ATP utilization and force during intermittent and continuous muscle contractions

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Chasiotis, D., M. Bergström, and E. Hultman. ATP utilization and force during intermittent and continuous muscle contractions. J. Appl. Physiol. 63(1): 167-174, 1987.—Energy utilization and force generation under anaerobic conditions were studied in electrically stimulated quadriceps femoris muscle of four volunteers. To investigate the effects of intermittent vs. continuous stimulation one leg was stimulated intermittently and the other continuously during 50 s. The same initial force was produced, and biopsy samples were obtained before the stimulation and after 10, 20, and 50 s and analyzed for energy-rich phosphagens, glycolytic intermediates, and phosphorylase. The ATP utilization and glycolysis were greater during intermittent contraction, but glycogenolysis was equal. ATP content decreased to lower values after intermittent contraction (16.4 compared with 19.6 mmol/kg dry muscle after continuous contraction). Force generation was well preserved during continuous contraction but successively decreased after 20 s of intermittent stimulation down to 50% of initial at end of work. The energy cost per unit work was greater during intermittent contraction and increased with contraction time, whereas it decreased with time during continuous stimulation. The decrease in force generation in intermittent exercise is suggested to be due to the higher energy cost for contraction resulting in greater changes in the intracellular environment with lower ATP and increased H+ and F-. These changes would decrease both activation of the contractile system and the crossbridge turnover rate resulting from activation.

electrical stimulation; energy consumption; glycolysis; quadriceps femoris; phosphorylase

Muscle work involves the transformation of stored chemical energy to mechanical work. Since heat is also produced, muscle energetics can be studied by the classical myothermic methods as well as by chemical methods. Heat production during a contraction-relaxation cycle can be divided into activation, maintenance, relaxation, and recovery heat. One component, the heat of shortening, is defined as the heat produced during a contraction where the muscle shortens after subtraction of the heat produced by a maintained isometric tetanus at the appropriate muscle length (maintenance heat). During an in vivo isometric contraction shortening heat could occur initially, when there is axial displacement of the filaments and stretching of elastic elements in the muscle-tendon apparatus.

Much of the relations between myothermic and biochemical findings remain unclear, but activation heat is probably related to the Ca²⁺ movement during activation of the contractile system. Maintenance heat, which is proportional to force, is a reflection of several ATP-consuming processes, such as actomyosin turnover and activity of the Ca²⁺- and Na⁺-K⁺-adenosinetriphosphatases (ATPases) (19).

During muscle contraction under anaerobic conditions the main energy sources are phosphocreatine (PCr) and glycogen. PCr breakdown during exercise not only provides energy to maintain contraction but also liberates inorganic phosphate (Pᵢ), and it has been suggested that this phosphate increase serves as a link between PCr breakdown and glycogen utilization (4).

The relation of fatigue to energy utilization and/or production is unclear. In previous papers from this laboratory, the biochemical changes in fatiguing muscle have been studied by use of electrical stimulation. Under these conditions the stimulus to contract is constant and fatigue can be defined as decreasing force. Earlier studies have shown that force is well preserved even after 1 min of continuous electrical stimulation of the quadriceps muscle (15), whereas intermittent stimulation produces earlier fatigue and, in relation to the work performed, more profound chemical changes (16, 25).

The present study was designed to compare, in the same individual, energy utilization and fatigue development during continuous and intermittent work. The chemical energy used will be related to the isometric work produced and the activation of phosphorylase will be compared with the observed rate of glycogenolysis during these two patterns of work.

METHODS

Subjects. Four healthy volunteers (3 males and 1 female) participated in this study. Their mean (range) age, height, and weight were 35 (29-40) yr, 173 (162-184) cm, and 69 (63-73) kg, respectively. The subjects were not well trained but regularly took part in some form of physical activity. They were informed of the possible risks involved in the experiments before their voluntary consent was obtained. The study was part of a larger project approved by the Ethical Committee of the Karolinska Institute, Stockholm.

