Reserve capacity for ATP consumption during isometric contraction in human skeletal muscle fibers

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Han, Young-Soo, David N. Proctor, Paige C. Geiger, and Gary C. Sieck. Reserve capacity for ATP consumption during isometric contraction in human skeletal muscle fibers. J Appl Physiol 90: 657–664, 2001.—Maximum velocity of the actomyosin ATPase reaction ($V_{\text{max}}$ ATPase) and ATP consumption rate during maximum isometric activation ($\text{ATP}_{\text{iso}}$) were determined in human vastus lateralis (VL) muscle fibers expressing different MHC isoforms. We hypothesized that the reserve capacity for ATP consumption (1 - (ratio of $\text{ATP}_{\text{iso}}$ to $V_{\text{max}}$ ATPase)) varies across VL muscle fibers expressing different MHC isoforms. Biopsies were obtained from 12 subjects (10 men and 2 women; age 21–66 yr). A quantitative histochemical procedure was used to measure $V_{\text{max}}$ ATPase. In permeabilized fibers, ATP$_{\text{iso}}$ was measured using an NADH-linked fluorometric procedure. The reserve capacity for ATP consumption was lower for fibers coexpressing MHC$_{2A}$ and MHC$_{2X}$ compared with fibers singularly expressing MHC$_{2A}$ and MHC$_{2X}$ (39 vs. 52 and 56%, respectively). Tension cost (ratio of ATP$_{\text{iso}}$ to generated force) also varied with fiber type, being highest in fibers coexpressing MHC$_{2X}$ and MHC$_{2D}$. We conclude that fiber-type differences in the reserve capacity for ATP consumption and tension cost reflect functional differences such as susceptibility to fatigue.

quantitative histochemistry; immunohistochemistry; muscle biopsy; sodium dodecyl sulfate-polyacrylamide electrophoresis; adenosine triphosphate

MYOSIN HEAVY CHAIN (MHC) is the site of ATP hydrolysis during cross-bridge cycling, and ATP consumption rate during cross-bridge cycling is a major determinant of the mechanical performance of skeletal muscle fibers. This is evident by the close relationship between the maximum velocity of the actomyosin ATPase reaction ($V_{\text{max}}$ ATPase), measured biochemically, and the fiber-type composition and contractile properties of various skeletal muscles (3).

Several recent studies have used an NADH-linked fluorometric technique to measure the rate of ATP consumption in single permeabilized muscle fibers during maximum isometric activation ($\text{ATP}_{\text{iso}}$) (6, 22, 23, 25). In both animal (6, 22, 23) and human (25) studies, muscle fibers expressing the MHC$_{\text{slow}}$ isoform were found to have a slower rate $\text{ATP}_{\text{iso}}$ compared with fibers expressing fast MHC isoforms (MHC$_{2A}$, MHC$_{2X}$, and MHC$_{2B}$). However, ATP$_{\text{iso}}$ is submaximal, and therefore this measure does not establish the maximum capacity for ATP hydrolysis (22, 23). It is well established that ATP consumption increases with power output and work performance (11, 12, 22). The $V_{\text{max}}$ ATPase establishes the upper limit for ATP consumption during work performance for each fiber type in skeletal muscle. In this respect, it is important to establish the range of ATP consumption rates (from ATP$_{\text{iso}}$ to $V_{\text{max}}$ ATPase) because this provides a measurement of the reserve capacity for ATP consumption. In the rat diaphragm muscle, we found that the reserve capacity for ATP consumption [calculated as 1 - (ratio of ATP$_{\text{iso}}$ to $V_{\text{max}}$ ATPase)] was ~64, ~54, and ~52% for fibers expressing MHC$_{\text{slow}}$, MHC$_{2A}$, and MHC$_{2X}$. respectively (23). Unfortunately, values obtained in laboratory animals cannot be necessarily extrapolated to human muscle fibers. Therefore, the purpose of the present study was to determine the reserve capacity for ATP consumption of single permeabilized fibers from the human vastus lateralis (VL) muscle. Therefore, the purpose of the present study was to determine the reserve capacity for ATP consumption of single permeabilized fibers from the human vastus lateralis (VL) muscle. We hypothesized that the reserve capacity for ATP consumption varies across VL muscle fibers expressing different MHC isoforms.

