Metabolic costs of isometric force generation and maintenance of human skeletal muscle

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Received 2 October 2001; accepted in final form 18 October 2001

Russ, David W., Mark A. Elliott, Krista Vandeborne, Glenn A. Walter, and Stuart A. Binder-Macleod. Metabolic costs of isometric force generation and maintenance of human skeletal muscle. Am J Physiol Endocrinol Metab 282: E448–E457, 2002; 10.1152/ajpendo.00285.2001.—During isometric contractions, no true work is performed, so the force-time integral (FTI) is often used to approximate isometric work. However, the relationship between FTI and metabolic cost is not as linear. We tested the hypothesis that this nonlinearity was due to the cost of attaining a given force being greater than that of maintaining it. The ATP consumed per contraction in the human medial gastrocnemius muscle (n = 6) was determined by use of 31P-NMR spectroscopy during eight different electrical stimulation protocols. Each protocol consisted of 8 trains of a single frequency (20 or 80 Hz) and duration (300, 600, 1,200, or 1,800 ms) performed under ischemic conditions. The cost of force generation was determined from the ATP turnover during the tetanus, despite the fact that the 0.1-s contractions produced greater ATP consumption than a single 5-s tetanus, even though the number of pulses and contractions were equal and the 10-Hz trains produced lower FTIs (4, 8, 36). Newham et al. (28) demonstrated, in human adductor pollicis muscles, that 50 0.1-s contractions produced greater ATP consumption than a single 5-s tetanus, despite the fact that the 0.1-s contractions produced smaller peak forces. Ratkevičius et al. (30), studying human triceps surae muscles, found that an intermittent stimulation protocol consumed more ATP as a continuous stimulation protocol, despite the fact that the intermittent protocol produced only 20% of the total contraction time. Finally, pilot work from our laboratory demonstrated that stimulation with 12-pulse 50-Hz trains produced more fatigue than stimulation with 12-pulse 10-Hz trains, even though the number of pulses and contractions were equal and the 10-Hz trains produced higher FTIs (32). None of these studies, however, specifically addressed the different costs of force generation and force maintenance in human muscle.

TOTAL ATP CONSUMPTION by skeletal muscle increases with increased work and power (1, 12, 13, 33). In addition, ATP consumption in response to single, isometric twitch is proportional to the area under the force-time curve, or force-time integral (FTI) (17). Because twitch FTI and work appear to relate to ATP consumption in a similar manner, FTI is often used as an approximation of work (8, 11, 21) during isometric contractions that produce no true physical work. During electrically elicited isometric twitches, ATP consumption increases linearly over the range of frequencies below that at which summation of force occurs and the ATP consumption per twitch remains constant (27).

During an isometric tetanus, however, it has been suggested that metabolic cost is not a linear function of contraction duration, because the cost of achieving a level of force is greater than that of maintaining it (14). If the cost of attaining a given force level is greater than that of maintaining it, then the net rate of ATP hydrolysis should decrease as the duration of a tetanus increases. This effect has been observed in fast-twitch mouse extensor digitorum longus (EDL) (11) and rat gastrocnemius muscles (35). Moreover, He et al. (20) found that the rate of actin-myosin ATPase (AM-ATPase) activity was highest at the onset of contraction and progressively decreased with subsequent cross-bridge cycles in skinned, mammalian fibers. In addition, a number of studies in human skeletal muscle have demonstrated that, during intermittent isometric electrically elicited contractions, shorter contractions produce greater total ATP utilization than longer contractions when force, total contraction time, FTI, and number of stimulation pulses are controlled (4, 8, 36). Newham et al. (28) demonstrated, in human adductor pollicis muscles, that 50 0.1-s contractions produced greater ATP consumption than a single 5-s tetanus, despite the fact that the 0.1-s contractions produced smaller peak forces. Ratkevičius et al. (30), studying human triceps surae muscles, found that an intermittent stimulation protocol consumed >60% as much ATP as a continuous stimulation protocol, despite the fact that the intermittent protocol produced only 20% of the total contraction time. Finally, pilot work from our laboratory demonstrated that stimulation with 12-pulse 50-Hz trains produced more fatigue than stimulation with 12-pulse 10-Hz trains, even though the number of pulses and contractions were equal and the 10-Hz trains produced higher FTIs (32). None of these studies, however, specifically addressed the different costs of force generation and force maintenance in human muscle.
The purpose of this study was to determine the metabolic cost and ATPase rates, by use of in vivo $^{31}$P-NMR spectroscopy, associated with electrical stimulation of the human medial gastrocnemius, with trains of different frequencies (20 and 80 Hz) and durations (300, 600, 1,200, and 1,800 ms), and to use these values to estimate the different costs of force generation and force maintenance. We hypothesized that, for a given contraction duration, 80-Hz stimulation would involve a greater metabolic cost than 20-Hz stimulation, consistent with the greater force associated with the higher frequency. We further hypothesized that, within a given frequency, increasing duration would decrease the mean ATPase rate, as ATP turnover would be greatest at the onset of contraction. As a result, we expected to observe a relatively lower ATP turnover than would be anticipated for a linear increase in ATPase rate with increasing contraction duration.

