Progressive decrease of intramyocellular accumulation of H\(^+\) and Pi in human skeletal muscle during repeated isotonic exercise

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Rico-Sanz, J. Progressive decrease of intramyocellular accumulation of H\(^+\) and Pi in human skeletal muscle during repeated isotonic exercise. Am J Physiol Cell Physiol 284: C1490–C1496, 2003. First published February 5, 2003; 10.1152/ajpcell.00419.2002.—The purpose of this study was to evaluate the hypotheses that accumulation of hydrogen ions and/or inorganic phosphate (Pi) in skeletal muscle increases with repeated bouts of isotonic exercise. \(^{31}\)P-Magnetic resonance spectroscopy was used to examine the gastrocnemius muscle of seven highly aerobically trained females during four bouts of isotonic plantar flexion. The exercise bouts (EX1–4) of 3 min and 18 s were separated by 3 min and 54 s of complete rest. Muscle ATP did not change during the four bouts. Phosphocreatine (PCr) degradation during EX1 (13.3 ± 2.4 mmol/kg wet weight) was higher (P < 0.01) compared with EX3–4 (9.7 ± 1.6 and 9.6 ± 1.8 mmol/kg wet weight, respectively). The intramyocellular pH at the end of EX1 (6.87 ± 0.05) was significantly lower (P < 0.001) than those of EX2 (6.97 ± 0.02), EX3 (7.02 ± 0.01), and EX4 (7.02 ± 0.02). Total Pi and diprotonated Pi were significantly higher (P < 0.001) at the end of EX1 (17.3 ± 2.7 and 7.8 ± 1.6 mmol/kg wet weight, respectively) compared with the values at the end of EX3 and EX4. The monoprotonated Pi at the end of EX1 (9.5 ± 1.2 mmol/kg wet weight) was also significantly higher (P < 0.001) than that after EX4 (7.5 ± 1.1 mmol/kg wet weight). Subjects’ rating of perceived exertion increased (P < 0.001) toward exhaustion as the number of exercises progressed (7.1 ± 0.4, EX1; 8.0 ± 0.3, EX2; 8.5 ± 0.3, EX3; and 9.0 ± 0.4, EX4; scale from 0 to 10). The present results indicate that human muscle fatigue during repeated intense isotonic exercise is not due to progressive depletion of high energy phosphates nor to intracellular accumulation of hydrogen ions, total, mono-, or diprotonated Pi.

MUSCULAR CONTRACTION sustained for a period of time eventually leads to muscle fatigue or failure to maintain the required power output; that is, the inability to produce force at a determined velocity. Among the possible contributory factors associated with fatigue are 1) depletion of the muscular energy deposits and 2) accumulation of their product metabolites, which might negatively affect excitation-contraction-relaxation processes (15, 44, 45). At the onset of muscle contraction, phosphocreatine (PCr) breakdown is the primary energy source to resynthesize ATP, the direct energy donor for contraction (4, 7). Glycogen is also broken down to provide ATP to the contracting muscle fibers (18, 21). Intramyocellular accumulation of inorganic phosphate (Pi), lactate, and H\(^+\) ions occurs as a consequence of the elevated degradation rate of PCr and glycogen during high-intensity muscle contraction (13, 32, 46). Thus, as intense muscle contraction continues, a reduction in intramyocellular PCr and glycogen levels and accumulation of Pi and H\(^+\) take place, events that have been associated with fatigue (15, 44, 45).

As the supply of oxygen and substrates from blood augments during prolonged exercise, the relative contribution to the total energy demand from these anaerobic energy sources decreases, whereas mitochondrial oxidative phosphorylation from carbohydrate and fat metabolism increases (5, 34). It is thus possible that during intense aerobic muscle contractions, the contribution of Pi and H\(^+\) ion accumulations might play a lesser role on fatigue. The total work performed during one prolonged bout of muscular contractions until the point of fatigue can be partitioned into repeated bouts of contractions at higher intensity separated by periods of rest (14, 23, 37). In the present experiment, \(^{31}\)P-magnetic resonance spectroscopy (\(^{31}\)P-MRS) was used to evaluate the dynamic changes in muscle high-energy phosphates, Pi, and pH during four bouts of intense isotonic exercise. The number of contractions was maintained identical in all bouts while subjects progressively became fatigued, as they were unable to complete a fifth exercise bout. This protocol permits evaluation of the metabolic changes in muscle without confounding factors such as lower number of contractions per bout, decreased duration of exercise bouts, or the intensity of the previous exercise bout. It was hypothesized that there would be a progressive degradation of high-energy phosphates and a progressive accumulation of Pi and H\(^+\) with the number of bouts. On the contrary, the results of the experiment showed that muscle fatigue during repeated bouts of isotonic contractions can be dissociated from the progressive degradation of high-energy phosphates and intracellular accumulations of total, mono-, and diprotonated Pi and H\(^+\) ions.
METHODS