METHODS

Muscle biopsies. Needle-biopsy samples (~50–100 mg) were obtained from the superficial portion of the VL muscle in 12 healthy but sedentary volunteers (10 men, 2 women; age 21–66 yr). Muscle samples used for measurement of $\text{ATP}_{\text{iso}}$ were cleaned of visible fat and connective tissue and placed in a relaxing solution, at 5°C for 24 h, consisting of 85 mM K+, 1 mM free Mg$^{2+}$, 5 mM MgATP, 7 mM EGTA, propionate as the major anion, and 10$^{-9}$ M free Ca$^{2+}$ [log Ca$^{2+}$ concentration (pCa) 9.0]; imidazole was used to maintain the pH at 7.0 ± 0.02 and to adjust the ionic strength to 150 mM. The fiber bundles were then transferred to relaxing solution containing 50% glycerol (vol/vol) and stored at −20°C for no more than 4 wk before subsequent analysis.

Muscle samples used for quantitative histochemical measurements of $V_{\text{max}}$ ATPase were cleaned of visible fat and connective tissue, oriented vertically in embedding medium, frozen in isopentane cooled by liquid nitrogen, and stored at −80°C for no more than 4 wk before subsequent analysis.
and then stopped again, and extinction of the NADH fluorescence signal (Fig. 1). Flow through the cuvette was then reinitiated for 1 s extending the rate of extinction of the NADH fluorescence signal due to the activity of the mitochondrial ATPases and sarcoplasmic reticulum ATPase make no contribution to the control of ATP consumption rate during maximum isometric activation (ATP$_{iso}$). Permeabilized single fibers, ∼3 mm in length, were mounted between force and displacement transducers in a quartz cuvette that was perfused with solutions containing free ionized Ca$^{2+}$ concentrations of either 1 nM (pCa 9.0) or 100 μM (pCa 4.0) maintained at 15°C. Muscle fiber length was adjusted so that average sarcomere length was 2.5 μm.

Measurement of maximum isometric force and ATP$_{iso}$. Maximum isometric force (F$_{max}$) and ATP$_{iso}$ were measured concurrently at 15°C in a Gou ˆ th Scientific Instruments Muscle Research System (16, 17, 20). The procedures for measuring isometric force in single permeabilized muscle fibers have been previously reported (14, 15, 20, 22, 23). In preliminary studies on human fibers, we confirmed that F$_{max}$ is obtained at pCa 4.0 and that no active force was obtained at pCa of 9.0.

The NADH-linked fluorometric technique for measuring ATP$_{iso}$ has been previously described in detail (16, 17, 20, 22, 23). Using this procedure, it was confirmed that mitochondrial ATPases and sarcoplasmic reticulum ATPase make no detectable contribution to the observed ATPase activity (17). Measurements of ATP$_{iso}$ were made while fibers were mounted in the quartz cuvette and perfused with either relaxing (pCa 9.0) or activating (pCa 4.0) solutions. NADH fluorescence was excited at 340 nm using a mercury lamp and an interposed band-pass filter. Emitted fluorescence was measured at 450 nm using a photomultiplier tube. The ATP solutions consisted of relaxing (pCa 9.0) and activating (pCa 4.0) solutions, both containing 5 mM phospho(enol)pyruvate (PEP), 0.2 mM NADH, 100 U/ml pyruvate kinase (PK), 140 U/ml lactate dehydrogenase (LDH), and 0.2 mM 1P$_1$P$_2$di(adenosine-5')pentaphosphate. The NADH-linked enzymatic assay involves the following reactions