**METHODS**

**Subjects**

Six healthy subjects (3 males), ranging from 25 to 42 yr of age (mean 33.3 ± 5.54 (SE) yr), with no history of muscle or joint problems, participated in this study. All subjects were informed of the purpose and procedures of the study and gave written, informed consent to their participation. The experimental protocols were approved by the Human Subjects Review Boards of the Universities of Delaware and Pennsylvania.

**Experimental Procedures**

**Experimental setup.** Before any testing, one of the investigators (D. W. Russ) located the motor point of the medial gastrocnemius muscle (MG) on each subject and recorded its relationship to the following anatomic landmarks: the posterior fibular head, the medial tibial plateau, and the popliteal crease. These measurements were used to assist the investigators in finding the motor point and placing the surface coil during subsequent testing sessions. One carbonized rubber electrode (4.5 × 4 cm), coated with a conductive gel and held in place with a paper-tape patch, was placed directly over the anterior surface of the knee joint. This electrode placement produced comfortable contractions at the same force level and improved the NMR signal-to-noise ratio compared with the more traditional placement, with both electrodes over the muscle (31). The subject lay supine, with the foot at a 90° angle to the shank, in a custom-built nonmagnetic plantar-/dorsi-flexion ergometer, locked in neutral position. A 3 × 7-cm oblong surface coil with a shallow penetration profile (see Localization of $^{31}$P-NMR spectra), double-tuned to both $^1$H and $^{31}$P, was placed over the upper one-third of the MG. An inflatable blood pressure cuff was placed over the middle of the subject’s thigh. The subject was stabilized using nonelastic nylon straps with Velcro closures across the foot, ankle, shank, thigh, and trunk. The subject’s plantarflexion maximum volitional isometric contraction (MVIC) was assessed by using the mean of three maximal-effort plantar flexions. Electrical stimulation of the muscle was delivered using a Grass S48 stimulator with a Grass model SIU8T stimulus isolation unit (Astro-Med, West Warwick, RI). All stimulation pulses were 600 μs in duration. Stimulation intensity was set such that a 1-s, 100-Hz train produced 50% of the subject’s MVIC. Once the stimulation intensity was set, the force transducer was disconnected, as it was found that simultaneous operation of the transducer, the spectrometer, and the stimulator introduced an unacceptable amount of noise into the NMR spectra.

**Testing session.** All experiments involved isometric testing of the MG in a 1-m-bore 2.0-Tesla superconducting magnet, interfaced with a custom-built spectrometer (39). An adaptation of the protocol previously described by Blei et al. (5) was used to measure the metabolic cost associated with each train. Changes in the energy-rich phosphate content were measured with $^{31}$P-NMR spectroscopy during electrically induced contractions in the absence of oxygen. For this purpose, a blood pressure cuff was placed around the thigh and inflated to 230 mmHg for 5 min before stimulation. It has been demonstrated that a 5-min period of ischemia is sufficient to eliminate the oxygen supplying the muscles (5). $^{31}$P-NMR spectroscopy was performed during eight ischemic stimulation protocols. Each protocol consisted of 8 trains, delivered at a rate of 1 every 32 s. Only one train frequency and duration was used per protocol (Table 1), and the order in which these protocols were delivered was randomly determined for each subject. At both frequencies, the bulk of force generation appeared to have been achieved by the 600-ms duration trains (Fig. 1). Two protocols were delivered per experimental session, and the order in which the protocols were delivered was randomly determined. Approximately 20 min of rest (5 min of recovery data plus an additional 15 min of rest) were given between protocols.