Subjects. Seven healthy, highly trained females (five highly competitive soccer players and two aerobically trained athletes) volunteered to participate in this study. The study was approved by the Local Ethics Committee. Informed written consent was obtained from all subjects after receiving a detailed explanation of the procedures and the risks and discomforts of the experiment.

Procedures. Subjects came to the laboratory on at least three occasions to become familiarized with the equipment setup (30) and the exercise protocol. Subjects assumed a sitting position, and the pedal and chair were well secured with straps to prevent any movement during the exercise protocol. Recruitment from other muscles was eliminated by fixing the knee joint in a semieverted position. A computer program was used to trigger a light every 2 s, which prompted the subjects to start the contraction. Psychological factors were avoided by having subjects trained to focus on the light turning on, and the experimenter made sure there was 100% compliance with the cycle “light on-contraction.” No other visual or verbal clues were allowed during the entire exercise protocol. Each bout consisted of 99 plantar flexions at the same load and was completed in 3 min and 18 s. The subjects performed the test with exquisite concentration to complete exactly 99 contractions in each exercise period. The repeated exercise protocol was intended to have subjects complete four identical bouts of intense plantar flexion at a rate of 0.5 Hz with contraction duration of ∼0.5 s and displacement of the pedal of 3 cm. The load in subsequent visits was increased or decreased depending on whether subjects were able or not to complete the four exercise bouts, which were separated by 3 min and 54 s of complete rest. Once the maximal load they were able to sustain for four bouts was identified, they were asked to come to the laboratory for the final experimental visit. The magnetic resonance data acquisition was realized just before each contraction.

31P-MRS measurements. A two-turn inductively driven surface RF coil, 39 mm in diameter, was located under the Otsuka VivoSpec spectrometer. For spectra acquisition, a signal from muscle water and signal were multiplied by 5 Hz exponential line broadening.

RESULTS

Figure 1, A and B, shows a stack and contour plot, respectively, of a representative nuclear magnetic resonance (NMR) spectrum from one of the subjects. Whereas muscle ATP remained the same during the four exercise bouts, the net amount of PCr degraded in EX1 (13.3 ± 2.4 mmol/kg wet weight) was larger (P < 0.01) compared with EX3 (9.7 ± 1.6 mmol/kg wet weight) and EX4 (9.6 ± 1.8 mmol/kg wet weight) (Fig. 2). The PCr/ATP ratio at the end of EX3 (2.02 ± 0.30) and EX4 (2.97 ± 0.52) was also larger (P < 0.01) than that at the end of EX1 (1.47 ± 0.30). After exercise, the half time of PCr recovery was slightly longer after EX1 (29.6 ± 3.6) compared with those of EX2–EX4 (19.5 ± 1.2, 17.5 ± 1.9 and 23.0 ± 2.6 s, respectively), but the difference was not statistically significant. The PCr/ATP ratio at the end of the recovery from the four exercise bouts was not different from the resting PCr/ATP ratio (3.95 ± 0.36).

Figure 3 shows the changes in muscle pH during the four exercise bouts. At around 50 s and throughout the exercise, muscle pH was higher in EX2–4 compared with EX1. The end-exercise muscle pH of EX1 (6.87 ± 0.05) was significantly lower (P < 0.001) than end-exercise pH of EX2 (6.97 ± 0.02), EX3 (7.02 ± 0.01), and EX4 (7.02 ± 0.02). The total Pi at the end of EX1 (17.3 ± 2.7 mmol/kg wet weight) was significantly larger (P < 0.001) compared with the values at the end of EX3 and EX4 (12.3 ± 1.7 and 11.7 ± 1.8 mmol/kg wet weight, respectively) (Fig. 4). The diprotonated Pi concentration was also larger (P < 0.001) at the end of EX1 (7.8 ± 1.6 mmol/kg wet weight) compared with those of EX3 and EX4 (4.4 ± 0.7 and 4.2 ± 0.7 mmol/kg wet weight, respectively) (Fig. 5). The monoprotonated Pi at the end of EX4 (7.5 ± 1.1 mmol/kg wet weight) was significantly lower (P < 0.01) compared with that of EX1 (9.5 ± 1.2 mmol/kg wet weight) (Fig. 6). The rating of perceived exertion increased (P < 0.001) progressively from 7.1 ± 0.4 after EX1 to 7.9 ± 0.3 (EX2), 8.5 ± 0.3 (EX3), and 9.0 ± 0.4 (EX4) in a scale from 0 to 10, reflecting signs of exhaustion and 10 reflecting when subjects could not continue. Subjects were unable to complete a fifth bout.

