Skeletal muscle energy metabolism during prolonged, fatiguing exercise

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Febbraio, Mark A., and Jane Dancey. Skeletal muscle energy metabolism during prolonged, fatiguing exercise. J. Appl. Physiol. 87(6): 2341–2347, 1999.—A depletion of phosphocreatine (PCr), fall in the total adenine nucleotide pool (TAN = ATP + ADP + AMP), and increase in TAN degradation products inosine 5′-monophosphate (IMP) and hypoxanthine are observed at fatigue during prolonged exercise at 70% maximal O2 uptake in untrained subjects (J. Baldwin, R. J.; Snow, M. F. Carey, and M. A. Febbraio. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R295–R300, 1999). The present study aimed to examine whether these metabolic changes are also prevalent when exercise is performed below the blood lactate threshold (LT). Six healthy, untrained humans exercised on a cycle ergometer to voluntary exhaustion at an intensity equivalent to 93 ± 3% of LT (−65% peak O2 uptake). Muscle biopsy samples were obtained at rest, at 10 min of exercise, ~40 min before fatigue (F = 143 ± 13 min), and at fatigue (F = 186 ± 31 min). Glycogen concentration progressively declined (P < 0.01) to very low levels at fatigue (28 ± 6 mmol glucosyl U/kg dry wt). Despite this, PCr content was not different when F = 40 was compared with F and was only reduced by 40% when F was compared with rest (52.8 ± 3.7 vs. 87.8 ± 2.0 mmol/kg dry wt; P < 0.01). In addition, TAN concentration was not reduced, IMP did not increase significantly throughout exercise, and hypoxanthine was not detected in any muscle samples. A significant correlation (r = 0.95; P < 0.05) was observed between exercise time and glycogen use, indicating that glycogen availability is a limiting factor during prolonged exercise below LT. However, because TAN was not reduced, PCr was not depleted, and no correlation was observed between glycogen content and IMP when glycogen stores were compromised, fatigue may be related to processes other than those involved in muscle high-energy phosphagen metabolism.

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between glycogen availability and muscle energy metabolism during fatiguing steady-state exercise, requires an ATP turnover rate that cannot be sufficiently met by oxidative metabolism in untrained individuals. The increase in PCR degradation and accumulation of IMP observed in the presence of low glycogen concentration may, therefore, be unrelated to glycogen availability but may be due to increased energy provision from the CPK and AK reactions throughout exercise. Thus the purpose of the present study was to examine muscle energy metabolism in untrained subjects throughout prolonged, fatiguing exercise at a workload where the ATP demand was met via oxidative sources. We hypothesized that although glycogen would be depleted at fatigue, there would be little, if any, disruption to the intracellular milieu.

METHODS

Subjects. Six healthy but untrained subjects [20.7 ± 2.4 yr; 62.7 ± 8.0 kg; peak O2 uptake (VO2peak) = 2.49 ± 0.5 l/min] volunteered for the experiment. The experimental procedures and possible risks of the study were explained to all subjects before they gave their informed, written consent. The experiment was approved by the Human Research Ethics Committee of The University of Melbourne.

VO2peak and lactate threshold (LT) determination. Each subject initially performed a cycling test to volitional fatigue on an electromagnetically braked cycle ergometer (Lode Instrument, Groningen, The Netherlands) to determine VO2peak and LT. Expired air was directed into Douglas bags via a Hans Rudolf valve and plastic tubing. Oxygen and carbon dioxide content of the Douglas bags were analyzed by using Applied Electrochemistry (Ametek, Pittsburgh, PA) S-3A/1i and CD-3A gas analyzers, calibrated before each test with a commercially prepared gas mixture of known composition. The volumes of expired gases were determined by using a gas meter (Parkinson-Cowan, Manchester, UK). VO2peak was calculated by using standard equations (8). During this test, venous blood samples were also obtained at rest and at the completion of every increment in the workload. Samples of whole blood were immediately mixed in a tube containing lithium heparin. A 125-µl aliquot of whole blood was added to 250 µl perchloric acid and spun in a centrifuge, and the supernatant was frozen and stored for subsequent lactate determination (26). Each subject’s LT was determined according to the methods of Coyle et al. (14). Briefly, the increase in blood lactate was plotted against O2 uptake (VO2). After determination of the lactate steady state during the initial incremental workloads, a value corresponding to 1 mmol/l above this point was taken as the LT. The corresponding VO2 at this point was multiplied by 0.95 to calculate 95% LT. The desired workload was then determined from the VO2 vs. workload regression equation.

Experimental procedure. At least 1 wk after the VO2peak test, subjects returned to the laboratory to perform a familiarization trial. This trial served to familiarize subjects with the cycling protocol and enabled us to check the workload and determine an approximate time to fatigue. Subjects were instructed to refrain from alcohol, caffeine, tobacco, and strenuous exercise and to consume their normal diet for the preceding 24 h. Subjects arrived in the laboratory in the morning after an overnight fast, were weighed, and then commenced cycling on the previously mentioned cycle ergometer at the predetermined workload. Expired gases were collected via Douglas bags during this trial and analyzed at 20-min intervals for VO2 to verify exercise intensity. Heart rate was also monitored during this trial via telemetry (Sports Tester, Polar). An electric fan was used to circulate air, and water was provided ad libitum. Subjects were instructed to cycle at the predetermined work rate, maintaining a pedal frequency of 80-90 rpm until fatigue. Fatigue was defined as the inability to complete one pedal revolution because the work rate on the electrically braked cycle ergometer was non-pedal-frequency dependent. All subjects were given strong verbal encouragement from the investigators to continue cycling.

