Energy metabolism in single human muscle fibers during contraction without and with epinephrine infusion

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GREENHAFF, PAUL L., JIAN-MING REN, KARIN SÖDERLUND, AND ERIC HULTMAN. Energy metabolism in single human muscle fibers during contraction without and with epinephrine infusion. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E713–E718, 1991.—The concentrations of glycogen, ATP, and phosphocreatine were analyzed in types I and II muscle fibers separated from biopsy samples of the quadriceps femoris muscle in five healthy volunteers. Muscle samples were obtained before and after 64 s of intermittent electrical stimulation. The experiment was carried out without and with epinephrine (Epi) infusion. Before stimulation the glycogen concentration was 11% higher in type II than in type I fibers (P < 0.05). During electrical stimulation, rapid glycogenolysis occurred in type II fibers with hardly any detectable glycogenolysis in type I fibers. The calculated rates of glycogenolysis were 0.16 ± 0.14 and 3.54 ± 0.53 mmol glucose·kg dry muscle⁻¹·s⁻¹ in types I and II fibers, respectively. Epi infusion increased the rate of glycogenolysis during electrical stimulation in type I fibers (10-fold) but did not enhance the rate in type II fibers (P > 0.05). It is considered that, during short-term maximal muscle contraction, rapid muscle glycogenolysis occurs predominantly in type II fibers even though types I and II fibers are recruited and that, when Epi stimulation of glycogenolysis occurs, this is predominantly limited to type I fibers.

Adenosine 3',5'-triphosphate; glycogenolysis; maximal muscle contraction; phosphocreatine

SKELETAL MUSCLE consists of at least two distinct muscle fiber types, each of which is characterized by its own individual mechanical (13) and biochemical (22) properties.

Maximal power and its subsequent rapid decline appear to be closely related to the contribution made by type II (fast-twitch) muscle fibers to force production. This has been demonstrated in animal whole muscle preparations (5) and in bundles of human skeletal muscle fibers (16). Despite this, due to technical difficulties, most scientists to date investigating the metabolic response of human skeletal muscle to maximal exercise have based their conclusions upon the biochemical analysis of whole muscle samples or upon the semiquantitative analysis of whole muscle sections, neither of which may reflect the true individual fiber type response.

Epinephrine (Epi) infusion has been shown to stimulate glycogenolysis in resting rat skeletal muscle containing a high proportion of fast-twitch fibers but has no effect on slow-twitch muscles (8, 30). Conversely, during intense electrical stimulation with Epi infusion, glycogenolysis is accelerated only in slow-twitch muscle (30). Little or no information is currently available concerning the influence of Epi upon glycogenolysis in individual human skeletal muscle fibers. The purpose of the present experiment was 1) to compare the glycogenolytic response of type I and type II human skeletal muscle fibers during electrical stimulation by biochemically analyzing pools of individual fibers for phosphocreatine (PCr), ATP, and glycogen concentrations and 2) to investigate the effect of Epi infusion upon this response.

MATERIALS AND METHODS

Subjects. Five healthy volunteers (3 men and 2 women) participated in the study. Their mean age, height, and weight was 28 yr (range 23–33 yr), 174 cm (range 158–185 cm), and 64 kg (range 51–79 kg), respectively. The subjects were active, since they regularly took part in some form of physical activity. Before commencement of the study, the experimental procedures were explained to all subjects, and their voluntary consent was obtained in writing. This study was part of a large project approved by the Ethical Committee of the Karolinska Institute, Sweden.

Experimental protocol. The experiments were performed in the morning or early afternoon with each subject lying in a semisupine position on a bed after fasting for at least 2 h. Both legs were flexed over the end of the bed at an angle of 90°. The leg being studied at any one time was attached by means of an ankle strap to a strain gauge that was secured to the frame of the bed. Immediately before the start of the study, the subject performed three maximal voluntary contractions (MVC) with each leg to determine the maximal isometric force of the knee extensors. There was little difference in MVC between legs. The force generated was displayed on an oscilloscope and recorded on ultraviolet-sensitive paper using a Medelec System (Medelec, Old Woking, Surrey, UK). The control leg was then prepared for electrical stimulation as described previously (23). The control and test legs were chosen at random. Briefly, the anterolateral region of the thigh muscle was stimulated to contract using square wave electrical impulses of 0.5 ms duration at a frequency of 50 Hz. The muscles were intermittently stimulated, resulting in 1.6 s of contraction and 1.6 s of rest for 64 s (total contraction time 32 s). Muscle biopsies were obtained from the vastus lateralis muscle of the control leg (1) at rest before stimulation.
and at the end of the stimulation period. The second leg was then prepared for stimulation, and muscle biopsies were obtained as in the control leg, with the exception that no resting biopsy was taken. Approximately 50–60 min separated the stimulation of the two legs. Three minutes before the stimulation of the second leg, a catheter was inserted into a peripheral arm vein, and Epi was infused via an infusion pump (IVAC 630 volume pump). Subjects received 2 ml/min of saline solution containing 5 µg Epi/ml, corresponding to 0.14 µg Epi·kg body wt⁻¹·min⁻¹, and infusion was continued throughout the experiment. The same dose rate of Epi has been shown previously to result in the near complete transformation of phosphorylase to the a form (9). Muscle samples were immediately frozen by immersion in liquid freon maintained at its melting point (-150°C) by liquid nitrogen. The time delay between insertion of the biopsy needle and freezing of the sample ranged from 3 to 5 s. The samples were freeze-dried and stored at -80°C until analyzed at a later date.