The session began with collecting NMR spectra for 5 min with the subject at rest, after which circulation was occluded by inflating the blood pressure cuff to 230 mmHg. Resting spectra were collected for an additional 5 min during circu-

![Fig. 1. Force responses from a typical subject to the different trains used in the stimulation protocols. Vertical lines represent, from left to right, the 300-, 600-, 1,200-, and 1,800-ms time points. Open symbols, 80-Hz trains; closed symbols, 20-Hz trains.](http://ajpendo.org)
latory occlusion. At the end of these 5 min, the first stimulation protocol commenced. The blood pressure cuff was released 30 s after the last train of the protocol was delivered, and spectra were collected throughout 5 min of recovery. The subject was allowed to rest for 15 min after the end of the protocol, and then the process was repeated using a second ischemic protocol.

**Data acquisition.** Phosphorous spectra were collected using an adiabatic 90° pulse with a sweep width of 3 KHz and 1,024 complex data points. Pulse repetition (TR) time was set at 4 s. Homogeneity of the magnetic field was adjusted using the proton signal (full width at half-maximal height /H11349 30 Hz), and the spectral data were filtered with an exponential filter corresponding to a line broadening of 5.1 Hz. These spectra were collected into 8-sum bins, providing a temporal resolution of 32 s. This allowed us to collect spectra that included one stimulation train per bin during exercise while improving our signal-to-noise ratio through signal averaging. In addition, fully relaxed spectra (TR time /H11005 30 s) were collected to provide appropriate saturation correction factors.

**Localization of 31P-NMR spectra.** Because NMR spectra reflect metabolic changes that occur in the volume of tissue "seen" by the coil, we performed two pilot experiments, one designed to determine localization of the phosphorous signals and the other designed to determine the activity of the muscle sampled within the field of view (FOV) of our experimental setup.

We determined the thickness of the MG of the subjects in the present study on the basis of transaxial surface-coil proton images of the subjects' plantarflexor muscles acquired at the center and the proximal and distal ends of the coil (FOV = 12.8 × 12.8 cm). Two phantoms simulating the shape and size of the MG and soleus muscles of the subject population were filled with 100 mM dibasic and 100 mM monobasic sodium phosphate solutions, respectively (39). 31P spectra (TR time = 4 s) were collected from the artificial "calf," and signal contamination was determined as the percentage of the total integrated phosphate area accounted for by the monobasic phosphate peak. Greater than 95% of the total 31P signal was from the gastrocnemius phantom (Fig. 2), indicating that the large majority of the tissue sampled in the current experiment consisted of the MG.

The second pilot study monitored signal changes in T2-weighted images taken before and after stimulation to determine whether the MG was active during the stimulation protocols. Changes in signal intensity in such images have been demonstrated to reflect recent muscle activation (23, 41). The imaging procedures were performed in a 1.5-Tesla clinical magnet (General Electric) with a birdcage extremity coil. Subjects (n = 3) were placed supine, with their leg in the coil. Multiple T2-weighted spin-echo images from the ankle plantarflexor muscles were acquired with echo times of 30 and 60 ms, TR = 2 s, 128 × 256 matrix, 18 cm FOV, and 7 mm slice thickness. Images were acquired before and after stimulation with one of the trains (80 Hz, 1,800 ms duration, stimulation intensity set to 50% MVIC) used in the present experiment. We chose this train because we expected it to be the most metabolically demanding, and changes in signal intensity seen in T2-weighted images have been related to exercise intensity (23). This gave us the best chance of observing a change, but use of the other lower-frequency shorter-duration trains in the study should not affect the volume of muscle activated, because the same stimulation intensity (50% MVIC) was used for every protocol. The response of a

![Fig. 2. A: 1H image from phantom simulating a human calf. Note that −95% of signal intensity is recorded from the bag simulating the medial gastrocnemius (MG). Sol, soleus. B: 31P spectrum recorded from the bag simulating the Sol, alone. C: 31P spectrum recorded from the bag simulating the MG, alone. D: 31P spectrum recorded from the two-bag phantom used in A. Again, −95% of the signal came from the MG phantom bag.](http://ajpendo.physiology.org/doi/fig/10.1152/ajpendo.00423.2001)
typical subject is presented in Fig. 3 and clearly indicates that the electrode placement and stimulation intensity used here activated the MG.