\[ \text{ATPase} \]

\[ \text{ATP} \rightarrow \text{ADP} + P_i \quad (1) \]

\[ \text{PK} \]

\[ \text{ADP} + P_i \rightarrow \text{pyruvate} + \text{ATP} \quad (2) \]

\[ \text{Pyruvate + NADH} \rightarrow \text{lactate + NAD}^+ \quad (3) \]

where, in reaction 1, ATP is hydrolyzed by actomyosin ATPase to ADP and P$_i$ during detachment of the myosin head from the myosin-binding domain of actin. In reaction 2, ATP is regenerated from ADP and P$_i$ by PK. In reaction 3, the resulting pyruvate is converted to lactate by LDH, which results in stoichiometric conversion of fluorescent NADH to nonfluorescent NAD$^+$. For each mole of ADP produced by the actomyosin ATPase-dependent hydrolysis of ATP, 1 mol of NADH is converted to NAD$^+$. Therefore, for a period of time when perfusion of the cuvette was stopped (15 s), the amount of ATP consumed by the ATP$_{iso}$ was determined by measuring the rate of extinction of the NADH fluorescence signal (Fig. 1). Flow through the cuvette was then reinitiated for 1 s and then stopped again, and extinction of the NADH fluorescence signal was remeasured. This cycling continued for ∼10 min at each pCa condition. Calibration involved measurements of fluorescence intensity for known amounts of NADH. On the basis of changes in the NADH fluorescence intensity, ATP$_{iso}$ was determined and expressed as nanomoles per cubic millimeter per second. The ATP$_{iso}$ was determined by subtracting the ATP consumption rate measured at a pCa 9.0 from that obtained at pCa 4.0.

In a subset of permeabilized muscle fibers, the temperature dependence of the ATP$_{iso}$ was determined by obtaining measurements at 15, 20, and 25°C. The temperature coefficient (Q$_{10}$) for the ATP$_{iso}$ was then calculated over this temperature range.

Gel electrophoretic determination of MHC isoform expression in single fibers. After completion of the force and ATP$_{iso}$ measurements, the MHC isoform composition of the muscle fiber was determined by SDS-PAGE using a previously described procedure (14, 15, 22, 23). Briefly, fibers were placed in 25 μl of SDS sample buffer containing 62.5 mM Tris-HCl, 2% (wt/vol) SDS, 10% (vol/vol) glycerol, and 0.001% (wt/vol) bromophenol blue at a pH of 6.8. The sample was denatured by boiling for 2 min, and 10-μl samples [∼125 ng as determined by the Lowry method (19)] were loaded per lane. The gels were silver stained to visualize the MHC migration bands. Two mixed muscle fiber samples were run on each gel to compare the migration patterns of identified MHC isoforms. In the case of coexpression of MHC isoforms within a single fiber, the relative expression of each MHC isoform was determined by densitometric analysis.

Quantitative histochemical measurement of fiber $V_{max}$ ATPase. The quantitative histochemical procedure for measuring the $V_{max}$ ATPase in type-identified muscle fibers has been previously described in detail (4, 24). Serial cross sections of muscle fibers were cut at 10-μm thickness using a cryostat kept at −20°C, and alternate sections were used to determine MHC isoform expression and the $V_{max}$ ATPase. In four alternate transverse sections, immunoreactivity against antibodies specific for anti-MHC$_{slow}$ (NCL), anti-MHC$_{2A}$ (SC-71), and anti-MHC$_{4}$ (BF-35) (MHC$_{4}$ means all but the MHC$_{2A}$ isoform) was evaluated. Primary antibodies were diluted in PBS (pH 7.6) containing 0.5% bovine serum albumin and were then applied to the muscle sections for ∼12 h at room temperature in a humidified chamber. Slides were then washed in PBS and incubated with a fluorescein-conjugated secondary antibody (goat anti-mouse IgG) for ∼60 min at room tempera-