DISCUSSION

The main purpose of this experiment was to examine the hypothesis that intramyocellular accumulation of metabolites of the anaerobic energy delivery pathways is associated with fatigue during repeated bouts of

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intense isotonic exercise in humans. The data showed that the accumulation of intracellular total, mono-, and diprotonated Pi and H⁺ decreased with repeated bouts of intense contraction.

Clearly, the muscle pH results do not support a role for intramyocellular H⁺ accumulation as the cause of fatigue during repeated intense exercise. Previously, it had been proposed that accumulation of H⁺ is associated with fatigue due to decreased Ca²⁺ sensitivity of force activation, peak force, and maximum velocity of shortening during maximal Ca²⁺ activation and prolonged sarcoplasmic reticulum Ca²⁺ reuptake and relaxation time (6, 9, 10, 25, 40). Furthermore, Metzger and Moss (25) suggested that accumulation of H⁺ causes decrements in the number of crossbridges in fast-twitch (FT) muscle fibers and reduces the force per crossbridge in both slow- and FT muscle fibers, as differential causes of fatigue in each fiber type. Taking into consideration that during submaximal exercise there is an orderly recruitment of slow-twitch (ST) and FT oxidative-glycolytic (FTa) and FT glycolytic (FTb) as contraction time progresses (12, 18, 42), it is un...
The accumulation of H⁺ was the cause of progressive fatigue in the present study, because during EX4, the accumulation of H⁺ was basically insignificant and similar to the resting H⁺ values. Also, during the first bout when the intramyocellular pH decreased 0.2 units, subjects exercised for an additional 2 min (total of 60 more contractions) without decreasing the rate of isotonic contractions. The present findings agree with some reports dissociating H⁺ from muscle fatigue in animals and humans (1, 3, 11, 13).

The Pi data of the present study also indicated that total Pi is not associated with fatigue during repeated, intense, isotonic exercise. It had been suggested that accumulation of total Pi during intense muscle contraction depresses force by reducing the crossbridge transition from the low- to the high-force state and slowing Ca²⁺ reuptake into the sarcoplasmic reticulum (20, 27, 44, 45). It has been proposed that intramyocellular concentrations of Pi between 15 and 30 mmol cause force decline in frog semitendinosus, skinned rabbit psoas fibers, and human muscles (13, 20, 27, 39, 43, 44).
The depressive effect of total Pi on force production appeared larger in FT muscle fibers than in ST muscle fibers already at a Pi concentration of 15 mmol (39). However, in the present study, muscle total Pi during \textit{EX1} was above 15 mmol/kg wet weight for over 2 min while subjects maintained the rate of contraction remarkably, and Pi concentration decreased with the number of bouts when the FT fibers are progressively recruited. Therefore, it is unlikely that Pi is the cause of fatigue because total Pi accumulation decreased progressively with the number of bouts.

Increments in H\(^+\) concentration augment the diprotonated levels of Pi, which might independently cause skeletal muscle force depression. Wilson et al. (46) indicated that fatigue in humans correlates with diprotonated Pi and pH during maximal contractions, but when maximal exercise is preceded by submaximal exercise, diprotonated Pi rather than H\(^+\) was believed to be the primary metabolic factor responsible for muscular fatigue. In their study, 4 min of maximal wrist flexion contractions caused a ninefold increase in diprotonated Pi and a 25\% reduction in force. However, an ~eightfold increase in diprotonated Pi during \textit{EX1} did not cause a drop in force generation in the present study. Nosek et al. (28) suggested that perhaps total and monoprotonated Pi cause fatigue in ST muscle fibers and total and diprotonated Pi in FT muscle fibers. However, in the present study, coinciding with progressive recruitment of FT fibers (12, 18, 42), the accumulation of total, mono-, and diprotonated Pi was progressively lower during repeated bouts. If the accumulation of total, mono-, and diprotonated Pi and H\(^+\) is a major cause of human muscle fatigue, a progressive rise in their concentration should have been observed with the number of exercise bouts as progressive increasing rates of perceived exertion were reported. The present findings agree with those of Adams et al. (1) in cat muscles, which indicated that force during repetitive contractions is not directly due to pH or diprotonated phosphate, and those of Cieslar and Dobson (11) in rat gastrocnemius, which showed that force decline is not due to increased pH and/or diprotonated Pi.