Experimental design. The experiments were performed with the subject semisupine on a bed. Both lower legs were flexed to 90° over the end of the bed. One leg, chosen at random, was then attached to a strain gauge by a strap around the ankle. The isometric force was...
displayed on an oscilloscope screen and recorded on ultraviolet-sensitive paper using the Medelec system (Medelec, Old Woking, Surrey, UK). Two aluminum foil electrodes were placed about 15 cm apart on the anterolateral part of the thigh. The subject then performed three maximal voluntary contractions (MVC), and the highest value was used as an estimate of MVC force. In this experimental setup there is usually little variation in the MVC measurement from time to time and from side to side. The mean value for MVC in this study was 524 N (range 387–765).

Electrical stimulation was performed as described earlier (15, 17). In this study we used stimulation pulses with a duration of 0.5 ms and a frequency of 20 Hz (Medelec IS/V stimulator). The voltage was increased until stimulation produced a force corresponding to ~25% of the previously determined MVC of that leg. At a voltage of 120–180 V the initial force was 96–162 N, corresponding to 21–28% of MVC. The stimulation mainly activates the vastus lateralis, and the use of a voltage producing ~25% of the MVC ensures that biopsies can be obtained from the contracting part of the muscle.

Two different stimulation models, one continuous and one intermittent, were used; two of the subjects started with the continuous model, the other two with the intermittent. At least 15 min were allowed to pass from the end of one stimulation until the experiment continued with the other stimulation model on the other leg.

Thirty seconds before stimulation a pneumatic cuff around the upper part of the thigh was inflated to ~300 mmHg. The cuff remained inflated during the entire experiment and ensured that the muscle was ischemic and after a few seconds of work also anaerobic, since the myoglobin-bound O2 would be rapidly depleted (11).

The continuous model consisted of a continuous 20-Hz stimulation lasting 52 s. In the intermittent model the muscle was stimulated by trains of 20-Hz pulses giving contractions of 1.6 s separated by pauses of 1.6 s. The total number of contractions was 31, and since three contractions were prolonged to 3.2 s to give time for biopsies, the total contraction time was 54.4 s.

Three muscle biopsies were taken during each stimulation by use of the needle biopsy technique (1). During continuous stimulation biopsies were obtained after 12, 22, and 52 s. From the muscles that were stimulated intermittently biopsies were taken during the prolonged contractions 6, 13, and 31, which correspond to 10, 22, and 53 s of contraction. A resting biopsy was also taken immediately before stimulation. Muscle samples were immediately frozen by immersing them in Freon maintained at its freezing point (~150°C) by liquid N2, freeze-dried, and stored at ~80°C until further treatment.

Analytical methods. The freeze-dried samples were dissected free from blood, fat, and all visible connective tissue and pulverized. The muscle powder was divided in two parts, one for determination of glycogen phosphorylase and one for metabolites.

Muscle tissue was homogenized in a Potter-Elvehjem-type homogenizer at −33°C in an aqueous solution of 60% (vol/vol) glycerol, containing 30 mM sodium \( \beta- \) glycerophosphate, 50 mM sodium fluoride, 5 mM ethylendiaminetetraacetic acid, 0.5 g/l bovine serum albumin, and 20 mM 1,4-dithiothreitol; pH was adjusted to 7.0; 100 \( \mu l \) of the homogenizing reagents were used per milligram of dry muscle.

Glycogen phosphorylase was assayed at 35°C (pH 7.0) in the direction of glycogen breakdown by use of a method adapted from Holmes and Mansour (13) as previously described (4). The reaction was started by addition of AMP-free glycogen, and formation of hexose phosphates was measured enzymatically according to the technique of Harris et al. (12). Phosphorylase \( \alpha \) was assayed in the absence of AMP, and phosphorylase \( \alpha + b \) in the presence of 2 mM AMP. Values are reported as millimoles glycogen units per kilogram of dry muscle per second. The analytical error (coefficient of variation) for measurement of phosphorylase \( \alpha \) and \( \alpha + b \) has been determined from duplicate analyses of the same muscle homogenate to be 8.6 and 2.9%, respectively.