![Fig. 1. Typical trace of the simultaneous measurement of force and ATP consumption rate during maximum isometric activation (ATP$_{iso}$) using the NADH-linked fluorometric procedure in a single permeabilized fiber from the human vastus lateralis muscle [slow myosin heavy chain (MHC$_{slow}$) expression]. Fiber size: length, 3.0 mm; diameter, 78.3 μm.](Image)}}
ture in a humidified chamber. The slides were then washed in PBS, coverslipped with Permount, and viewed through a microscope (model BH2, Olympus) equipped with epifluorescence. An additional four alternate sections were stained for myofibrillar ATPase (mATPase) after preincubation at pH 4.3, 4.6, 9.0, and 10.4 (after 4% paraformaldehyde fixation) (7, 24).

The $V_{\text{max}}$ ATPase was measured in a series of 28 alternate sections of the same muscle fibers. The values for ATPase in a given fiber were the average of measurements across the four sections at a given ATP concentration, and the same fiber was measured at each of seven ATP concentrations (see below). In a previous study, our laboratory verified that the quantitative histochemical method is specific for the $V_{\text{max}}$ ATPase (4) and that the deposition of the reaction product is localized at the site of cross-bridge cycling (i.e., A band). In this procedure, an image-processing system (MegaVision 1024 XM), mounted on an Olympus BH-2 microscope and calibrated for microdensitometry using a set of neutral density filters [0.1–2.0 optical density (OD) units], was used. Microscopic images of muscle fiber cross sections were digitized (pixel) resolution into a $1,024 \times 1,024$ picture element (pixel) array using a video scanner and then stored in a computer file. In the digitizing procedure, 16 separate scans were averaged to reduce electronic noise, and a shading algorithm was employed to reduce errors attributed to uneven illumination of the tissue. Previously, our laboratory estimated that, when using this imaging system, the measurement error for microdensitometry was <4% (4, 24). The boundaries of individual fibers within the digitized images were delineated, and the average OD of all pixels within the fiber was calculated.

Four replicate sections of the same muscle fibers were reacted at 22°C in an incubation medium containing one of a series of ATP concentrations (0.0, 0.5, 0.75, 1.0, 2.0, 4.0, 5.0 mM ATP). The use of several ATP concentrations was necessary because sufficient ATP could not be dissolved in the incubation medium to avoid substrate-limiting the $V_{\text{max}}$ ATPase (4). In the $V_{\text{max}}$ ATPase, the $P_i$ ions produced by the enzymatic hydrolysis of ATP were precipitated within the muscle fiber cross section by complexing with lead ions (lead ammonium citrate-acetate complex) to form a lead phosphate precipitate. The precipitated lead ions were subsequently converted to nanomoles per cubic millimeter per minute by 10.220.33.5 on October 23, 2017 http://jap.physiology.org/ Downloaded from

