In situ rat fast skeletal muscle is more efficient at submaximal than at maximal activation levels

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Received 24 May 2001; accepted in final form 9 November 2001

Abbate, F., C. J. De Ruiter, C. Offringa, A. J. Sargeant, and A. De Haan. In situ rat fast skeletal muscle is more efficient at submaximal than at maximal activation levels. J Appl Physiol 92: 2089–2096, 2002—The influence of stimulation frequency on efficiency (total work output/high-energy phosphate consumption) was studied using in situ medial gastrocnemius muscle tendon complexes of the rat. The muscles performed 20 repeated concentric contractions (2/s) at 34°C. During these repeated contractions, the muscle was stimulated via the severed sciatic nerve with either 60, 90, or 150 Hz. The muscle was freeze-clamped immediately after these contractions, and high-energy phosphate consumption was determined by measuring intramuscular chemical change relative to control muscles. The average values (±SD) of efficiency calculated for 60, 90, and 150 Hz were 18.5 ± 1.5 (n = 7), 18.6 ± 1.5 (n = 9), and 14.7 ± 1.3 mJ/μmol phosphate (n = 9). The results indicate that the efficiency of the muscles that were submaximally activated (60 or 90 Hz) was higher (+26%, P < 0.05) than that of those maximally activated (150 Hz). Additional experiments showed that the low efficiency at maximal activation levels is unlikely to be the result of a higher energy turnover by the Ca2+-ATPase relative to the total energy turnover. Therefore, alternative explanations are discussed.

DURING DYNAMIC CONTRACTIONS, skeletal muscles convert energy into work. The extent to which chemical energy is converted into work is termed efficiency. Efficiency has been investigated in humans as well as in animals under various conditions. However, reported values for efficiency show a large variation. Furthermore, the values found in human whole body movements are too high to be explained by the efficiency values described in single-fiber or isolated muscle studies (22). Van Ingen Schenau et al. (22) suggested that an important reason for the relatively low efficiencies found in single-fiber and isolated muscle experiments was the use of “energy wasting” protocols during these experiments.

Examination of the experimental protocols indicates that the activation pattern differs markedly between experiments performed in single fibers and isolated muscles compared with whole body exercise. Maximal activation levels, which are highly reproducible, have most often been used in single fibers or isolated muscles, whereas submaximal activation levels are used in whole body studies of efficiency in which energy utilization is calculated from O2 uptake during steady-state exercise.

In vivo, higher activation levels result, according to the size principle of Henneman et al. (15), in the recruitment of progressively larger and faster motor units and, hence, increasingly faster muscle fibers. Although the fiber types may operate at different efficiencies (3, 7, 8), higher activation levels are also reflected by the increasing stimulation frequencies of the activated fibers (rate coding).

It is generally known that high-stimulation frequencies result in an improved mechanical performance (e.g., peak power/force) of the muscle (fibers) through an increased Ca2+ release. Moreover, Blinks et al. (6) have shown that Ca2+ release may further increase with higher stimulation frequencies, even while there is no further augmentation in the generated isometric force. This suggests that a decreasing fraction of the intracellular Ca2+ concentration is used for crossbridge formation and, hence, work generation at higher stimulation frequencies. As all released Ca2+ needs to be reaccumulated into the sarcoplasmic reticulum (SR) at the expense of ATP, higher efficiencies may be expected at submaximal activation frequencies compared with maximal activation frequencies.

Therefore, to study whether the different efficiency values found between whole body movements and in situ and in vitro experiments are the result of the difference in the level of activation, we examined the effect of stimulation frequency on efficiency. The study was performed using in situ medial gastrocnemius muscle tendon complexes of the rat, which performed 20 repeated concentric contractions while activated at either 60, 90, or 150 Hz. To study whether the effects of
stimulation frequency on efficiency could be related to a relatively high-energy cost used for the reuptake of Ca\(^{2+}\) at high-stimulation frequencies, additional experiments were performed in which the energy turnover of the Ca\(^{2+}\)-ATPase was estimated during 60 or 150 Hz of stimulation.