ATP, ADP, phosphocreatine (PCr), creatine (Cr), glucose 1-phosphate (G-1-P), glucose 6-phosphate (G-6-P), fructose 6-phosphate (Fru-6-P), glycerol 1-phosphate (Glyc-1-P), pyruvate (Pyr), and lactate (La) were analyzed in perchloric acid extracts of freeze-dried muscle with the enzymatic methods previously described by Harris et al. (12). Values are reported as millimoles per kilogram of dry muscle.

The contents of AMP and IMP in muscle were analyzed with high-performance liquid chromatography as previously described (18). The system consisted of a high-pressure pump (Waters model M6500A), a loop injector (Waters V6K), a column packed with Supelcosil LC-18 (C-18 reversed phase), and an ultraviolet detector (Waters model 440) used at 254 nm. The mobile phase was 220 mM KH2PO4 with 1% (vol/vol) methanol (pH 6.9). IMP and AMP obtained from Boehringer Mannheim were used for standardization. The injection volume was usually 10 \( \mu l \). Values are reported as millimoles per kilogram of dry muscle.

Calculations. The force produced by the knee extensor during stimulation was measured on paper recordings. Since the actual force in newtons will be influenced by such individual factors as quadriceps mass, leg length, and stimulation voltage, force was always related to the MVC and expressed as a fraction. Thus force measurements will be more logically related to biochemical measures expressed per kilogram working muscle.

The force-time integral was estimated by measuring the area below the force tracings. Again, the values were related to MVC giving a measure of isometric work where one unit corresponds to the work of holding an MVC for 1 s. The unit for this quantity would be seconds, but since it is specific for the experimental arrangements used here, it will be called an arbitrary unit (AU).

The net ATP utilization rate ("ATP turnover rate") was calculated from the changes in metabolite values assuming that the circulatory occlusion had provided a closed system and an aerobic metabolism

\[
\text{ATP utilization} = 1.5\Delta[\text{La}] + \Delta[\text{PCr}] + 2\Delta[\text{ATP}]
\]

\[
- \Delta[\text{ADP}] \text{ (mmol/kg dry muscle)}
\]
The value was divided by the stimulation time between biopsies to give the ATP utilization rate (mmol·kg dry muscle·s⁻¹).

The glycolytic rate [post-phosphofructokinase (PFK) metabolite accumulation] was calculated from the formula

\[ 0.5(\Delta[Glyc-1-P] + \Delta[Pyr] + \Delta[La]) \]

The value obtained was divided by time to give millimole glucosyl units per kilogram of dry muscle per second.

The relative contributions to the ATP utilization from ATP and PCr degradation were calculated from the concentration changes during the period, whereas the amount of ATP resynthesized through glycolysis was calculated from the lactate and pyruvate formed assuming 1.5 mmol ATP/mmol.

The maximal activity of phosphorylase a \( (V_{max}a) \) and the maximal activity of phosphorylase \( a + b \) \( (V_{max}a + b) \) were calculated from the measured phosphorylase activities at 11 mmol P₃/l, using a Michaelis constant \( (K_m) \) of 26.2 and 6.8 mmol P₃/l for \( a \) and \( a + b \), respectively (3). The percentage of phosphorylase in the \( a \) form was calculated as \( (V_{max}a/V_{max}a + b) \times 100 \). This constitutes a minimal estimate of the fraction of phosphorylase in the \( a \) form and is independent of the P₃ concentration used in the assay system (4). The activity of phosphorylase \( a \) in muscle was calculated from the P₃ content in each biopsy, assuming a \( K_m \) value of 6.8 mmol/l. It was also assumed that the AMP concentration was high enough to lower the \( K_m \) of phosphorylase \( a \) to 6.8 mmol/l but not so high as to activate the \( b \) form.