### RESULTS

**Electrophoretic determination of MHC isoform expression in single fibers.** The VL muscle displayed three distinct MHC migration bands. On the basis of Western blot analysis, these MHC migration bands were found to correspond with the expression of MHC$_{\text{slow}}$, MHC$_{2A}$, and MHC$_{2x}$ isoforms. To evaluate MHC isoform expression in single VL fibers, a larger number of fibers were sampled in addition to those used in the mechanical and energetic studies (see Classification of fiber types in muscle cross sections). Among human VL fibers, MHC$_{\text{slow}}$ ($n = 64$) and MHC$_{2A}$ isoforms ($n = 65$) were found to be singularly expressed. However, the MHC$_{2x}$ isoform was not found to be singularly expressed and was coexpressed predominantly with the MHC$_{2A}$ isoform ($n = 24$; Fig. 3). Within fibers coexpressing MHC$_{2x}$ and MHC$_{2A}$, the relative expression of each isoform ranged from 20 to 80%, but the mean relative expression was 54.8 ± 1.2% MHC$_{2x}$ and 45.2 ± 1.2% MHC$_{2A}$. In these preliminary studies, it was determined that the fiber-type composition of the VL muscle could not be characterized from a single biopsy. It was estimated that up to seven biopsies would be required, and such repeated biopsies were not possible in these subjects. In addition, there was a relatively low abundance of fibers expressing the MHC$_{2x}$ isoform in the biopsies that were obtained. Indeed, it was necessary to obtain biopsies from 12 subjects to obtain a sufficient sample of fibers expressing the MHC$_{2x}$ isoform. For these reasons, it was not possible to characterize the overall population of this or other fiber types in the VL of subjects. This raised the important issues of whether across-subject variability may have influenced the results. For fibers expressing the MHC$_{2x}$ isoform, this issue could not be addressed. However, in comparing values for fibers expressing MHC$_{\text{slow}}$ and MHC$_{2A}$ isoforms, there were no significant differences across subjects. The coefficient of variation for ATP$_{\text{iso}}$ of fibers expressing MHC$_{2A}$ across subjects was 6.26%.

![Fig. 2. Lineweaver-Burke plot displaying the dependence of the velocity (V) of the maximum velocity of the actomyosin ATPase reaction (V$_{\text{max}}$ ATPase) on ATP concentration in the incubation medium for 3 human vastus lateralis muscle fibers expressing different (MHC) isoforms. Slope and y-intercept of each line were calculated by linear regression analysis, from which the V$_{\text{max}}$ ATPase reaction was determined. OD$_{570\text{nm}}$, optical density at 570 nm. Brackets denote concentration.](http://jap.physiology.org/Downloaded_from)
Classification of fiber types in muscle cross sections.

The patterns of immunoreactivity against specific MHC antibodies in the VL muscle generally corresponded with the histochemical classification of fiber types. VL fibers classified histochemically as type I displayed immunoreactivity for the anti-MHCslow antibody, and fibers classified histochemically as type IIa displayed immunoreactivity for the anti-MHC2A antibody. However, the anti-MHCall-2X antibody (BF-35), specific for all MHC isoforms except for MHC2X, was less reactive with fibers classified histochemically as type IIb, indicating expression of the MHC2X isoform. In these fibers, immunoreactivity for the MHCall-2X antibody varied from faint to moderate, and most of these fibers were also immunoreactive for the anti-MHC2A antibody in varying degrees. Therefore, these immunohistochemical results were consistent with the coexpression of MHC2A and MHC2X.

\[ F_{\text{max}} \] (n=46). \( F_{\text{max}} \) of human VL fibers was significantly lower for fibers expressing MHC2A (n=21; range 10–20 N/cm²) compared with fibers expressing MHCslow (n=19; range 12–22 N/cm²; Table 1; \( P < 0.05 \)). For fibers coexpressing MHC2X and MHC2A (n=6), there was a considerable range in \( F_{\text{max}} \) (11–27 N/cm²). Unfortunately, there were an insufficient number of fibers sampled in this group to determine whether \( F_{\text{max}} \) depended on the relative expression of MHC2X and MHC2A.

\[ ATP_{\text{iso}} \] (n=46). The \( ATP_{\text{iso}} \) of human VL fibers expressing the MHCslow isoform was significantly lower than that of fibers expressing MHC2A as well as fibers coexpressing MHC2X and MHC2A (\( P < 0.05 \); Fig. 4). In fibers singularly expressing MHCslow or MHC2A, the reserve capacity for ATP consumption was only 39% (\( P < 0.05 \)) compared with fibers singularly expressing MHCslow or MHC2A.

\[ \text{Isometric tension cost} \] (n=46). For a measurement of ATP cost for generating force, the isometric tension

Table 1. Cross-sectional area, maximum isometric force, and tension cost of human vastus lateralis muscle fibers at 15°C