**Data analysis.** Spectra were manually phased, and the areas of ATP, phosphocreatine (PCr), phosphomonoester (PME), and Pi peaks were manually integrated by use of customized software (38). Resting [PME] was below the noise threshold in the spectrum. The changes in [PME] reported for each protocol were therefore based on the assumption that the resting [PME] was zero. Intracellular pH was calculated from the chemical shift of Pi on the basis of the equation,

\[
\text{pH} = -\log \left( \frac{10^{\text{pH} - 6.75}}{10^{\text{pH} - 6.75} + 10^{\text{pH} - 6.75}} \right)
\]

where \(\delta\) is the chemical shift of the Pi peak in parts per million (ppm) relative to PCr. Absolute concentrations of phosphorous metabolites were calculated on the basis of a resting [ATP] of 8.2 mM (18).

The ATPase rate during ischemic exercise was determined by the equation

\[
\text{ATPase} = 1.5L + \frac{\text{dPCr}}{\text{dt}} + \frac{\text{dATP}}{\text{dt}}
\]

where \(L\) represents anaerobic glycolysis. \(L\) can in turn be calculated as follows

\[
L = \beta_{\text{tot}} \frac{\text{dpH}}{\text{dt}} - \theta \frac{\text{dPCr}}{\text{dt}}
\]

where \(\beta_{\text{tot}}\) is the apparent buffer capacity of the muscle in millimoles of acid added per unit change in pH (slykes) and is determined initially from \(\Delta \text{[PCr]} / \Delta \text{pH}\) during ischemic exercise. \(\theta\) represents the millimolar concentration of protons released by PCr when coupled to Pi formation by functional ATPases and is calculated as

\[
\beta_{\text{tot}} = \frac{2.303 \cdot [P_i]}{(1 + 10^{\text{pH} - 6.75})(1 + 10^{\text{pH} - 6.75})} \quad (4)
\]

\[\beta_{\text{G-6-P}} = \frac{2.303 \cdot [G-6-P]}{(1 + 10^{\text{pH} - 6.20})(1 + 10^{\text{pH} - 6.20})} \quad (5)\]

\[\beta_{\text{CO}_2} = \frac{2.303 \cdot S \cdot \text{PCO}_2 \cdot 10^{\text{pH} - 6.1}}{(1 + 10^{\text{pH} - 6.1})(1 + 10^{\text{pH} - 6.1})} \quad (6)\]

where \(S\) in Eq. 6 is the solubility constant of \(\text{CO}_2\) in a closed system. These equations have been used in previous work on intense voluntary exercise of human skeletal muscle (24, 38). Once these different buffer capacities have been determined, \(\beta\) can then be calculated by subtracting \(\beta_{\text{Pi}}, \beta_{\text{G-6-P}},\) and \(\beta_{\text{CO}_2}\) from \(\beta_{\text{tot}}\).

After the ATPase rate associated with each protocol was calculated, the ATP consumed per train was determined by multiplying the mean ATPase rate by the duration of the stimulation trains delivered during that protocol. Then the

![Fig. 3. A: prestimulation, T2-weighted image of the lower leg from a typical subject. Mg, medial gastrocnemius; SOL, soleus; LG, lateral gastrocnemius. B: poststimulation, T2-weighted image of lower leg from the same subject. Note increased signal from MG, evidenced by lighter shading of muscle, indicating that the entire muscle was recruited during the stimulation protocol. C: surface coil proton image of the calf of the same subject. Note that the majority of the volume consists of the MG. Therefore, spectra collected during ischemic stimulation protocol reflected changes occurring predominantly in the MG.](image-url)
relative costs (mM ATP consumed) of attaining and maintaining force for both the 20- and 80-Hz protocols were determined by subtracting the mean ATP consumed by the 600-ms protocols from the mean ATP consumed by the 1,800-ms protocols at each frequency. In addition, we subtracted the ATP turnover during the 600-ms protocols from that during the 1,200-ms protocols, and the ATP turnover from the 1,200-ms protocols from that during the 1,800-ms protocols, to compare the metabolic costs of 600-ms blocks during the tetanic stimulation trains. Finally, the percentage of the total ATP turnover during each protocol associated with the breakdown of PCr was calculated by dividing the change in [PCr] by the total ATP consumption and multiplying by 100.