The intracellular phosphate (P₃) concentration during stimulation was calculated from the changes in ATP, ADP, PCr, and hexose monophosphates (HMP) using the formula


The metabolite concentrations were divided by 3.0, assuming 3 liters intracellular water/kg dry muscle to give values of millimoles P₃ per liter. A resting value of 2.9 mmol/l was used (3, 6).

The observed glycogenolytic rate was calculated as

\[ \Delta[HMP] + 0.5(\Delta[Glyc-1-P] + \Delta[Pyr] + \Delta[La]) \]

The value was divided by time to give millimole glucosyl units per kilogram per second.

Hydrogen ion concentrations \([H^+]\) during work were calculated from [La] and [Pyr] according to Sahlin et al. (23). The resting value is from the same reference.

Statistics. Differences between stimulation models were tested using Student’s \( t \) test for paired observations. To test changes with time during the stimulation linear regression was performed, and the regression coefficient was tested for significance (24). A probability level of 0.05 was used to indicate significance.

RESULTS

In this study, the quadriceps muscles were stimulated electrically by continuous stimulation of one leg and intermittent stimulation of the other. The stimulation was adjusted to give initial isometric forces of ~25% MVC. Absolute initial force was 121 ± 30 and 125 ± 31 N, respectively, in the legs stimulated continuously and intermittently; these values correspond to relative forces of 24 ± 3 and 23 ± 2% MVC, respectively. The initial force was well maintained during the first 20 s of contraction in both work models, but after that the force declined rapidly during intermittent stimulation, reaching a value of about 50% of initial force after 50 s. In contrast to this, force during continuous stimulation was about 90% after 50 s (Fig. 1). These differences in the development of fatigue are also evident from the estimates of isometric work, calculated as described in METHODS. The total amount of work performed was significantly larger during continuous stimulation (10.4 ± 1.3 AU) compared with intermittent (6.5 ± 0.5 AU). The experiments can be divided into three parts, since biopsies were taken after about 10, 20, and 50 s of work (exact times are given in METHODS). If the values for estimated isometric work are divided by the duration of each period, the values for work per unit time presented in Table 3 are obtained. It is obvious that the amount of work per second was constant during continuous stimulation, whereas it decreased to about half the initial value during the last 30 s of intermittent stimulation. This difference between stimulation models is significant.

The results from the metabolite determinations are presented in Tables 1 and 2. The most striking differences between the two work patterns are that ATP and

![Fig. 1. Contraction force (filled circles) and ATP utilization rate during intermittent (A) and continuous (B) electrical stimulation. Values are means ± SD. Energy contributions from ATP, phosphocreatine (PCr), and glycolysis are indicated.](image-url)
TABLE 1. Muscle content of ATP, ADP, AMP, IMP, PCr, Cr, and P_i at rest and during intermittent and continuous electrical stimulation

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Intermittent</th>
<th>Continuous</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 s</td>
<td>10 s</td>
<td>22 s</td>
<td>53 s</td>
</tr>
<tr>
<td>ATP</td>
<td>26.0±1.6</td>
<td>23.2±2.3</td>
<td>22.9±2.4</td>
<td>16.4±2.3</td>
</tr>
<tr>
<td>ADP</td>
<td>3.4±0.3</td>
<td>3.7±0.4</td>
<td>4.2±0.2</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>AMP</td>
<td>0.13±0.04</td>
<td>0.21±0.13</td>
<td>0.23±0.10</td>
<td>0.54±0.37</td>
</tr>
<tr>
<td>ATP + ADP + AMP</td>
<td>29.7±1.7</td>
<td>27.1±2.8</td>
<td>26.4±2.5</td>
<td>21.8±2.6</td>
</tr>
<tr>
<td>IMP</td>
<td>0.00±0.00</td>
<td>0.1±0.1</td>
<td>1.5±1.3</td>
<td>7.7±4.0</td>
</tr>
<tr>
<td>PCr</td>
<td>75.3±1.9</td>
<td>41.4±4.1</td>
<td>20.2±4.8</td>
<td>3.4±1.7</td>
</tr>
<tr>
<td>Cr</td>
<td>58.7±6.6</td>
<td>90.1±9.9</td>
<td>117.9±5.8</td>
<td>138.1±9.0</td>
</tr>
<tr>
<td>PCr + Cr</td>
<td>134.0±9.9</td>
<td>131.2±11.9</td>
<td>138.1±4.3</td>
<td>141.3±7.3</td>
</tr>
<tr>
<td>ATP/ADP</td>
<td>7.7±0.9</td>
<td>6.3±0.4</td>
<td>5.4±0.6</td>
<td>3.5±0.6</td>
</tr>
<tr>
<td>P_i</td>
<td>2.9</td>
<td>14.3±2.1</td>
<td>20.6±2.0</td>
<td>27.7±1.7</td>
</tr>
</tbody>
</table>