MATERIALS AND METHODS

Muscle Preparation

Experiments were performed using male Wistar rats (\(n = 51\); body mass 275 ± 12 g) anesthetized with urethane (1.5 g/kg body mass ip). Supplemental injections of 0.63 g/kg body mass ip were given if necessary. Experimental procedures and type of contractions used have been described previously (1, 13) and will be summarized below. The right medial gastrocnemius muscle tendon complex was prepared free from surrounding muscles without compromising the blood supply. The animal was placed prone on a heated pad (35°C) with the femur of the operated leg clamped in a vertical position and the muscle held horizontally. The tendon was connected to a force transducer, which was part of an isovelocity measuring system. Stimulation was performed through the severed sciatic nerve with only the branch to the medial gastrocnemius left intact. The current of each stimulation pulse was set on 1 mA, which was 30% higher than needed for maximal force development. Pulse duration was 0.05 ms. Motor movements and stimulation were computer controlled. Force and length data were digitized (1,000 Hz) and stored on disk for later analyses. At the end of the experiments, anesthetized rats were killed by cervical dislocation.

Muscle Optimum Length and Temperature

Tetanus optimum length (\(L_o\)) was first estimated by determining twitch \(L_o\). \(L_o\) was usually ~1 mm below twitch \(L_o\), and, therefore, only two or three tetani (120 Hz, 150-ms duration) were needed to determine \(L_o\). After the assessment of \(L_o\) of the muscle, there was a resting period of 15 min before the actual experiments. In this way, the assessment of \(L_o\) hardly compromised energy metabolism before the actual exercise. Muscle temperature was controlled by a water-saturated airflow around the muscle of 34 ± 0.5°C. A previous study showed that muscle temperature was within 1°C of the temperature of the airflow (10).

Isovelocity Concentric Contractions

Before each contraction, the muscle was first passively stretched to \(L_o + 2.5\) mm. Then, before the start of shortening, stimulation was started, and the force was allowed to build up to the level that the muscle could approximately maintain during the concentric contraction. When that force level was reached, the shortening was started. This procedure resulted in isovelocity concentric contractions that were near isotonic (e.g., Refs. 1, 12). Examples of force traces are shown in Fig. 1. During the contractions, the muscles were stimulated with 60, 90, or 150 Hz. A study by de Haan (10) showed that, during concentric contractions, lower relative forces are generated than under isometric conditions using the same stimulation frequency. The extent of this effect increases with higher shortening velocities. Therefore, 60, 90, and 150 Hz lead to ~35, 50, and 95% of maximal isometric force output under the present experimental concentric conditions.

In the experiments, the duration of dynamic force generation by the muscle was held equal (~70 ms) for all three stimulation frequencies used. Because high-stimulation frequencies have a faster force buildup rate, the start of shortening could be commenced earlier than during low-frequency stimulation. Therefore, the stimulation duration was longer at lower stimulation frequencies: 90 ms for 60 Hz, 80 ms for 90 Hz, and 60 ms for 150 Hz. Care was taken that the relaxation was completed before the end of the motor move-
ment. The motor performed a movement from $L_o + 2.5$ mm to $L_o - 5.5$ mm during all three stimulation frequencies. In the present study, the movement interval was kept similar in the three experimental conditions, i.e., two per second.

**Choice of Shortening Velocity**

During in vivo locomotion, high-stimulation frequencies are used when high-power output needs to be generated. Therefore, to assess the relevance for in vivo movement, the possible effects of stimulation frequency on efficiency were studied at those shortening velocities at which maximal power output was generated by each stimulation frequency (65, 80, and 100 mm/s for 60, 90, and 150 Hz, respectively). Furthermore, studies by, for example, Barclay et al. (4) and Buschman et al. (7), have shown that, in fast skeletal muscle fibers, the maximum efficiency was reached at a shortening velocity close to optimal velocity (the velocity at which peak power is reached in the power-velocity curve). The velocity of shortening is reported in millimeters per second, which refers to the contraction velocity of the whole muscle tendon complex. The fiber length of the distal part of the medial gastrocnemius muscle was $\sim 14$ mm. Thus the shortening velocities correspond with $\sim 4.6, 5.7$, and $7.1$ fiber lengths/s at 60, 90, and 150 Hz, respectively.

**Experimental Procedures**

Just before the start of experiments, the blood supply to the muscle was occluded to minimize aerobic metabolism and prevent lactate removal from the muscle. Then 20 repetitive concentric contractions (2 contractions/s) were started, after which the muscle was immediately freeze-clamped with a pair of tongs precooled in liquid nitrogen. Note that relaxation was completed when the muscles were freeze-clamped. The time between the end of the contractions and freezing was $< 2$ s. After weighing, the muscle was stored in liquid nitrogen. The contralateral (resting) medial gastrocnemius was subsequently prepared free and freeze-clamped to serve as control.