<table>
<thead>
<tr>
<th>MHC Isoform</th>
<th>n</th>
<th>CSA, μm²</th>
<th>( F_{\text{max}}, ) N/cm²</th>
<th>Tension Cost, pmol·mm⁻¹·s⁻¹·mN⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>19</td>
<td>4972 ± 42</td>
<td>17.5 ± 0.6</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>2A</td>
<td>21</td>
<td>4368 ± 261</td>
<td>13.6 ± 0.6*</td>
<td>5.5 ± 0.2**</td>
</tr>
<tr>
<td>2X/2A</td>
<td>6</td>
<td>4651 ± 719</td>
<td>15.7 ± 0.1</td>
<td>6.7 ± 0.9**†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of muscle fibers. CSA, fiber cross-sectional area; \( F_{\text{max}} \), maximum isometric force; MHC, myosin heavy chain. *Significant difference from fibers expressing MHCslow, \( P < 0.05 \). †Significant difference from fibers expressing MHC2A, \( P < 0.05 \).

The ATPiso of single fibers singularly expressing MHC2A was also significantly lower than that of fibers coexpressing MHC2X and MHC2A (\( P < 0.05 \); Fig. 4). \( V_{\text{max, ATPase}} \) (n=525). The \( V_{\text{max, ATPase}} \) of VL muscle fibers expressing MHCslow was significantly lower than that of fibers expressing MHC2A either alone or coexpressed with MHC2X (\( P < 0.05 \); Fig. 4). The \( V_{\text{max, ATPase}} \) of fibers expressing MHC2A alone was significantly lower than that of fibers coexpressing MHC2X and MHC2A (\( P < 0.05 \); Fig. 4).

Reserve capacity of ATP consumption. For human VL muscle fibers, the reserve capacity for ATP consumption was calculated as

\[ \text{Reserve capacity for ATP consumption} = 1 - \left( \frac{\text{ATP}_{\text{iso}}}{V_{\text{max, ATPase}}} \right) \]

In fibers singularly expressing MHCslow or MHC2A, the reserve capacity for ATP consumption was 56 and 52%, respectively. For fibers coexpressing MHC2X and MHC2A, the reserve capacity for ATP consumption was only 39% (\( P < 0.05 \)) compared with fibers singularly expressing MHCslow or MHC2A.

Fig. 4. Comparison of ATPiso and \( V_{\text{max, ATPase}} \) for human vastus lateralis fibers expressing different MHC isoforms. *Significantly different from fibers expressing MHCslow, \( P < 0.05 \). †Significantly different from fibers expressing MHC2A, \( P < 0.05 \).
The cost of human VL fibers was determined by dividing the ATP$_{iso}$ by the corresponding isometric force. The tension cost of human VL fibers expressing the MHC$_{slow}$ isoform was significantly lower than that of fibers expressing MHC$_{2A}$ ($P < 0.05$; Fig. 5). There were an insufficient number of fibers sampled in this group to determine whether tension cost depended on the relative expression of MHC$_{2X}$ and MHC$_{2A}$. Comparison of tension cost determined at 15°C between human VL fibers and rat diaphragm fibers in our laboratory's previous study (22) is shown in Fig. 5. The relationship between $V_{max}$ ATPase and tension cost is displayed in Fig. 6.

Temperature dependence of ATP$_{iso}$ ($n = 6$). The dependence of ATP$_{iso}$ was determined by comparing measurements at 15, 20, and 25°C. As temperature increased, ATP$_{iso}$ of fibers also increased. The Q$_{10}$ value of the ATP$_{iso}$ was 1.72 ± 0.16 (Fig. 7).

