fibers expressing MHCslow remains lower even after normalization for half-sarcomere MHC content (16). These results suggested that there are intrinsic differences in force per cross bridge between fibers expressing fast and slow MHC isoforms. In the present study, ATP consumption rate of rat Dia fibers was normalized for fiber MHC concentration to test the hypothesis that intrinsic differences in ATP consumption rate per cross bridge exist across different MHC isoforms.

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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 Dia was excised. Muscle fiber bundles were then stretched (~20% of relaxed length approximating optimal length, L0), 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 single fiber dissection, a fiber bundle was placed in relaxing solution containing 1% Triton X-100 to permeabilize the plasma membrane. While in the skinnning solution (~20 min), single fibers were dissected under a dissecting microscope. Before measurements of ATP consumption rate, the fibers were transferred from the skinnning solution to a relaxing solution (pCa 9.0).

Measurement of ATP consumption rate and maximum isotonic 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 μM (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 P1, Pd-adenosine-5′-pentaphosphate (Ap5P). 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 established by the Bradford method and used to calculate fiber cross-sectional area (CSA) and volume.

Baseline force and ATP consumption rate measurements were obtained while fibers were perfused with a pCa 9.0 solution. The perfusate was subsequently 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 isotonic 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% L0) 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 αmax (Fig. 2). The gapp was calculated by using Eq. 1 on the basis of the measured parameters of b, n, αmax, 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 Dia fibers have been previously described (14–17). Single dissected Dia 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 Sugiura 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 Dia fibers in a 1:200 dilution of SDS sample buffer [−9.0 ng/μl MHC concentration determined by the Bradford method (41)] 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, 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.

Dia, 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 MHC2B; 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).

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>
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<tbody>
<tr>
<td>MHCSlow</td>
<td>36.9 ± 3.7⁺</td>
<td>33</td>
</tr>
<tr>
<td>MHC2A</td>
<td>36.2 ± 4.1⁺</td>
<td>22</td>
</tr>
<tr>
<td>MHC2X</td>
<td>95.1 ± 8.6</td>
<td>26</td>
</tr>
<tr>
<td>MHC2B/2X</td>
<td>108.2 ± 11.2</td>
<td>37</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of cross bridges. ⁺Significantly different (P < 0.05) from fibers expressing myosin heavy chain (MHC)₂X and/or MHC₂B/₂X.
and visualized with alkaline-phosphatase (Vectastain ABC-kit, Vector Labs).

To determine the MHC concentration of single Dia<sub>m</sub> 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 Dia<sub>m</sub> 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 Dia<sub>m</sub> 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 Dia<sub>m</sub> fibers. In addition, maximum isometric force was measured in another 41 Dia<sub>m</sub> 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 F<sub>max</sub> (Table 2). Fibers expressing MHC<sub>2X</sub>, either alone or together with MHC<sub>2B</sub>, exhibited the greatest specific force, followed by fibers expressing MHC<sub>2A</sub> and MHC<sub>Slow</sub>. Fibers expressing MHC<sub>2A</sub> generated greater specific force compared with fibers expressing MHC<sub>Slow</sub>.

Isometric ATP consumption rate varied across Dia<sub>m</sub> fibers expressing different MHC isoforms (Fig. 4). Fibers expressing MHC<sub>2X</sub> either alone or together with MHC<sub>2B</sub> displayed the highest ATP consumption rate, followed by fibers expressing MHC<sub>2A</sub> and MHC<sub>Slow</sub>. The isometric ATP consumption rate of fibers expressing MHC<sub>2A</sub> was higher than that of fibers expressing MHC<sub>Slow</sub>. The ratio of fiber stiffness determined in a pCa 4.0 activating solution with or without ATP (rigor) provided an estimate of the α<sub>fs</sub>. The α<sub>fs</sub> was similar across all Dia<sub>m</sub> fibers regardless of MHC isoform expression (Table 2).

g<sub>app</sub>. The g<sub>app</sub> was derived from the simultaneous measurements of force and ATP consumption rate (see

![Fig. 3. A: MHC isoforms in the rat Dia<sub>m</sub> identified by SDS-PAGE. B: graph representing the linear relationship between brightness-area product and MHC concentration. Δ, Known amounts of myosin loaded in 25-ng increments; ■, rat Dia<sub>m</sub> single fibers loaded in 10-μl volumes and assayed for MHC content on the basis of the standard curve.](image-url)
Eq. 1). Similar to force and ATP consumption rates, $g_{ \text{app} }$ was found to vary across Diam fibers expressing different MHC isoforms, being fastest in fibers expressing MHC2X either alone or together with MHC2B. The $g_{ \text{app} }$ of fibers expressing MHC2A and MHC Slow was ~30% and 50% slower than that of fibers expressing MHC2X and/or MHC2B (Fig. 5). The $g_{ \text{app} }$ of fibers expressing MHC2A 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 to the corresponding isometric force. Similar to force and ATP consumption rates, $g_{ \text{app} }$ was found to vary across Diam fibers expressing different MHC isoforms. As previously reported (1, 37, 38), isometric ATP consumption rate was found to vary across Diam fibers expressing different MHC isoforms, being slowest in fibers expressing MHC Slow followed in rank order by fibers expressing MHC2A and MHC2X (Fig. 6). The ATP consumption rate per myosin head of fibers expressing MHC2A was significantly higher than that of fibers expressing MHC Slow.