Under the present experimental conditions, aerobic metabolism using stored oxygen was calculated to be maximally 9% of total energy utilization (25).

**Sham Experiments**

To determine possible effects of the assessment of twitch and tetanus $L_o$ on the metabolic state of the muscle, six rats were used to perform sham experiments. After the determination of $L_o$, these muscles were allowed to recover for 15 min and were then freeze-clamped and subsequently analyzed for high-energy phosphates and lactate.

**Energy Usage by the $\text{Ca}^{2+}$-ATPase**

The overlap between actin and myosin filament decreases with increasing muscle length. Therefore, a decreasing force-time integral (FTI), which is calculated as the sum of the areas under the force-time curve of the 20 repeated isometric contractions, and a diminishing contribution of cross-bridge cycling to the high-energy phosphate consumption (HEPC) is found at higher muscle lengths. By extrapolating the relation between FTI and HEPC to FTI $= 0$, a HEPC value is obtained at which there is no overlap between the actin and myosin filaments. This HEPC value can be taken as the amount of energy turnover related to activation processes, predominantly by reactions related to calcium movements (17, 21).

For the stimulation protocols used in the present study, the energy turnover related to the reaccumulation of Ca$^{2+}$ by the SR was estimated by measuring the HEPC during 20 isometric contractions performed at several muscle lengths varying from $L_o$ to $L_o + 10$ mm. During these contractions, the muscle was stimulated with either 60 Hz ($n = 9$) or 150 Hz ($n = 11$) using the same duration and movement interval (2 per second) as during the efficiency measurements.

**Peak Power and Work**

Peak power was calculated for the first contraction as the product of shortening velocity and force, which was obtained directly from the force records and measured when the muscle passed $L_o$. Work per contraction was calculated by integrating force over the distance of shortening, whereas total work output was calculated as the sum of work of the 20 individual contractions. Passive forces were $\sim 300$ mN at $L_o$, 2 N at $L_o + 5$ mm, and 7 N at $L_o + 10$ mm for which power, FTI, and work output were corrected.

**Analysis of Metabolites**

The frozen muscles were ground in a mortar under the constant addition of liquid nitrogen. Then the muscle powder was freeze-dried and stored at $-80^\circ$C until further analysis. Separation and quantification of metabolites occurred using a HPLC system that consisted of a binary LC pump (model 250, Perkin-Elmer), an autosampler with cooling tray and automatic injector (Basic Marathon, Spark Holland), and a variable-wavelength ultraviolet spectrophotometric detector (model 759A, Applied Biosystems). The size of the injection loop was 20 µL. For ATP-IMP and phosphocreatine (PCr)-creatinine (Cr) separations, the ultraviolet absorption was measured at wavelengths = 254 and 210 nm, respectively. Peaks were identified and quantified by a chromatography data system (model 717, Axxiom Chromatography) by comparing sample peak heights to those of external standards. Lactate was measured enzymatically using a Beckman DU 640 spectrophotometer.

**Nucleotides and Cr compounds.** Before HPLC analysis, metabolites were extracted by adding 1 µL methanol (60%, vol/vol) per microgram muscle powder (200 µg). Extraction occurred overnight at $-80^\circ$C. Preliminary work by Karatzafieri et al. (18) did not identify any significant loss of metabolites from muscle after prolonged storage at $-80^\circ$C.

Analysis was carried out as described previously for single fibers (18). Separation occurred at controlled room temperature ($20^\circ$C), under isocratic conditions, using a reversed-phase 125 × 4-mm analytic column protected by a 4 × 4-mm guard cartridge (both 5-µm particle size, RP-18 LiChrospher 100, Hewlett-Packard). The mobile phase was pumped at a flow rate of 1 mL/min. For ATP and IMP analysis, the mobile phase consisted of 215 mM KH$_2$PO$_4$, 2.3 mM tetrabutylammonium hydrogen sulfate, and 2% acetonitrile aqueous solution adjusted to pH 6.5 with 5 M KOH. For PCr and Cr analysis, the mobile phase consisted of 14.7 mM KH$_2$PO$_4$ and 1.15 mM tetrabutylammonium hydrogen sulfate aqueous solution adjusted to pH 5.3 with 5 M KOH. The eluents were degassed before use and were kept under helium during analysis. Before each assay, the column was washed and equilibrated with the mobile phase.