**DISCUSSION**

The present study examined both maximal ($V_{max}$ ATPase) and submaximal (ATP$_{iso}$) values for ATP consumption in human VL muscle fibers. The $V_{max}$ ATPase establishes the upper limit for ATP consumption during work performance for each fiber type in skeletal muscle (23). In this respect, it is important to determine the range of ATP consumption rates (from $V_{max}$ ATPase to ATP$_{iso}$), because this provides a measure of the reserve capacity for ATP consumption. The present study reports important new information in this regard. The reserve capacity for ATP consumption was lower for fibers expressing MHC$_{2X}$ (coexpressed with MHC$_{2A}$) compared with fibers expressing MHC$_{2A}$ and MHC$_{slow}$. This is consistent with the lower energy efficiency of fibers expressing the MHC$_{2X}$ isoform as reflected by the higher tension cost of these fibers.

Similar to previous reports (10, 30), we found that three MHC isoforms were expressed in the VL muscle of healthy adult humans, MHC$_{slow}$, MHC$_{2A}$, and MHC$_{2X}$. Other studies evaluating MHC isoform expression in the human VL muscle also found three isoforms, although MHC$_{2B}$ expression was reported rather than MHC$_{2X}$ (18, 25). Because there is now strong evidence to indicate that MHC$_{2B}$ is not expressed in human fibers, it is likely that expression of MHC$_{2B}$ was confused with MHC$_{2X}$ expression. On the basis of single-fiber gel electrophoresis and Western blot analysis, it appears that MHC$_{slow}$ and MHC$_{2A}$ are singularly expressed in VL fibers, whereas the MHC$_{2X}$ isoform is only coexpressed, predominantly with MHC$_{2A}$. The coexpression of MHC$_{2X}$ and MHC$_{2A}$ in human VL muscle fibers has also been reported in other studies (10, 30). The pattern of MHC isoform expression in single human VL muscle fibers, as determined by SDS-PAGE, generally corresponded with the pattern of immunoreactivity against specific MHC antibodies, as well as the histochemical classification of fiber types based on the pH lability of myofibrillar mATPase staining. These results are consistent with other studies showing a relationship between histo-
similar to the previous results of Stienen and colleagues (25) for the human rectus abdominis and VL muscles, we found that ATP iso varied across fibers expressing different MHC isoforms in human VL muscle. These results are also in general agreement with our laboratory’s previous observations in the rat diaphragm muscle (22, 23). However, the values for ATP iso in human muscle fibers reported by Stienen and colleagues were lower than those found in the present study. These investigators measured ATP iso at 20°C rather than 15°C. The dependence of ATP iso on temperature was measured in both studies; a Q10 of 1.72 was found in the present study vs. a Q10 of 2.34 in the study of Stienen et al. Even when corrected for differences in temperature, the ATP iso values found in the present study were ~30–40% higher than those reported by Stienen et al. It should be noted that Stienen and colleagues measured NADH concentration by absorbency rather than fluorometry, and these technical differences may have accounted for the discrepancies in reported values.

31P-nuclear magnetic resonance (NMR) spectroscopy has also been used to measure ATP consumption in human muscle fibers in vivo on the basis of the dynamics of creatine phosphate content. Using this procedure, Blei and colleagues (5) reported an average ATP consumption rate of 0.15 ± 0.03 nmol-mm–3-s–1 during single-twitch stimulation in the human forearm flexor musculature, whereas Turner and colleagues (27) reported an average value of 4.4 ± 0.4 nmol-mm–3-s–1 in the human adductor pollicis muscle during maximum isometric activation. After corrections for the higher in vivo temperature were made, the ATP iso for VL muscle fibers in the present study would range from 1.02 ± 0.07 nmol-mm–3-s–1 for fibers expressing MHC slow to 3.06 ± 0.15 nmol-mm–3-s–1 for fibers coexpressing MHC 2X and MHC 2A. However, when we normalized our ATP iso values compared with fibers expressing MHC 2X and MHC 2B (14). Furthermore, our laboratory found that, during maximum isometric activation, the fraction of cross bridges in the force-generating state was comparable across fiber types (14). Thus, with a lower MHC concentration, lower ATP iso and V max ATPase would be expected for fibers expressing MHC slow and MHC 2A. However, when we normalized our V max ATPase for previously reported myofibrillar volume densities in the VL muscle (28), fiber-type differences in ATP iso and V max ATPase persisted. Thus it is likely that the lower ATP iso and V max ATPase values seen in VL fibers expressing the MHC slow isoform primarily reflect phenotypic differences in the capacity for ATP consumption of MHC slow vs. MHC 2A or MHC 2X molecules. The lower ATP iso and V max ATPase of fibers expressing MHC slow are also likely to be reflected by a slower maximum rate constant for cross-bridge detachment compared with fibers expressing MHC 2A or MHC 2X (23).