Statistical analysis. Two-way repeated-measures ANOVAs were used to test for the effects of frequency and duration (2 × 4) on the changes in [PCr], [Pi], [ATP], [PME], and pH produced by each protocol. If significant main effects were detected, post hoc comparisons were made using paired sample t-tests, corrected for multiple comparisons by use of Holm's sequentially rejective Bonferroni test (25). A priori, we chose to compare differences in the changes in [PCr], [Pi], [ATP], [PME], and pH between the 80- and 20-Hz protocols at each duration. Within each frequency, we chose, a priori, to compare changes in these variables resulting from a given protocol with changes from the protocol with trains of the next highest duration. One-way repeated-measures ANOVAs were performed on the ATP consumed per contraction within each protocol to determine whether the cost per contraction changed over the course of the eight contractions. If no significant effect was detected, the mean ATP cost of the eight contractions was used for subsequent comparisons. The mean ATP consumption and percentage of ATP turnover associated with PCr breakdown were compared using a two-way (frequency × duration) repeated-measures ANOVA. Post hoc comparisons again were made using paired sample t-tests modified for multiple comparisons with Holm's sequentially rejective Bonferroni correction. Significance for all tests was set at P ≤ 0.05.

RESULTS

Muscle Metabolites

There were no significant changes in ATP concentration throughout any of the protocols tested (Figs. 4 and 5). The other metabolites examined ([PCr], [Pi], [PME], and pH) all exhibited marked changes in response to the different stimulation protocols (see Figs. 4-7). Generally, for a given frequency, increasing the duration of stimulation increased the change in metabolites. Likewise, for a given duration, increasing the frequency increased the change in metabolites (Table 2).

ATP Cost of Contraction

Increasing contraction duration at a given frequency increased the ATP consumed per contraction, as did increasing the frequency for a given duration (Fig. 8A). If this cost per contraction was divided by the duration of that contraction, however, the ATPase rate (mM/s) decreased with increasing duration. At 20 Hz, the mean ATPase rates were 4.23 ± 0.62, 2.60 ± 0.25, 2.01 ± 0.19, and 1.94 ± 0.20 mM/s for the 300-ms, 600-ms, 1,200-ms, and 1,800-ms protocols, respectively. At 80 Hz, the mean ATPase rates were 7.00 ± 0.86, 5.35 ± 0.54, 3.80 ± 0.29, and 3.05 ± 0.20 mM/s for the 300-ms, 600-ms, 1,200-ms, and 1,800-ms protocols, respectively. One-way repeated-measures ANOVAs detected no significant effect of contraction number on the ATP cost per contraction for any of the protocols.
Thus the ATPase rates reported were calculated from the mean of the eight contractions in each protocol.

Although the costs per contraction in response to the 20- and 80-Hz protocols were significantly different, there were no significant differences between the 20- and 80-Hz protocols for the cost of force maintenance during the 600- to 1,200-ms and 1,200- to 1,800-ms time periods (Fig. 8C). Thus differences in the ATP cost per contraction between the two protocols appeared to be a function of the initial force-generating portion of the train.

For each protocol, the total ATP consumption was the sum of the PCr breakdown by creatine kinase (CK) and the glycolysis that occurred, because there was no net decline in ATP. Interestingly, the percentage of ATP consumption due to PCr breakdown varied across the 20- and 80-Hz protocols (Fig. 9). For the 20-Hz protocols, there were no significant differences in this percentage for any of the different durations, although it had decreased by 1,800 ms. For the 80-Hz protocols, the percentage of ATP cost accounted for by CK was greatest for the protocol using the shortest trains, and it declined as the duration of the trains increased (Fig. 9). In addition, for the 1,200- and 1,800-ms protocols, the percent ATP cost associated with PCr breakdown was greater for the 20-Hz than for the 80-Hz protocols.

DISCUSSION

This study used in vivo $^{31}$P-NMR to analyze the metabolic cost associated with stimulation trains of different frequencies and durations in the human MG. As we hypothesized, attaining force appeared to be metabolically more costly than maintaining it, resulting in higher ATPase rates during short contractions than during long contractions. These results are consistent with earlier work demonstrating that shorter contractions were more metabolically costly than longer, sustained contractions in both human (4, 8, 36) and animal (21, 35) muscle. As a consequence of the lower metabolic cost of force maintenance, the net ATPase rate declined as contraction duration increased.