**Lactate.** Duplicate extractions occurred by homogenizing ~5 mg of dry muscle tissue in 0.5 mL cold perchloric acid (5% vol/vol). The muscle tissue was sonicated for 1 min, and the homogenate was centrifuged at 0°C (Biofuge 22R, Heraeus Sepatech), after which 400 µL of the supernatant were neutralized with 50 µL K$_2$CO$_3$-Tris solution (2.8 M K$_2$CO$_3$, 0.1 M Tris). The neutralized homogenate was centrifuged during 20 min (17,000 rpm at 4°C). The supernatant was taken and...
HEPC and Efficiency

To correct for small errors in sample weighing, the concentrations of ATP, IMP, PCr, and Cr in each muscle were normalized to the average total amount of Cr (PCr + Cr) of all muscles. HEPC was calculated from the differences in metabolite concentrations between the experimental and the contralateral muscles using the following formula (HEPC = 1.5 \times \Delta \text{lactate} – \Delta \text{PCr} – \Delta \text{ATP} + \Delta \text{IMP}, where \Delta is change) (24). HEPC was then multiplied by 0.23 (dry-to-wet mass ratio) (11) and by its muscle mass to obtain the HEPC per muscle. Efficiency was calculated from the total work output and HEPC per muscle and expressed as millijoules per micromole phosphate usage.

Statistics

Errors are expressed as SD. A one-way ANOVA was used to test for differences among the three groups (60, 90, or 150 Hz of stimulation). Bonferroni post hoc tests were used to test for significant differences among the group means (P < 0.05). With respect to the measurements of the energy turnover by the Ca\(^{2+}\)-ATPase, a linear regression line was fitted through the data points of each stimulation frequency (60 or 150 Hz). The difference between the two regression lines at FTI = 0 was tested for significance (P < 0.05) using an analysis of covariance as described by Armitage (2).

RESULTS

Peak Power/Work Output

The peak power output for the first contraction was 109.1 ± 33.4 mW (n = 7) at 60 Hz, 191.9 ± 22.3 mW (n = 9) for 90 Hz, and 276.7 ± 31.2 mW (n = 9) for 150 Hz and was significantly different (P < 0.05) among all three stimulation frequencies (Fig. 2A). (Note that peak power output is calculated as the product of force and shortening velocity. Force was measured when the muscle passed Lₒ. Because force was similar but the shortening velocity was higher at 150 Hz than at 90 Hz, peak power output was increased, whereas work output was similar.)

Figure 2B shows the average work generated per contraction. Work output first increased significantly during the series of contractions with all three stimulation frequencies, but the maximal increase in work output was higher during 60 Hz (~4.5 mJ) and 90 Hz (~3.8 mJ) than at 150 Hz (~1.8 mJ) (P < 0.05).

The total work output generated at 60, 90, and 150 Hz was 234.5 ± 56.6 mJ (n = 7), 335.8 ± 25.6 mJ (n = 9), and 344.9 ± 35.2 mJ (n = 9), respectively (Fig. 2C). The total work output generated at 60 Hz was significantly lower (P < 0.05) compared with that at 90 and 150 Hz, whereas there was no significant difference in total work output between 90 and 150 Hz.

As described in MATERIALS AND METHODS, a few tetani were needed to set the muscle at tetanus Lₒ. During these tetani, the muscles were stimulated at 120 Hz, which resulted in peak isometric force. Then, after a rest period, the muscles would perform a series of dynamic contractions at either 60, 90, or 150 Hz. The muscles were randomly divided in these groups. Consequently, there were no statistical differences in isometric peak tetanic tension (at 120 Hz), and all differences in mechanical output during the dynamic contractions (work, power) must come from the differences in stimulation frequency.