Values are means ± SD and are given as mmol/kg dry muscle, except for P_i, which is mmol/l intracellular water. For calculation of P_i see METHODS. * Significant difference between corresponding values during continuous and intermittent stimulation.

PCr were significantly lower and lactate significantly higher during intermittent compared with continuous stimulation. As a result of this, the calculated ATP/ADP ratios during intermittent stimulation are lower. Calculation of phosphate and [H+] gives higher values for the intermittent stimulation.

Net ATP utilization rates are listed in Table 3. The values decrease with contraction time in both types of work but are significantly higher during intermittent stimulation when the respective values are compared for each time period. The total ATP utilization over the whole stimulation period was 234 ± 28 mmol/kg dry muscle during intermittent work and 186 ± 19 during continuous (P < 0.05). The glycolytic rate was also higher during intermittent stimulation, a difference that is significant during the last 30 s. The fraction of ATP resynthesis derived from glycolysis increased from ~50% of the ATP utilization during the first 10 s to ~80-90% at the end of stimulation. There are no major differences in this respect between the two work models (Fig. 1).

The increased glycolytic rate in contracting muscle is regulated by the activity of phosphorylase. In the present study the observed glycolytic rates were not significantly different during continuous and intermittent work (Table 4). In the resting samples 26% of phosphorylase was in the α form (Table 5). The α form increased about threefold during the first 20 s, and the increase was similar in both types of stimulation. There was a slight decline during the final 30 s of contraction. More than 70% of phosphorylase was in the α form at the end of the 50 s contraction, whereas phosphorylase α + β was almost unchanged. The measured phosphorylase α activities were also used, as described in METHODS, to calculate the glycogenolytic rates in vivo at the appropriate P_i concentrations. There is a good agreement between calculated and observed glycogenolytic rates (Fig. 2).

The ATP utilization rate in relation to units of work are presented in Table 3. These values represent millimoles of ATP utilized per kilogram of dry muscle per arbitrary unit of isometric work. The values increase significantly during intermittent work, from an initial value of 26.6 ± 4.2 mmol ATP kg dry muscle⁻¹ AU⁻¹ to 35.1 ± 4.5 during the third period of stimulation. On the other hand there are decreasing values during continuous stimulation, from 21.4 ± 2.3 to 17.4 ± 2.7 mmol ATP kg dry muscle⁻¹ AU⁻¹. Moreover, if the values during intermittent and continuous stimulation are compared for each time period, the values for intermittent work are significantly higher during work periods at 10-20 and 20-50 s.
TABLE 3. ATP utilization rates, isometric work, and ATP utilization per unit work during intermittent and continuous electrical stimulation

<table>
<thead>
<tr>
<th>Electrical Stimulation</th>
<th>0-10 s</th>
<th>10-22 s</th>
<th>22-55 s</th>
<th>0-12 s</th>
<th>12-22 s</th>
<th>22-52 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP utilization rate,</td>
<td>6.9±0.9</td>
<td>5.3±0.9</td>
<td>4.5±0.4</td>
<td>4.3±0.3*</td>
<td>4.1±0.5*</td>
<td>3.5±0.4*</td>
</tr>
<tr>
<td>mmol·kg⁻¹·s⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isometric work, AU/s</td>
<td>0.22±0.01</td>
<td>0.19±0.02</td>
<td>0.13±0.01</td>
<td>0.20±0.03</td>
<td>0.21±0.03</td>
<td>0.20±0.02*</td>
</tr>
<tr>
<td>ATP utilization/unit work,</td>
<td>36.6±4.2</td>
<td>28.6±4.1</td>
<td>35.1±4.5</td>
<td>21.4±2.3</td>
<td>20.0±1.8*</td>
<td>17.4±2.7*</td>
</tr>
<tr>
<td>mmol ATP·kg⁻¹·AU⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. AU, arbitrary units (see text). * Significant difference between corresponding values during continuous and intermittent stimulation.