These findings are consistent with work in single muscle fibers that demonstrated a markedly greater...

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**Fig. 6.** $\text{Pi}$ and phosphomonoester (PME) concentrations (means ± SE) from 6 subjects during 80-Hz stimulation protocols (A) and 20-Hz stimulation protocols (B). Before onset of stimulation protocols, points for PME represent fluctuations of random noise about zero, as no consistent peak was observable above noise in spectra. Shaded areas, duration of stimulation protocol.

**Fig. 7.** pH values (means ± SE) from 6 subjects during 80-Hz stimulation protocols (A) and 20-Hz stimulation protocols (B). Shaded area, duration of stimulation protocol. Note: increasing variance during recovery of pH is due to loss of area under $\text{Pi}$ peak in some subjects, a phenomenon that has been noted previously (6).
Fig. 8. A: ATP cost per contraction associated with the stimulation protocols. Bars, means ± SE. *P < 0.05: significantly different from 20-Hz protocol corresponding train duration; †significantly different from protocol with next highest duration train at 20 Hz; ‡significantly different from protocol with next highest duration train at 80 Hz. B: ATP cost associated with 1st 600 ms (force generation) and final 1,200 ms (force maintenance). *P < 0.05, significantly different from all other costs of contraction. C: ATP cost associated with 600-ms blocks corresponding to beginning, middle, and end of an 1,800-ms tetanus. Bars represent means ± SE. *P significantly different from 80-Hz train for the same time frame (P < 0.05); †significantly different from cost of 0- to 600-ms block for the same frequency (P < 0.01); ‡trend toward a difference in cost of 0- to 600-ms block for the same frequency (P < 0.10).

Changes in measured metabolites in response to the different stimulation protocols. Values are changes that occurred over the course of each protocol (8 contractions) and are presented as means ± SE. ∆PME, change in concentration of phosphomonoesters; PCr, phosphocreatine. ANOVA for PCr: effect of frequency, F = 9.00, P = 0.030; effect of duration, F = 14.28, P < 0.01; frequency × duration, F = 0.084, P = 0.882. ANOVA for Pi, effect of frequency, F = 8.46, P = 0.030; effect of duration, F = 17.44, P < 0.01; frequency × duration, F = 2.07, P = 0.147. ANOVA for ATP: effect of frequency, F = 4.34, P = 0.529; effect of duration, F = 0.994, P = 0.423; frequency × duration, F = 0.614, P = 0.617. ANOVA for pH: effect of frequency, F = 29.89, P < 0.01; effect of duration, F = 42.41, P < 0.01; frequency × duration, F = 11.07, P < 0.01. ANOVA for PME: effect of frequency, F = 4.79, P = 0.080; effect of duration, F = 4.70, P = 0.02; frequency × duration, F = 0.883, P = 0.472. *Significantly different from 80 Hz at corresponding train duration, P < 0.05; †significantly different from next longest duration at the same frequency, P < 0.05; ‡different from 80-Hz at corresponding train duration, P < 0.10.

rate of ATP hydrolysis at the onset of a twitch (9) and during initiation of a contraction than during the steady-force phase (20). They also may explain the observation that there is a force-dependent component of the labile heat associated with the onset of stimulation in single muscle fibers (7). The greater ATP turnover associated with attaining a high force would account for the differences in total labile heat observed.
with an increase in force (7). Moreover, although our results were obtained from electrically stimulated muscle, evidence suggests that the anaerobic ATP cost of such contractions is comparable to brief high-intensity voluntary contractions (30).