J Appl Physiol • VOL 92 • MAY 2002 • www.jap.org
Higher Efficiency of Muscle at Lower Activation Levels

Table 1. Concentrations of Intracellular High-Energy Phosphates and Lactate at Rest (Contralateral) and After 20 Repeated Concentric Contractions with a Stimulation Frequency of 60, 90, or 150 Hz

<table>
<thead>
<tr>
<th>Stimulation, Hz</th>
<th>PCr (umol/g dry wt)</th>
<th>Cr (umol/g dry wt)</th>
<th>PCr/Cr</th>
<th>Lactate (umol/g dry wt)</th>
<th>ATP (umol/g dry wt)</th>
<th>IMP (umol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 51)</td>
<td>90.2 ± 9.6</td>
<td>41.9 ± 6.7</td>
<td>2.2 ± 0.4</td>
<td>12.6 ± 2.6</td>
<td>26.4 ± 3.1</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>60</td>
<td>54.0 ± 9.2†</td>
<td>75.5 ± 9.3‡</td>
<td>0.68 ± 0.21†</td>
<td>37.9 ± 7.5‡</td>
<td>27.8 ± 9.3</td>
<td>0.6 ± 0.6*</td>
</tr>
<tr>
<td>90</td>
<td>42.9 ± 6.5</td>
<td>85.0 ± 6.1</td>
<td>0.48 ± 0.11</td>
<td>48.7 ± 4.1*</td>
<td>25.9 ± 4.8</td>
<td>1.3 ± 0.6*</td>
</tr>
<tr>
<td>150</td>
<td>38.0 ± 7.1</td>
<td>91.9 ± 6.9</td>
<td>0.37 ± 0.11</td>
<td>65.1 ± 5.3</td>
<td>26.4 ± 1.8</td>
<td>2.9 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD in umol/g dry wt; n, no. of animals. PCr, phosphocreatine; Cr, creatine; PCr/Cr, ratio of PCr to Cr. Significant difference from †150 Hz and ‡90 Hz, P < 0.05.

Sham Experiments

The average metabolite levels (in umol/g dry wt) found in the control muscles were 12.6 ± 2.6 lactate, 90.2 ± 9.6 PCr, 26.4 ± 3.1 ATP, and 0.4 ± 0.3 umol/g dry wt IMP (n = 51). The metabolite levels found in the sham muscles were 10.7 ± 2.2 lactate, 87.5 ± 8.7 PCr, 24.3 ± 3.3 ATP, and 0.5 ± 0.6 umol/g dry wt IMP (n = 6). There were no significant differences in metabolite levels between the control (resting) values and the sham-operated animals, indicating that any activity as a result of the assessment of twitch and tetanus L_o had no influence on the metabolite levels before the series of repeated contractions.

Metabolites and Efficiency

The concentrations of high-energy phosphates and lactate at rest and after 20 repeated concentric contractions at either 60, 90, or 150 Hz of stimulation are shown in Table 1. From the difference in concentrations of lactate, PCr, ATP, and IMP between experimental and contralateral muscles, HEPC was calculated and was highest at 150 Hz (23.5 ± 1.8 umol P/muscle; n = 9) and decreased from 18.1 ± 1.7 umol P/muscle at 90 Hz (n = 9) to 12.8 ± 3.5 umol P/muscle at 60 Hz (n = 7) (Fig. 2C). When total work output was divided by the HEPC in each condition, the calculated efficiency values for 150, 90, and 60 Hz were, respectively, 14.7 ± 1.3 (n = 9), 18.6 ± 1.5 (n = 9), and 18.5 ± 1.5 mJ/umol P (n = 7) (Fig. 3). Efficiency at 150 Hz was significantly lower (P < 0.05) compared with that at both 90 and 60 Hz.

Energy Utilization by the Ca2+-ATPase

Examples of force traces measured at different muscle lengths during stimulation at either 60 or 150 Hz are shown in Fig. 4. Figure 5 shows the significant relation between FTI and HEPC after 20 repeated isometric contractions performed at muscle lengths ranging from L_o to L_o + 10 mm. (Note: the duration of stimulation and movement interval were the same as those for the efficiency measurements.) To estimate the HEPC for the reaccumulation of Ca2+, a linear regression line was fitted through the data points and extrapolated to FTI = 0, at which there is no overlap between actin and myosin. The correlation coefficient r was 0.86 for the regression line of the experiments using 60 Hz of stimulation and 0.94 for the regression line using 150 Hz of stimulation. The HEPC values at FTI = 0 were 2.90 ± 1.75 umol P/muscle for 150 Hz and 3.65 ± 2.0 umol P/muscle for 60 Hz. There was no significant difference in the estimated HEPC at FTI = 0 between muscles stimulated at 60 or 150 Hz.