TABLE 4. Observed glycogenolytic and glycolytic rates in vivo and calculated glycogenolytic rate during intermittent and continuous electrical stimulation

<table>
<thead>
<tr>
<th>Electrical Stimulation</th>
<th>0-10 s</th>
<th>10-22 s</th>
<th>22-55 s</th>
<th>0-12 s</th>
<th>12-22 s</th>
<th>22-52 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obs glycogenolytic rate</td>
<td>1.3±0.5</td>
<td>1.5±0.4</td>
<td>1.5±0.2</td>
<td>0.9±0.1</td>
<td>1.2±0.3*</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Calc glycogenolytic rate</td>
<td>1.6±0.3</td>
<td>1.6±0.2</td>
<td>1.6±0.2</td>
<td>1.2±0.3*</td>
<td>1.3±0.3</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Obs glycolytic rate</td>
<td>0.9±0.1</td>
<td>1.2±0.3</td>
<td>1.3±0.2</td>
<td>0.9±0.2</td>
<td>0.9±0.1*</td>
<td>0.9±0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SD based on phosphorylase activity and are given as mmol glucosyl units·kg dry muscle⁻¹·s⁻¹. * Significant difference between corresponding values during continuous and intermittent stimulation.

TABLE 5. Effect of intermittent and continuous electrical stimulation on phosphorylase activity

<table>
<thead>
<tr>
<th>Electrical Stimulation</th>
<th>0 s</th>
<th>10 s</th>
<th>22 s</th>
<th>53 s</th>
<th>12 s</th>
<th>22 s</th>
<th>52 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase a</td>
<td>0.19±0.04</td>
<td>0.68±0.11</td>
<td>0.64±0.08</td>
<td>0.54±0.06</td>
<td>0.54±0.12</td>
<td>0.53±0.15</td>
<td>0.50±0.10</td>
</tr>
<tr>
<td>Phosphorylase a + b</td>
<td>1.52±0.26</td>
<td>1.06±0.23</td>
<td>1.57±0.22</td>
<td>1.59±0.13</td>
<td>1.40±0.15</td>
<td>1.45±0.17</td>
<td>1.43±0.19</td>
</tr>
<tr>
<td>Vmax,a</td>
<td>0.84±0.15</td>
<td>2.32±0.38</td>
<td>2.34±0.28</td>
<td>2.58±0.22</td>
<td>2.46±0.23</td>
<td>2.58±0.27</td>
<td>2.51±0.31</td>
</tr>
<tr>
<td>Vmax,a + b</td>
<td>2.45±0.41</td>
<td>2.67±0.38</td>
<td>2.54±0.35</td>
<td>2.58±0.22</td>
<td>2.26±0.23</td>
<td>2.34±0.27</td>
<td>2.31±0.31</td>
</tr>
<tr>
<td>(Vmax,a/Vmax,a + b) x 100</td>
<td>23.9±4.8</td>
<td>87.3±2.9</td>
<td>84.7±5.6</td>
<td>71.4±3.8</td>
<td>76.4±8.9</td>
<td>75.4±14.0</td>
<td>74.0±6.0</td>
</tr>
</tbody>
</table>

Values are means ± SD and are given as mmol glucosyl units·kg dry muscle⁻¹·s⁻¹. Vmax, maximal enzyme activity. For calculations see METHODS.