This study examined the ATP turnover in human muscle associated with a wide range of stimulation trains. Responses to the specific trains tested here can be compared with those in a number of separate, previous experiments. Meyer and Foley (27), in comparing human and animal ATPase rates during brief tetani (~1 s), suggested that the maximum ATPase rate for human muscle was ~2.0 μmol·g⁻¹·s⁻¹. The value of 7.00 mM/s found for the 300-ms, 80-Hz train in the present study can be converted to similar units (3), resulting in a value of 4.69 μmol·g wet wt⁻¹·s⁻¹, a much higher value than that predicted. However, the rate for the 1,800-ms, 80-Hz train, when similarly converted, becomes 2.03 μmol·g wet wt⁻¹·s⁻¹, nearly the expected value. Newham et al. (28) reported an ATP turnover of 9.24 mM/s for 50-Hz, 100-ms contractions in the human adductor pollicis muscle. This is greater than the highest ATPase rate found in the present experiment, but the 100-ms contraction time is also shorter than any used here. Because we observed that mean ATPase rate declined as contraction duration increased, it seems reasonable to assume that the rate would increase if contraction duration were smaller than durations used here. Walter et al. (38) found peak ATP turnover rates of 4.78 mM/s during voluntary maximal-effort plantarflexion exercise performed at a rate of 2–3 Hz. This is similar to the ATPase rate seen for the 80-Hz, 600-ms trains (5.35 mM/s) in the present study. Our results are also comparable to those obtained from biopsy studies of the human quadriceps femoris muscle. Chasidis and colleagues (8) and Bergström and Hultman (4) reported ATPase rates of 6.9 and 6.6 mmol·kg dry wt⁻¹·s⁻¹ in response to 20-Hz, 1,600-ms and 20-Hz, 800-ms trains, respectively. In the present experiments, values of 6.1 and 7.85 mmol·kg⁻¹·s⁻¹ (after conversion from mM/s) were obtained for the 20-Hz, 1,200 ms and 20-Hz, 600-ms trains, respectively. Thus our results appear to be compatible with previous work that used both NMR and biopsy methods.

In addition to confirming findings of earlier energy cost studies, the present work also allowed us to separate the costs of force generation and force maintenance. Surprisingly, the cost of maintaining force during the final 1,200 ms of the 1,800-ms trains was comparable for 20- and 80-Hz stimulation, despite the greater force produced at 80 Hz. The difference in metabolic cost at longer-contraction durations (1,200 and 1,800 ms) appeared to be a function of the greater cost of generating force at the onset of contraction. Because AM-ATPase activity has been shown to be proportional to force (3, 6, 29, 37), the lack of a difference in the cost of force maintenance at the two frequencies was unexpected.

The overall metabolic costs observed in this study are the result of the AM-ATPase and the noncontractile ATPases (Na⁻–K⁺ pump and SR-Ca²⁺ pump) that are thought to account for 20–40% of the total ATP consumption (2, 21, 22). The Na⁺–K⁺-ATPase is believed to contribute to the total ATP demand to a small degree (22, 27). Thus the bulk of the noncontractile cost is thought to result from the activity of the SR-Ca²⁺-ATPase. It is possible that differences in SR-Ca²⁺-ATPase activity could account for some of the differences in ATPase rate that we observed for the different protocols. However, there is evidence from single-fiber experiments (37) to suggest that the SR-Ca²⁺-ATPase was running at its maximum rate during all of the protocols used in the present experiments. Based on the force-pCa and ATPase-pCa curves presented in the work of Stienen et al. (37), the SR-ATPase rate plateaued at a pCa that corresponded to a force of ~30% maximum. Even the 300-ms, 20-Hz train in the present study, which produced the lowest forces, surpassed this level. Thus if the SR-Ca²⁺-ATPase was running at its maximum rate for all of the protocols, the differences we observed in total ATPase rate were probably primarily the result of differences in the AM-ATPase activity. We did not, however, make any attempt to separate the metabolic costs of the different ATPases in our experiments. Because of this, and because of differences in species, preparations (single fiber vs. in vivo), and activation methods (direct Ca²⁺ activation vs. nerve stimulation) between the work by Steinen et al. and our own, we cannot rule out the possibility that differences in the cost of Ca²⁺ handling may have contributed to our results. We believe that any such potential effect is likely to be small. In fact, differences in SR-ATPase activity would bias against our finding that ATPase rates were greater for short-duration trains than for long-duration trains. Thus, we think that differences in AM-ATPase rate account for the bulk of our findings.
The mechanisms behind the greater rate of ATP turnover at the onset of contraction vs. during the steady-force state remain unclear, even if we attribute them to differences in AM-ATPase rate. It may be due to sarcomere shortening that is occurring at the onset of the contraction (19, 29), cross-bridge cooperativity decreasing the rate of detachment as the contraction progresses (15), or transient buildup of P_i due to the rapid PCr breakdown at the initiation of contraction (9). Recent studies of the cross-bridge cycle, however, have suggested that the rate-limiting step in the cross-bridge cycle (and thus ATP turnover) is a strain-dependent isomerization that occurs after the power stroke and P_i release (for recent reviews see Refs. 15 and 16).