The experimental trial was conducted at least 7 days after the familiarization trial. The protocol was identical to the familiarization trial but included venous blood and muscle sampling at various stages throughout exercise. In addition to VO2 measurements, pulmonary gases were analyzed for the respiratory exchange ratio during this trial.

Venous blood samples were obtained by using a 20-gauge Teflon catheter (Terumo, Tokyo, Japan) inserted into a vein in the antecubital space. The vein was kept patent by flushing with 0.5 ml sodium chloride-5 U heparin after each sample collection. Muscle samples were obtained from the vastus lateralis by using the percutaneous needle biopsy technique with suction. Briefly, local anesthetide was injected ~10 cm and 13 cm proximal to the lateral epicondyle of the femur of both legs. Four separate incisions (2 in each leg) were then made over the anesthetized areas, and muscle samples were obtained at rest, at 10 min of exercise (10 min), ~40 min before fatigue (F = 40 = 143 ± 14 min), and at fatigue (F = 186 ± 31 min). F = 40 was estimated from the results obtained during the familiarization trial. On sampling, the muscle was rapidly frozen in liquid nitrogen for later metabolite analysis. The time from the cessation of exercise to freezing was ~10 s.

Tissue treatment and analysis. After each blood sample collection, blood was placed in fluoride heparin, mixed, and spun for 3 min at 8,000 rpm. The plasma supernatant was then removed, stored on ice until completion of the trial, and then frozen until later analysis of plasma glucose and lactate by using an automated method (EMI-105, Electrolyte Metabolite Laboratory, Radiometer, Copenhagen, Denmark). A further 1.5 ml of whole blood were placed in tubes containing 30 µl of EGTA/GSH. This tube was placed on ice until the completion of the trial and spun as previously described. The plasma was then frozen for later analysis of free fatty acids by using an enzymatic colorimetric method (Nephe L kit, Wako Pure Chemicals) according to the methods of Miles et al. (27).

Muscle samples were freeze-dried for 24 h, disected free of any blood and connective tissue, powdered, extracted, and analyzed for glycogen, lactate, adenine nucleotides (ATP, ADP, AMP) and their degradation products (IMP and hypoxanthine), PCR, and creatine (Cr) as previously described (17). The concentrations of ATP, ADP, AMP, IMP, PCR, and Cr were adjusted to the peak total PCR + Cr concentration for each subject. This procedure minimized the error in measuring nonmuscle components of the tissue not visible in the sample. Lactate and glucose were not corrected because of their extracellular presence.

Statistical analyses. A one-way ANOVA with repeated measures on the time factor was used to compare blood and muscle metabolite data throughout the trial. A Newman-Keuls post hoc test was used to locate difference when the ANOVA revealed a significant interaction. Correlation coefficients were determined by using Pearson’s product moments. All data are reported as means ± SE unless otherwise stated. The level of significance for all tests was set at P < 0.05.
RESULTS

Subjects cycled for 186 ± 31 min at a workload that corresponded to 93 ± 8% LT. This was equivalent to 1.60 ± 0.1 l/min or ~64% V̇O₂peak. Both muscle and plasma lactate accumulation increased (P < 0.05) in the initial period of exercise, but concentrations returned to resting levels thereafter, indicating that the contribution to energy metabolism via anaerobic glycolysis was minimal (Fig. 1).

Heart rate initially increased (P < 0.05) but reached a plateau after 80 min, whereas V̇O₂ did not alter throughout exercise (data not shown). Although the respiratory exchange ratio progressively fell (P < 0.05) during the first 80 min, it was maintained thereafter (Fig. 2). In addition, plasma glucose concentration did not alter throughout exercise, indicating that circulating glucose availability was not compromised at fatigue (Fig. 2). Plasma free fatty acid concentrations increased (P < 0.05) after 60 min of exercise (Fig. 2).