It is not surprising that the ATP iso of muscle fibers was only a fraction of the V max ATPase (i.e., maximum capacity for ATP consumption). In 1923, Fenn observed that energy utilization of skeletal muscle increases in proportion to work (Fenn effect; Refs. 11, 12). Thus, as muscle fibers reach maximum power during shortening, ATP consumption rate should increase (22). In a previous study on the rat diaphragm muscle, our laboratory found that the maximum rate of ATP consumption was achieved at a shortening velocity corresponding to peak power output of fibers (22). Although the maximum rate of ATP consumption achieved at peak power output was closer to the V max ATPase, it was still less. This may reflect differences in the number of cross bridges contributing to the measured ATP consumption rate during active shortening vs. those contributing to the measurements of V max ATPase.
The $F_{\text{max}}$ values for human VL muscle fibers measured in the present study were comparable to those reported in previous studies in single fibers (9, 18, 25, 29) as well as whole human muscle in vivo (13). We found that the $F_{\text{max}}$ of VL fibers expressing MHC slow was slightly greater than that for fibers expressing MHC2A. In contrast, Stienen et al. (25) reported that VL fibers expressing MHC slow generated lower $F_{\text{max}}$ compared with fibers expressing fast MHC isoforms. Larsson and Moss (18) reported no significant differences in $F_{\text{max}}$ across human VL muscle fibers expressing different MHC isoforms. In the rat diaphragm muscle, our laboratory found that fibers expressing MHC slow generated lower $F_{\text{max}}$ compared with fibers expressing fast MHC isoforms (14, 15, 22, 23).

The tension cost (the ratio of ATP isometric to isometric force; Fig. 5) of human VL muscle fibers reported in the present study is in general agreement with that reported by Steinen et al. (25). Fibers expressing MHC slow had the lowest values of tension cost followed by fibers expressing MHC2A and fibers coexpressing MHC2X and MHC2A. Therefore, fibers expressing MHC slow are the most energy efficient. Compared with values of tension cost reported for rat diaphragm muscle fibers (22), the tension cost of human VL muscle fibers was significantly lower. These results generally agree with the principle that energetic costs of generating muscular force are lower in larger animals (26).

In conclusion, measurement of submaximal and maximal rates of ATP consumption in the present study indicates that a substantial reserve capacity for ATP consumption exists in human muscle fibers. In addition, fiber-type differences in the reserve capacity for ATP consumption exist, with fibers expressing MHC2X (coexpressed with MHC2A) displaying a significantly lower reserve capacity compared with fibers singularly expressing MHC2A and MHC slow. These measurements provide new information that is important in determining the balance between energy supply and demand. Certainly this reserve capacity for ATP consumption becomes important under conditions where ATP production may be insufficient to meet the demands for cross-bridge cycling. Such an energetic imbalance has been suggested as an underlying mechanism of muscle fatigue. The lower reserve capacity for ATP consumption, together with the higher ATP consumption rates, may explain, at least in part, the greater fatigue susceptibility of fibers expressing MHC2X (21). Under conditions of greater workloads, as energy utilization increases in proportion to work (Fenn effect; Refs. 11, 12), reserve capacity for ATP consumption decreases and susceptibility to fatigue increases (2). Therefore, these novel results regarding the reserve capacity of ATP consumption in human VL muscle fibers have important functional implications.

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