**Analytical methods.** The freeze-dried muscle samples were divided into two parts. One portion was dissected free from blood and all visible connective tissue and was pulverized. The powdered muscle was used for the determination of mixed-fiber muscle metabolites (21). The calculation of mixed-fiber muscle glycogenolysis from the accumulation of lactate and glucose 6-phosphate (G-6-P) was achieved using the equation; glycogenolysis = \((\Delta\text{lactate}/2) + \Delta\text{G-6-P}\). The second portion of muscle was placed under a dissection microscope (magnification x10), and fragments of single muscle fibers were dissected free. The ends of individual fragments were then cut off and stained for myofibrillar adenosinetriphosphatase, for the identification of type I (slow-twitch) or type II (fast-twitch) fibers using a modification of the method of Brooke and Kaiser (4). In total, 30 fiber fragments were dissected free from each biopsy sample. After the removal of two pieces for fiber characterization, each fragment was weighed on a quartz-fiber fishpole balance that had been previously calibrated by spectrophotometrically determining the weight of p-nitrophenol crystals that had also been weighed on the balance. Repeated weighing over a 24-h period demonstrated that single fibers do not measurably change in weight when exposed to the atmosphere. After fiber characterization, 8–10 fiber fragments of each type were pooled. The weights of the pooled fibers averaged 25 µg. Glycogen was then extracted from types I and II fibers by adding 20 µl KOH (1 mol/l). Samples were agitated on a Vortex mixer and was assayed for glucose using a fluorometric modification of the method by Harris et al. (21).

The remaining fibers (5–10) were used for the lumi-nometric determination of ATP and PCr using a modification of the method of Lundin et al. (26). Fibers were pooled and extracted in 200 µl trichloroacetic acid (2.5%, wt/vol) and neutralized with 20 µl of KHCO₃ (2.2 mol/l). The determinations of ATP and PCr were based upon the quantitative measurement of light produced as a result of two coupled reactions

\[
\text{ADP + PCr} \rightleftharpoons \text{ATP + creatine} \quad (1)
\]

\[
\text{ATP} + \text{luciferin} + \text{O}_2 \overset{\text{FL}}{\rightleftharpoons} \text{AMP} + \text{pyrophosphate} + \text{oxyluciferin} + \text{CO}_2 + \text{light} \quad (2)
\]

where CK is creatine kinase and FL is firefly luciferase. Before the assay was initiated, 925 µl of firefly luciferase reagent (Pharmacia; Turku, Finland) was prepared and left at room temperature (25°C) for 10 min. The reaction was started by adding 50 µl of muscle extract. The light emission was recorded, and the ATP concentration was calculated from the change in light emission of an internal ATP standard. PCr was measured by adding 15 µl of ADP and 10 µl CK. The coefficient of variation was 3% for ATP and 1% for PCr.

**Statistical methods.** For statistical evaluation, a one-way analysis of variance (ANOVA) with repeated measures was employed. When the ANOVA resulted in a significant F value \((P < 0.05)\), the difference between means was located with the Newman-Keul test. Values in the text and figures 1 and 2 refer to means ± SE.

**RESULTS**

**Muscle contraction force.** At the onset of electrical stimulation, muscle contraction force was 195.4 ± 18.3 N in the control leg. It decreased successively during the whole stimulation period and was only 59% of the initial value at the end of the stimulation (Fig. 1). Similar results were obtained in the Epi leg, which demonstrated no effect of Epi infusion on muscle contraction force.