DISCUSSION

In this study, force and ATP utilization in human quadriceps muscle were measured during intermittent and continuous electrical stimulation. Muscle blood flow was interrupted during the experiments to provide anaerobic conditions and a closed metabolic system. The important findings are 1) that there was an earlier and more pronounced decrease in force production in the muscles stimulated intermittently and 2) that there was a greater ATP utilization during intermittent work. The calculated energy cost is consequently greater during intermittent work.

The energy consumed during these experiments was calculated from adenine nucleotide degradation and anaerobic glycolysis. Activation of the phosphorylase system, mediated by the Ca²⁺ increase during contraction, will provide HMP, the substrate for glycolysis. There was no significant difference in the observed glycogenolytic rates between the two stimulation models, suggesting that activation of the phosphorylase system was equal. This conclusion is supported by the fact that estimates of glycogenolytic rates, based on phosphorylase
determinations, agree with the rates obtained from measured metabolite accumulations.

The rate-determining step in glycolysis is generally considered to be PFK. The accumulation of HMP indicates that the activity of PFK was always lower compared with that of phosphorylase. The higher glycolytic rate during intermittent stimulation means that PFK activity varies between the two stimulation patterns. This is probably caused by the differences in intracellular environment. Among the various factors affecting PFK activity ADP, AMP, Pi, and Fru-6-P are known to activate PFK, whereas ATP and H+ inhibit this enzyme (21, 27). ATP was significantly lower during intermittent compared with continuous stimulation. [H+] was not measured but estimated in this study, and there was a tendency toward higher values during intermittent stimulation. Despite this, the glycolytic rate was higher. Possibly the higher contents of ADP, AMP, and Pi or some other factor, e.g., NH3 released by AMP deaminase activity, counteracted the effect of H+ resulting in a maintained glycolysis during intermittent contractions (26).

The cause of muscle fatigue is unknown, although many biochemical and electrophysiological changes that accompany fatigue have been described. In connection with this study it is of particular interest to consider whether the earlier decrease in force generation during one of our stimulation models could be the result of a failure of the electrical stimulation to activate the muscle. Since failure of the transmission along motor nerves has been reported for higher stimulation frequencies (10), the frequency used here, 20 Hz, has been chosen to avoid this problem. It is also difficult to see why such a mechanism should affect only an intermittent protocol. In a recent study by Duchateau and Hainaut (9), comparisons of electrical and mechanical failure during continuous and intermittent contractions lead these authors to conclude that intracellular processes are most important in the development of fatigue. The same study also demonstrated, with short contractions and rest periods, that intermittent contractions are more fatiguing than a continuous contraction.

Fast-twitch fibers are known to be more easily fatiguable than slow-twitch fibers, and a selective recruitment of fast-twitch fibers during intermittent stimulation could explain a faster decline in force. However, this would mean that the energy cost for intermittent work should decrease with time. The reason for this is that the remaining slow-twitch fibers have a lower energy cost during short tetani (7). Such a process might contribute to the small decline in force during continuous stimulation, when there was also a decline in ATP utilization, but our results do not support further conclusions regarding a selective recruitment or fatigue of fiber types.

In this paper we have demonstrated a greater energy cost for intermittent work than for continuous, and the difference seems to increase with contraction time. Using rat gastrocnemius muscle in situ, de Haan et al. (8) have obtained similar results for short contraction times (up to 6.3 s). In those experiments the differences in economy between stimulation protocols were caused mainly by a greater energy consumption during intermittent contractions rather than differences in the force-time integral.

Why do intermittent contractions consume more chemical energy than a continuous contraction producing the same force-time integral? This question can be addressed intuitively in thermodynamic terms. If each contraction during intermittent work is associated with activation heat and if maintenance heat, i.e., the force-related cost of cross-bridge turnover, is equal during intermittent and continuous work, then one could expect a higher total energy consumption during intermittent work. Moreover, if the contractions are not strictly isometric, there would be heat of shortening and the difference would increase. This is in several ways an oversimplification, but it still leads to some possibly involved mechanisms.