When force on the cross bridge is high, as it is during the force plateau of isometric contractions, the forward rate of this reaction step slows, and the rate constant of cross-bridge detachment \( g_{\text{app}} \) also slows. This is in agreement with recent studies by Sieck and colleagues (33, 34), who employ a model suggesting that isometric ATPase rate is a function of the isometric force \( F \) and \( g_{\text{app}} \), as presented in Eq. 7, where \( k \) is a constant representing the number of half sarcomeres per fiber divided by the mean force per cross bridge.

\[
\text{ATPase} = kFg_{\text{app}} \quad (7)
\]

If, at the onset of contraction, \( g_{\text{app}} \) is very high, a high ATPase rate will result. As force reaches a plateau, \( g_{\text{app}} \) decreases and, although the force remains high, the total ATPase rate will decrease. Such a decline in \( g_{\text{app}} \) with force development has been observed in single muscle fibers (G. C. Sieck, personal communication). If the relative decline in \( g_{\text{app}} \) during the plateau of the 80- vs. the 20-Hz stimulation trains was proportional to the differences in forces produced at the two frequencies, it could explain the comparable costs of force maintenance observed during the 20- and 80-Hz trains used in the present study. Of course, some direct measure of cross-bridge cycling is needed to confirm this hypothesis.

We also found that there was a progressive decline in the percentage of the ATP turnover associated with PCr breakdown, because contraction duration increased during the 80-Hz stimulation protocols (Fig. 9). This decline was not apparent during the 20-Hz protocols, although there was a trend toward a decrease when the 600-ms protocol was compared with the 1,800-ms protocol \( (P < 0.07) \). No net change in [ATP] was observed during any of the protocols, and so the remainder of the ATP turnover in each case was the result of glycolysis. Together, these findings suggest that a greater proportion of the ATP synthesis during the stimulation protocols was taken on by glycolysis as the duration of the contraction increased. Conley et al. (10) demonstrated that activation of glycolysis was a function of the number of stimulation pulses during twitch stimulation and that the glycolytic rate was dependent on muscle activation frequency, suggesting that glycolysis was regulated in a feed-forward manner by Ca^{2+} and not by a feedback mechanism related to metabolic by-products of stimulation. These observations may explain the present observation that the decline in the percentage of ATP resynthesis due to PCr breakdown with increasing contraction duration was more rapid at 80 Hz. At 20 Hz, the total number of stimulation pulses would accumulate more slowly, delaying the onset of glycolysis, and the lower frequency would produce a lower glycolytic rate.

Conclusions

Our results confirm the assertion that attaining force is more costly, in metabolic terms, than maintaining that force \( (14) \). This finding helps to explain previous studies that showed greater ATP turnover during brief intermittent contractions vs. longer sustained contractions when either the total contraction time \( (4, 8, 21) \) or total number of contractions \( (32) \) was kept constant. It may also help to explain why the FTI \( (32) \) does not always predict fatigue during isometric contractions. From the difference in the cost between force generation and force maintenance, it appears that the mean ATPase rate declines during contraction, consistent with single-fiber experiments that examine both twitch \( (9) \) and tetanic \( (10) \) contractions. Finally, the observation that maintaining force with 80-Hz stimulation was no more metabolically costly than maintaining a lower force with 20-Hz stimulation suggests an increased economy of contraction at higher frequencies. This finding may be related to current models of the cross-bridge cycle and ATP consumption.

We thank Dr. Samuel C. K. Lee and Michael Vardaro for their assistance in data collection.

Partial funding for this study was provided by the University of Delaware Office of Graduate Studies and the Foundation for Physical Therapy (D. W. Russ), and National Institutes of Health Grants HD-37789 (K. Vandenborne), HD-42164 (S. A. Binder-Macleod), and RR-2305.

Portions of these data were previously presented at the American Physiological Society Meeting on the Integrative Biology of Exercise, held in Portland, ME, in September, 2000.

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