**ATP and PCr changes.** At rest, ATP and PCr concentrations were not different when comparing type I and type II fibers (Table 1). During electrical stimulation, ATP and PCr concentrations decreased in both fiber types, and the magnitude of decline was not different when comparing fiber types \((P > 0.05)\). At the end of the stimulation period the mean muscle PCr concentration was 18.6 and 15.2 mmol/kg dry muscle in the types I and II fibers, respectively, and was totally depleted in the type II fibers of two subjects. Epi infusion had no influ-

**Fig. 1.** Changes (%) in maximal force production during 20 contractions (1.6 s stimulation at 50 Hz, 1.6 s rest) with and without epinephrine (Epi) infusion. Values are means ± SE [no. of subjects (n) = 5]. No difference was seen when comparing treatments.
GLYCOGENOLYSIS IN SINGLE MUSCLE FIBERS

TABLE 1. ATP and phosphocreatine concentrations in type I and type II skeletal muscle fibers

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Rest</th>
<th>Control</th>
<th>Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>24.1</td>
<td>21.6</td>
<td>22.2</td>
</tr>
<tr>
<td>(23.4-25.6)</td>
<td>(20.3-25.6)</td>
<td>(21.6-22.2)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>25.5</td>
<td>21.5</td>
<td>22.8</td>
</tr>
<tr>
<td>(22.4-28.2)</td>
<td>(17.4-24.2)</td>
<td>(18.0-27.0)</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>67.7</td>
<td>18.6</td>
<td>15.2</td>
</tr>
<tr>
<td>(63.6-71.8)</td>
<td>(10.0-30.2)</td>
<td>(11.4-18.9)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>71.2</td>
<td>13.2</td>
<td>9.0</td>
</tr>
<tr>
<td>(62.4-84.2)</td>
<td>(2.2-24.0)</td>
<td>(1.6-16.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Ranges are in parentheses. Concentrations are expressed as mmol/kg dry muscle and were measured before (rest) and after electrical stimulation for 20 contractions (1.6 s stimulation and 1.6 s rest at a frequency of 50 Hz) without (control) and with Epi infusion. PCr, phosphocreatine.

Values are means ± SE for 5 subjects. Concentrations are expressed as mmol/kg dry muscle and were obtained before (rest) and after electrical stimulation for 20 contractions (1.6 s stimulation at a frequency of 50 Hz, 1.6 s rest) without (control) and with Epi infusion. PCr, phosphocreatine; G-6-P, glucose 6-phosphate. * Significant difference (P < 0.05) between poststimulation control and Epi values.

Mixed-fiber muscle metabolites. During electrical stimulation in the control leg, the muscle G-6-P concentration increased from 2.1 ± 0.2 to 14.8 ± 1.9 mmol/kg dry muscle and the muscle lactate concentration from 2.7 ± 0.5 to 58.9 ± 3.8 mmol/kg dry muscle. Whole muscle glycogen concentration decreased from 428 ± 40 to 384 ± 31 mmol glucose/kg dry muscle (Table 2). In the Epi leg, the increase in muscle lactate concentration was similar to that of the control leg, although the increase in G-6-P was greater (P < 0.05). The decrease in muscle glycogen concentration was greater in the Epi leg compared with the control leg (P < 0.05).

Glycogen utilization in type I and type II fibers. Muscle glycogen concentration at rest was higher in type II fibers (445 ± 47 mmol glucose/kg dry muscle) when compared with type I fibers (390 ± 47 mmol glucose/kg dry muscle, P < 0.05). Individual glycogen degradation patterns for each subject during electrical stimulation are shown in Figs. 2 and 3. In the control leg, exercise had a major influence on the rate of glycogenolysis in type II fibers (3.54 ± 0.53 mmol glucose·kg dry muscle⁻¹·s⁻¹). However, very little glycogenolysis occurred in type I fibers (0.18 ± 0.14 mmol glucose·kg dry muscle⁻¹·s⁻¹). The rate of glycogenolysis was twenty times higher in type II fibers when compared with type I fibers (Fig. 2). Epi infusion did not increase the rate of glycogenolysis during exercise in type II fibers above that found in the control leg (3.71 ± 0.62 mmol glucose·kg dry muscle⁻¹·s⁻¹) but did enhance glycogenolysis in type I fibers (1.08 ± 0.33 mmol glucose·kg dry muscle⁻¹·s⁻¹). The rate of glycogenolysis after Epi infusion was only three and one-half times higher in type II when compared with type I fibers (Fig. 3).