Please note that, although the average FTI generated at L_o during 60 Hz (15.9 ± 0.6 N·s) was significantly smaller than that at 150 Hz (18.8 ± 0.4 N·s), the difference in FTI was relatively small compared with the difference in isometric peak force as was determined in an earlier study (11). This is explained by the longer stimulation duration at 60 Hz that allowed the muscle to develop a greater fraction of its maximal force than at 150 Hz.

Discussion

The main result of the present study was that efficiency (= total work output/HEPC) of skeletal muscle was significantly higher for submaximal stimulation frequencies (60 and 90 Hz) compared with the maximal stimulation frequency (150 Hz). It was found that reducing the stimulation frequency from 150 to 90 Hz resulted in a similar work output but a significantly lower energetic cost. Lowering the stimulation frequency further from 90 to 60 Hz resulted in a proportional decrease in work output and energetic cost and thus a similar efficiency. The efficiency measured at
Fig. 4. Example of force traces of iso-
metric contractions at different muscle
lengths during 60- (A) or 150-Hz (B)
stimulation. In both examples, the top
record (solid line) indicates force while
muscle length was at \( L_o \), the middle
record (dotted line) indicates force at
muscle length \( L_o + 5 \) mm, and the bottom
record (dashed line) indicates force at muscle length \( L_o + 10 \) mm. All
force signals were scaled such that
force was 0 at the start of a contraction.
The (higher) passive forces at
\( L_o - 2 \) N and \( L_o + 10 \) mm (7 N) were
scaled equal to the passive force at \( L_o 
\) (300 mN).

submaximal activation levels (60 and 90 Hz) was \(~26\%\) higher than the efficiency measured at the maximal
activation level (150 Hz).

As the activation level may vary considerably in
studies of efficiency on different levels of organization
(e.g., whole body vs. single-fiber studies), the findings
of the present study may help in understanding why
the efficiency values found in whole body movements
are higher than could be expected from the single-fiber
and isolated muscle data (for discussion, see Ref. 22).

During in vivo locomotion, increasing stimulation
frequencies are accompanied by the participation of
fast(er) fiber types as the intensity of exercise becomes
higher. Although it is presently not clear to what extent
variation in fiber-type recruitment affects efficiency
exactly, most studies found a higher efficiency for
slow-twitch than for fast-twitch fibers (e.g., Refs. 7,
8). However, in the present preparation, the muscle
was electrically stimulated, and, therefore, all muscle
fibers were simultaneously activated. Moreover, possible
differences in energy turnover and efficiency of the
fiber types will be minimal as >90% of the fibers in the
medial gastrocnemius muscle of the rat are fast twitch.
It, therefore, seems unlikely that the higher efficiency
found at submaximal stimulation frequencies compared
with maximal can be explained by differences in
recruitment and/or high-energy phosphate usage be-
tween fiber types.