Rather than being a direct cause of muscle fatigue, the elevation of intramyocellular total Pi, diprotonated Pi, and H\(^+\) concentrations during exercise might be metabolic consequences of the duration and intensity of the contraction, metabolism of the type of recruited muscle fibers, oxidative capacity of the fibers, and oxygen and energy substrate supply to the active fibers. Indeed, PCr and glycogen breakdown in human FT muscle fibers is higher than in ST fibers (8, 19), and their net breakdown is higher as the exercise intensity increases (21, 22, 36). High-intensity exercise provokes the accumulation of intracellular H\(^+\) and Pi in human muscle to levels above those during moderate-intensity exercise (22). It has also been shown that the intracellular Pi accumulation during muscle contraction is higher in FT compared with ST fibers (1). Moreover, when the duration of maximal contractions increase from 1 to 2 s, the increments of diprotonated Pi and H\(^+\) in human muscle are larger (46). Also, the FT fibers have lower oxidative potential than the ST fibers, as demonstrated by their lower density of capillaries, mitochondria, and enzymes of the oxidative energy pathways (35). Furthermore, Pi accumulation in human muscle is lower in a highly trained state (24). Many of the results associating hydrogen ion and Pi accumulation with fatigue have been obtained in animal muscle with the skinned fiber model, which does not provide circulating sources of energy to muscle (20, 26, 27, 39, 40). In addition, the experimental protocols in human experiments employed progressive intensity to fatigue, maximal contractions, or maximal contractions preceded by submaximal contractions that naturally increase the H\(^+\) and Pi accumulation in muscle as exercise intensity is elevated (13, 25, 42, 45).

In evaluating the entire data set of the present repeated exercise protocol, a change from relatively more anaerobic metabolism to aerobic metabolism presumably occurred from \textit{EX1} to \textit{EX2} and less progressively from \textit{EX2} to \textit{EX4}. The pH changes during the exercise periods indicate a larger glycolysis to lactate and hydrogen ion production in \textit{EX1} compared with \textit{EX2-4}. Evidence for decreased anaerobic glycolysis in \textit{EX2-4} compared with \textit{EX1} in the present study is further supported by the reduced accumulation of the phosphomonoesters resonance (see Fig. 1) and the higher pH. Decrements in anaerobic glycolysis in repeated exercise protocols have also been previously reported (5, 23, 31, 36). It is doubtful that accumulation of glycolytic intermediates and H\(^+\) is the cause of the reduction of anaerobic glycolysis in subsequent exercise bouts because PME and pH were similar to resting levels at the initiation of each repeated contraction. Because ATP was maintained the same throughout all the exercise bouts and the net PCr utilized during \textit{EX3-4} was lower than that used during \textit{EX1}, the data strongly suggest a net decrement in anaerobic energy production after the first exercise bout.

Changes in blood flow, oxygen uptake, and oxidative phosphorylation may account for the alterations in energy metabolism during repeated exercise bouts. The glycogen breakdown rate is significantly enhanced in ST fibers when circulation is occluded but not in FT fibers in which the rate is kept to about the same maximal level as when the circulation is intact (19). It is likely that oxidative phosphorylation was not fully optimized during \textit{EX1}. The blood flow in vastus lateralis after 3 and 10 min of passive recovery from intense exercise of similar duration as in this study was higher compared with resting blood flow (3). Whole body oxygen uptake is also enhanced during repeated bouts of intense exercise (5, 32). In addition, adipose tissue lipolysis is increased during a repeated bout of aerobic exercise (38). Thus, as a consequence of the increased blood flow and the delivery of oxygen and circulating substrates, it is expected that oxidative phosphorylation plays a more significant role than anaerobic metabolism during the repeated exercise bouts.
In conclusion, the results of this study show that repeated isotonic exercise does not cause a progressive accumulation of intramyocellular total, mono-, and diprotonated Pi and hydrogen ions nor depletion of high-energy phosphates in humans.

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REFERENCES