Mixed-fiber muscle glycogenolysis in the control and Epi legs was equal to 44 ± 20 and 68 ± 52 mmol glucose/kg dry muscle, respectively. This corresponds well with the average glycogenolysis in single fibers of 59 ± 27 and 77 ± 25 mmol glucose/kg dry muscle, assuming each biopsy is composed of 50% type I and 50% type II fibers. If one assumes each biopsy is composed of 60% type I and 40% type II fibers, the average glycogen decrease was 49 ± 22 and 68 ± 18 mmol glucose/kg dry muscle in the control and test legs, respectively. Mixed-fiber muscle glycogenolysis calculated from lactate and G-6-P concentrations was 43 ± 3 and 50 ± 2 mmol/kg dry muscle in the control and Epi legs, respectively. The failure of mixed-fiber muscle lactate and G-6-P concentrations to totally account for the decrease in glycogen concentration has been reported previously in mixed-
fiber muscle (12, 25) and single-fiber (22) experiments, and, in the present study, is at least partly due to the diffusion of lactate into the circulatory system and unstimulated muscle.

**DISCUSSION**

A major finding of the present experiment is that glycolgenolysis measured in vivo in human skeletal muscle during intense short-term contraction is nearly totally restricted to type II muscle fibers (Fig. 2). The glycolgenolytic rate of 3.54 ± 0.53 mmol glucose·kg dry muscle−1·s−1 in type II fibers of the present study is to our knowledge the highest recorded in human skeletal muscle. Previous histochemical studies on humans involving dynamic exercise have shown that, during repeated bouts of high-intensity exercise [100–150% maximal O2 uptake (VO2max)] of up to 1 min duration, glycolgenolysis is not restricted to one particular fiber type (15, 18). However, more recent histochemical evidence from the analysis of biopsy samples obtained after repeated bouts of short-term (60 × 8 s) maximal exercise (~200% VO2max) suggests that there is a differential pattern of glycolgenolysis between fiber types during this type of exercise (17). Due to the semiquantitative nature of histochemical analysis, the glycolgenolytic response of type I and type II muscle fibers to short-term maximal exercise has not been clearly established. The results of the present experiment clearly demonstrate that, during intense electrical stimulation in humans, glycolgenolysis is almost totally restricted to type II muscle fibers. This is in accordance with the results from animal studies that showed differential biochemical and physiological responses of fast- and slow-twitch muscles (6, 11) and single fibers (22) to electrical stimulation. As indicated in Fig. 4, due to the marked interfiber difference in glycolgenolysis, it is clear that previous estimates of the maximal rate of ATP turnover and glycolgenolysis in humans based upon the analysis of mixed-fiber muscle samples obtained after intense exercise do not accurately reflect the energy metabolism occurring in individual fiber types. The glycolgenolytic rate of 3.54 ± 0.53 mmol glucose·kg−1·s−1 observed in type II fibers of the present study is greater than the value of 1.37 ± 0.64 mmol glucose·kg−1·s−1 seen in mixed-fiber muscle from the same biopsy sample and is also greater than the value of ~2.3 mmol glucose·kg−1·s−1 measured in mixed-fiber muscle after 30 s of maximal dynamic exercise in humans (2, 3, 10). Furthermore, the glycolgenolytic rate observed in type II fibers during the present experiment is in excess of the maximal velocity of phosphorylase for glycogen (i.e., 1.9–3.4 mmol glucose·kg−1·s−1) measured in whole muscle by Chasiotis (7) but is in good agreement with the range of 2.1–4.0 mmol glucose·kg−1·s−1 calculated for type II fibers in human skeletal muscle by Harris et al. (20).

Most studies to date investigating force development in skeletal muscle have focused on the analysis of whole slow- or fast-twitch muscles from animal preparations (11). Little is known regarding the force-generating capacity and fatiguability of human skeletal muscle fibers during maximal exercise. Faulkner et al. (16) presented evidence showing that the ability to sustain a high-power output is very much dependent upon the contribution made by fast-twitch fibers to force production. Using bundles of human skeletal muscle fibers, the authors demonstrated that the peak isometric power output of fast-twitch fibers is approximately fourfold that of slow-twitch fibers. If one estimates that, during the present study, the hexose phosphate content of type II fibers at the end of contraction was ~20 mmol/kg dry muscle, it can be calculated that the total anaerobic ATP production of these fibers during exercise was ~306 mmol/kg dry muscle (~56 mmol/kg dry muscle from PCr degradation, ~280 mmol/kg dry muscle from anaerobic glycolysis). Assuming that the mechanical efficiency of type I fibers is similar to that of type II fibers, based on the conclusions of Faulkner et al. (16), it can be calculated that the total ATP production of type I fibers during the present experiment was ~84 mmol/kg dry muscle, of which ~49 mmol/kg dry muscle was produced by PCr degradation. Assuming that the remainder of the type I fiber ATP production is achieved by the oxidation of glucosyl units, it can be calculated that the glycogen concentration of type I fibers will decline by <2 mmol/kg dry muscle during stimulation. This value corresponds well with the observed decline in type I fibers of 5.6 ± 4.3 mmol/kg dry muscle.