Another possible explanation for the difference in
efficiency relates to the energy turnover by the \( \text{Ca}^{2+} \)-
ATPase. Blinks et al. (6) demonstrated that \( \text{Ca}^{2+} \) release might still increase with higher stimulation
frequencies, even when the maximal isometric force
output has been reached. Therefore, as more \( \text{Ca}^{2+} \) is
released without leading to an enhanced force produc-
tion, lower efficiencies would be expected, because this
extra amount of \( \text{Ca}^{2+} \) needs to be reaccumulated in the
SR at the expense of ATP and hence HEPC. Previous
studies have shown that \( \text{Ca}^{2+} \) reaccumulation by the
SR consumes between 25 and 40% of the total energetic
cost during isometric contractions at muscle \( L_o \) (e.g.,
Refs. 11, 17, 20, 23, 26). Clearly, changes in this com-
ponent of total energy turnover in the muscle could
have a significant impact on calculated efficiency. To
verify whether a relatively high HEPC by the \( \text{Ca}^{2+} \)-
ATPase could account for the present differences in
efficiency, additional experiments were performed in which the HEPC for the reaccumulation of Ca$^{2+}$ was estimated. Although those results showed that there was no statistical difference in the absolute values for energy turnover by the Ca$^{2+}$-ATPase at 60 and 150 Hz (Fig. 5), a direct comparison between the HEPC of the Ca$^{2+}$-ATPase at 60 and 150 Hz is not adequate, because the possible effects on efficiency relate more to the released Ca$^{2+}$ per time unit than to the total Ca$^{2+}$ release. After normalization to the same stimulation duration (100 ms) and 1 g of muscle wet weight, the HEPC at $L_0 + 10$ mm was 0.24 μmol P/g muscle for 60 Hz and 0.29 μmol P/g muscle for 150 Hz per contraction. According to Hochachka (16), two calcium ions are reaccumulated in the SR at the hydrolysis of one ATP, and, consequently, $2 \times 0.24 = 0.48$ and $2 \times 0.29 = 0.58$ μmol Ca$^{2+}$ would have been released at 60 and 150 Hz, respectively. The estimated Ca$^{2+}$ release under the present experimental conditions is very similar to the estimated Ca$^{2+}$ release calculated from other studies, such as Wendt and Barclay (23) (0.39 μmol Ca$^{2+}$) and de Haan et al. (11) (0.62 μmol Ca$^{2+}$), whose data were corrected to obtain the same units. In conclusion, our calculations indicate that, under the present experimental conditions, similar amounts of Ca$^{2+}$ were released as found in other studies and that substantially more Ca$^{2+}$ is released in the same time span during 150 Hz than during 60 Hz stimulation. However, FTI was ~26% higher at 150 Hz compared with 60 Hz (at $L_0$). Therefore, the estimated higher intracellular Ca$^{2+}$ level at 150 Hz led to a considerable increase in mechanical output. We, therefore, concluded that, under the present experimental conditions, the amount of Ca$^{2+}$ that is not used for cross-bridge formation is likely to be very small. Hence, it is unlikely that the low efficiency values at 150 Hz are the result of a high fraction of released Ca$^{2+}$ that is not used for mechanical output, and, therefore, other explanations should be sought.

Another possible explanation for a higher efficiency during submaximal stimulation is offered by a study of Buschman et al. (7). They found, in single fibers from Xenopus laevis, that lowering the activation level resulted in a decrease of force (by 20–30%) but increased cross-bridge efficiency (by a factor of 1.3). When we calculated power output (force × shortening velocity), it was found that power output was significantly lower at 60 and 90 Hz than at 150 Hz, but efficiency was ~26% higher. Similarly, Curtin and Woledge (9) also found a higher efficiency at lower power output. In contrast, the lower power output at 60 Hz compared with 90 Hz was not accompanied by a change in efficiency, which might imply that the mechanism responsible for the lower efficiency at maximal activation (e.g., altered cross-bridge efficiency) leads to substantial effects at high exercise intensities only. Although we are not able to determine whether cross-bridge efficiency is increased by a decrease in activation level in our preparation (whole mammalian skeletal muscle), such an explanation may account for the higher efficiencies at submaximal activation found in the present study.

It is known that muscle efficiency [= work/(HEPC × ΔG$_{\text{ATP}}$) × $e_p$ × 100%, where ΔG$_{\text{ATP}}$ is free energy of ATP hydrolysis and $e_p$ is phosphorylative efficiency] during whole body movements such as cycling is ~30% (using ΔG$_{\text{ATP}} = -52$ kJ and $e_p = 0.575$) (22). If a similar calculation were performed on the present results, muscle efficiency would increased from 16 to 21% if submaximal instead of maximal stimulation were used. Although these values are still lower than the estimated 30% in human whole body movements, they may contribute to the understanding of why efficiency values found in isolated and in situ muscles are lower than those found in whole body studies (in which muscles are always submaximally activated). Possibly, muscle efficiency could be further increased in vivo by asynchronous firing of the motor units, which allows the muscle to generate smooth force responses at even lower frequencies than those used in the present experiments.

In conclusion, the present results have shown that muscles can work more efficiently at submaximal stimulation frequencies compared with maximal stimulation frequencies, but this is accompanied by reduced power production. We have shown that the increased efficiency at the lower activation levels is very unlikely to be attributable to a reduction in the Ca$^{2+}$-ATPase-related HEPC, and thus the exact mechanism remains to be identified. As activation levels and hence stimulation frequencies differ among efficiency measurements in different preparations, the results of the present study may help to explain the relatively high efficiency found during whole body movements compared with single-fiber and isolated muscle experiments.

REFERENCES