Among the biochemical processes that consume energy during a contraction-relaxation cycle both Ca2+ transport and actomyosin ATPase activity could explain differences in energy consumption. Not only is the activation heat related to Ca2+ movements at the onset of contraction, but the Ca2+ transport over the sarcoplasmic reticular membranes will differ during intermittent and continuous work. When the muscle works intermittently, Ca2+ pumping has to restore the resting intracellular Ca2+ gradient once every contraction; this is much more energy consuming than the steady-state-like conditions of a continuous contraction. Kushmerick (19) has calculated from data on frog muscle that the Ca2+ uptake after a muscle twitch would utilize 0.1–0.3 mmol/kg wet wt of ATP, which should be compared with a utilization of 0.3–0.4 mmol·kg−1·s−1 by the actomyosin ATPase during an isometric tetanus. For short tetani, the contribution from Ca2+ pumping during relaxation would thus be very significant.

There is a tendency of both fast- and slow-twitch muscle fibers to increase their economy of contraction with increasing contraction time. This is most pronounced in fast-twitch fibers: mouse extensor digitorum longus muscles halve their energy consumption when tetanus duration increases from 3 to 15 s (7). This is thought to be a result of decreased actomyosin turnover. Since the human quadriceps muscle is a mixed muscle, such a mechanism could be responsible for the higher energy consumption during intermittent stimulation in this study. The contractions would not be long enough for the fast-twitch fibers to enter a more economical state of cross-bridge handling, and the resting periods could be long enough for the fibers to recover in this aspect. On the other hand, the continuous stimulation would lead to increased economy. The exact result of such a mechanism would of course depend on the fiber composition of the muscle and on contraction time as well as resting time. One factor studied in frog muscles, is the "start-up cost," which is paid during the onset of one contraction and, if the interval is short enough, need not be fully paid again during the next contraction. However, in anaerobic muscles poisoned with iodoacetate, the start-up cost was not influenced by the resting period between contractions and those experiments also
showed a higher energy consumption during intermittent contractions compared with a single tetanus (20, 22). Whereas the effects of one contraction on the next in a series of contractions are obviously complex, it seems probable that the energy consumption during the rest period does not contribute significantly to the difference between intermittent and continuous work. Estimates of the resting ATP utilization rate in quadriceps muscle during tourniquet ischemia have yielded values of <0.04 mmol ATP·kg dry muscle⁻¹·s⁻¹ (11). Furthermore, in another study, no changes in PCr and La were seen during the rest period between two contractions performed under ischemia (14).

Could the earlier fatigue during intermittent stimulation be caused by the increased energy consumption? Biochemical changes associated with fatigue include changes in phosphagen and [H⁺]. A lower ratio of ATP/ADP could result in a decreased cross-bridge turnover, and earlier research (16) has demonstrated a relation between ATP/ADP and decline in force. It is not known whether this relation holds for continuous stimulation. One effect of an increased [H⁺] is to decrease cross-bridge turnover, e.g. by inhibition of Ca²⁺ release from the sarcoplasmic reticulum and/or an effect on Ca²⁺-troponin binding (2). In this study the tendency is for ATP/ADP to be lower and for [H⁺] to be higher during intermittent stimulation, but the connection to the earlier fatigue remains speculative. F; could decrease force generation by affecting the power stroke of cross bridges (5). In our experiments calculated P; tends to be higher during intermittent stimulation. One of these factors or a combination of them could lead to earlier fatigue in the intermittent model. The indirect cause of the decrease in force during intermittent stimulation would then be that the contractile mechanism is negatively affected by the intracellular environment created by an increased energy consumption. This effect could be both on the activation of the contractile mechanism and on the level of cross-bridge cycling.

In summary, we have demonstrated in human muscle a higher energy cost and an earlier development of fatigue during intermittent compared with continuous work. This result is supported by earlier research and compatible with theoretical models of muscle energetics. To evaluate the energetics of intermittent work further, comparisons of different contraction times and work-to-rest ratios will be necessary.

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