**Isometric tension cost.** Isometric tension cost of rat Diam fibers was determined by the ratio of ATP consumption rate to the corresponding isometric force. Isometric tension cost varied across Diam fibers, being highest in fibers expressing MHC2X alone or together with MHC2B followed by fibers expressing MHC2A and MHC Slow (Fig. 7). The ATP consumption rate per myosin head of fibers expressing MHC2A was significantly higher than that of fibers expressing MHC Slow.

The results of the present study supported the hypothesis that intrinsic differences in ATP consumption rate per cross bridge (myosin head) exist across rat Diam fibers expressing different MHC isoforms. As previously reported (1, 37, 38), isometric ATP consumption rate was found to vary across Diam fibers expressing different MHC isoforms, being slowest in fibers expressing MHC Slow followed in rank order by fibers expressing MHC2A, MHC2X, and/or MHC2B. 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 expressed per cross bridge).

**Table 2. Summary of maximum specific force and fraction of cross bridges in the force-generating state**

<table>
<thead>
<tr>
<th>MHC Isoform</th>
<th>$F_{\text{max}}$ (N cm$^{-2}$)</th>
<th>$a_{\text{ps}}$</th>
<th>$xy$, μm</th>
<th>$xx$, μm</th>
<th>Corrected CSA, μm$^2$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC Slow</td>
<td>8.48 ± 0.44‡‡</td>
<td>0.77 ± 0.04</td>
<td>42.86 ± 2.08</td>
<td>21.31 ± 2.49</td>
<td>1.246 ± 175.3</td>
<td>26</td>
</tr>
<tr>
<td>MHC2A</td>
<td>11.07 ± 0.55‡‡</td>
<td>0.77 ± 0.03</td>
<td>47.09 ± 2.68</td>
<td>19.04 ± 1.09</td>
<td>1.316 ± 111.5</td>
<td>31</td>
</tr>
<tr>
<td>MHC2X</td>
<td>13.60 ± 0.57‡‡</td>
<td>0.75 ± 0.02</td>
<td>69.52 ± 1.85</td>
<td>27.51 ± 0.96</td>
<td>3.415 ± 186.3</td>
<td>51</td>
</tr>
<tr>
<td>MHC2B/2X</td>
<td>14.73 ± 0.45‡‡</td>
<td>0.77 ± 0.02</td>
<td>72.47 ± 1.95</td>
<td>27.68 ± 1.07</td>
<td>4.022 ± 271.1</td>
<td>46</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of cross-bridges. $F_{\text{max}}$, maximum specific force; CSA, cross-sectional area. ‡Significantly different ($P < 0.05$) from fibers expressing MHC Slow. ‡‡Significantly different ($P < 0.05$) from fibers expressing MHC2X; ‡‡Significantly different ($P < 0.05$) from fibers expressing MHC2A.
bridges per half-sarcomere), the rate constant for
cross-bridge detachment ($g_{\text{app}}$), and the fraction of
strongly bound cross bridges ($\alpha_f$) (5, 29, 37). Consis-
tent with previous studies (13, 16), we found that $\alpha_f$
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 MHC$_{2\text{X}}$ and/or MHC$_{2\text{B}}$ 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 measure-
ments, were consistent with these previous observa-
tions.

ATP consumption rate per myosin head. The esti-
mates 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 hydro-
lyzed 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 my-
osin head existed across different MHC isoforms. Dif-
fences 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 con-
sumption 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 MHC$_{2\text{X}}$ alone or together
with MHC$_{2\text{B}}$, followed by fibers expressing MHC$_{2\text{A}}$
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

Fig. 5. Differences in the apparent rate constant for
cross-bridge detachment ($g_{\text{app}}$) across $\text{Dia}_m$ fibers
expressing different MHC isoforms. Values are means ±
SE. *Significantly different ($P < 0.05$) from fibers
expressing MHC$_{\text{Slow}}$. #Significantly different ($P < 0.05$)
from fibers expressing MHC$_{2\text{A}}$. +Significantly different
($P < 0.05$) from fibers expressing MHC$_{2\text{X}}$.

Fig. 6. Differences in fiber MHC concentration of $\text{Dia}_m$
fibers expressing different MHC isoforms. Values are
means ± SE.

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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 ± 0.008 nmol·mm⁻³·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 Fmax as 0.045–0.230 nmol·mm⁻³·s⁻¹ and 6.8–11.4 N/cm², 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

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Fig. 7. Differences in ATP consumption rate per myosin head across Diam fibers expressing different MHC isoforms. Values are means ± SE. *Significantly different (P < 0.05) from fibers expressing MHCslow. #Significantly different (P < 0.05) from fibers expressing MHC2X.

Fig. 8. Differences in isometric tension cost across Diam fibers expressing different MHC isoforms. Values are means ± SE. *Significantly different (P < 0.05) from fibers expressing MHCslow. #Significantly different (P < 0.05) from fibers expressing MHC2X.
ATP consumption rate per myosin head

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 Ila MHC and IIX 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 IIB MHC, mixed, IIX MHC, Ila MHC, and I 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).

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