In contrast to slow-twitch fibers, fast-twitch fibers are known to demonstrate a rapid decline in power output during intense electrical stimulation (14). A similar rapid decline in force has been observed in the quadriceps muscle group of humans during prolonged electrical stimulation (24). In association with the decline in force was a marked decline in whole muscle lactate accumulation and glycolgenolysis. The authors concluded that these concomitant changes could be explained by fatigue development in type II fibers alone. The initial high rate of glycolgenolysis in type II fibers (Fig. 4) and the rapid decline in whole muscle force production (Fig. 1) during the present experiment support this suggestion. Clearly, the negligible rate of glycolgenolysis in type I fibers suggests that the contribution of these fibers to whole muscle force production is of a low enough magnitude.
for energy demand to be met by PCr degradation and aerobic metabolism alone. The low force generating capacity and fatigue-resistant nature of these fibers may also explain the previous observations that whole muscle force production rarely decreases below ~20% of maximal force (24). Few studies have considered the possibility that fatigue during maximal exercise in humans may be restricted to type II muscle fibers. The high rate of glycogenolysis and the rapid decrease in power output observed during maximal sprint exercise in humans (10) suggest that this phenomenon may also be implicated with fatigue during maximal dynamic exercise.

Figure 4 demonstrates that Epi infusion had no effect on the rate of glycogenolysis in type II fibers during the present experiment. The rate of type II fiber glycogenolysis without and with Epi infusion was equal to 3.54 + 0.53 and 3.71 ± 0.62 mmol glucose·kg⁻¹·s⁻¹, respectively, and therefore suggests that the glycogenolytic process in type II fibers is activated maximally during this type of contraction. This suggestion is in agreement with the data of Harris et al. (20), which demonstrated a maximal activity of phosphorylase in type II fibers ranging from 2.1-4.0 mmol glucose·kg⁻¹·s⁻¹. Figure 4 indicates that the increased rate of glycogenolysis seen in human mixed-fiber muscle during exercise after Epi infusion (7, 29) may be due solely to an increased rate of glycogenolysis in type I fibers. This is in agreement with Richter et al. (30) who demonstrated that Epi infusion enhanced glycogenolysis in rat soleus muscle during electrical stimulation but had no effect on gastrocnemius muscle. The higher than normal G-6-P content observed in mixed-fiber muscle after Epi infusion in the present study (Table 2) is in agreement with earlier studies (7) and is also in accordance with dynamic exercise studies that show a marked decline in whole muscle hexose phosphate levels after β-blockade (19).

In rat skeletal muscle, ATP, PCr, and glycogen concentrations have been reported to be markedly higher at rest in type II fibers compared with type I fibers (22, 27). Although this was found to be true for muscle glycogen concentrations, resting levels of ATP and PCr appeared to be only marginally higher when comparing fiber types in the present experiment. This is in agreement with previously published data on human skeletal muscle (28). In contrast with previously published data (29) is the finding that the decline in PCr and ATP concentrations in type II fibers during exercise was no different from that of type I fibers. However, this finding may have arisen because of an insufficient number of preexercise PCr and ATP determinations (Table 1).

It is generally considered that glycogen availability will not limit performance during high-intensity maximal exercise, since mixed-muscle values are still high at the point of fatigue. However, the results of the present experiment indicate that, during repeated bouts of maximal exercise, glycogen availability in type II fibers may limit the rate of ATP resynthesis.

Finally, depending upon the buffering capacity of type II fibers, the high rate of glycogenolysis in type II fibers could also result, at least initially, in a more pronounced accumulation of hydrogen ions (H⁺) in these fibers than has been previously observed in whole muscle (31). Such a rapid accumulation of II⁺ could have a suppressive effect on energy generation in type II fibers and may at least partly explain the observed decline in whole muscle force production during intense muscle contraction (24).

In conclusion, the results of the present study indicate that, although both type I and type II skeletal muscle fibers are recruited during short-term maximal exercise, glycogenolysis is predominantly restricted to type II fibers. Furthermore, the increased rate of glycogenolysis during exercise after Epi infusion appears to be restricted to type I fibers. The implications of these findings to the development of fatigue in humans during maximal exercise needs to be investigated further.

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