Muscle glycogen concentration decreased (P < 0.05) progressively throughout exercise, and concentrations were very low (<50 mmol glucosyl U/kg dw) at F (Fig. 2).
However, despite the fact that glycogen content was decreased by 50% in all subjects when F - 40 was compared with F, this decrease was not statistically significant. Muscle PCr was higher (P < 0.05) when rest was compared with 10 min, F - 40, and F. Of note is the fact that, although PCr declined (P < 0.05) as exercise progressed beyond 10 min, it did not decrease when F - 40 was compared with F (Table 1). Conversely, the concentration of intramuscular Cr increased (P < 0.05) throughout exercise and was different from rest at 10 min, F - 40, and F (Table 1). There was no change in muscle ATP, ADP, or AMP concentrations throughout exercise, and, therefore, the TAN pool remained unchanged throughout exercise (Table 1). Although there appeared to be a tendency for IMP to accumulate throughout exercise, this did not reach statistical significance (P > 0.05) (Table 1). Hypoxanthine was not detected in any sample despite an analytic detection limit of between 0.005 and 0.01 mmol/kg dry wt. Furthermore, the change in IMP throughout exercise was not different (Fig. 4), and there was no correlation (r = 0.056, P > 0.05) between IMP and glycogen content at either F - 40 (r = 0.083; P > 0.05) or F (r = 0.73; P > 0.05) (Fig. 4). In contrast, a significant correlation (r = 0.95; P < 0.05) was observed between time to exhaustion and glycogen use (Fig. 5).

**DISCUSSION**

This study is the first to measure muscle energy metabolism throughout exercise in untrained subjects at a workload below the LT, where ATP supply from oxidative metabolism is sufficient to meet the ATP demand. Unlike previous studies conducted in untrained subjects (2, 5, 32, 34), the results from this study suggest that despite compromised intramuscular glycogen stores, fatigue appears to be associated with factors other than those related to muscle high-energy phosphagen metabolism. The relationship between gly-
Previous studies where the workload was normalized to result in fatigue in the present study. In contrast, in which can disrupt contractile processes, could not have for ing that these untrained subjects were able to exercise supply from oxidative metabolism was sufficient in lactate production and removal, they suggest that ATP although these data only reflect a balance between concentration fell to resting levels thereafter (Fig. 1). The onset of exercise, this rise was transient, and concentration increased in both muscle and plasma at equally met by oxidative processes. Although lactate descreased such that the ATP requirement was ad-

phagen metabolism. The workload selected in the present study was 3 h. In addition, the low muscle glycogen levels cogen use and exercise duration (Fig. 5), as well as the very low levels of glycogen within the muscles at fatigue, supports previous studies (2, 3, 9, 12, 13, 25, 32, 34, 38) that suggest that glycogen availability may be a limiting factor during steady-state exercise. However, because TAN was not reduced, PCr degradation did not fall at fatigue, IMP did not significantly accumulate (Table 1), and no correlation was observed between glycogen content and IMP late in exercise (Fig. 4), there was little evidence that this reduced glycogen availability had a major influence on muscle high-energy phosphagen metabolism.

The workload selected in the present study was designed such that the ATP requirement was adequately met by oxidative processes. Although lactate concentration increased in both muscle and plasma at the onset of exercise, this rise was transient, and concentration fell to resting levels thereafter (Fig. 1). Although these data only reflect a balance between lactate production and removal, they suggest that ATP supply from oxidative metabolism was sufficient in meeting energy demand. Given this, it was not surprising that these untrained subjects were able to exercise for 3 h. In addition, the low muscle glycogen levels observed at fatigue were expected because carbohydrate has been demonstrated to provide −50% of the total energy metabolized during exercise at this intensity (31). Interestingly, the concentration of glycogen at F − 40 was lower than that previously observed at fatigue in some studies (2, 32, 35). This is probably due to the important fact that this is the only study to date that has normalized the exercise intensity to a marker of metabolic stress rather than to a percentage of \( V_{O_2\text{max}} \). Therefore, factors such as metabolic acidosis, which can disrupt contractile processes, could not have resulted in fatigue in the present study. In contrast, in previous studies where the workload was normalized to a percentage of \( V_{O_2\text{max}} \), factors such as metabolic acidosis may have contributed to fatigue before glycogen depletion.

It is important to note, however, that although glycogen was reduced by −50% in all subjects when \( F = 40 \) is compared with \( F \), the fall was not statistically significant. Although unlikely, because of the relationship between glycogen use and exercise duration (Fig. 5), the possibility cannot be ruled out that fatigue was related to factors other than glycogen availability, such as a decrease in the central drive to exercise. It has been proposed for a number of years (39) that the serotonergic system may play a crucial role in the central control of fatigue during prolonged exercise. In addition, prolactin has been proposed as a marker of central serotonergic activity, and increases in plasma prolactin concentration have been observed as exercise intensity increased (16). Recent evidence suggests that muscular contraction increases reactive oxygen species in skeletal muscle, which promote low-frequency fatigue in vitro (30). Therefore, the impairment of contractile function may be independent of glycogen availability. Further investigations into the role of the central nervous system and reactive oxygen species production during prolonged exercise to fatigue are needed.

In the present study, TAN did not fall, IMP did not significantly accumulate, PCr was not reduced when \( F \) was compared with \( F = 40 \) (Table 1), and no hypoxanthine was detected in the muscle samples. Taken together, these data demonstrate that the intracellular high-energy phosphagen pool was not affected to a great extent. Even though there was a tendency for IMP to accumulate, the important factor was that there was no correlation between IMP accumulation and glycogen concentration near the end of exercise, when glycogen levels were compromised. In fact, the subject with the lowest muscle glycogen content at fatigue displayed no detectable IMP accumulation, whereas the subject with the highest glycogen at fatigue had the highest IMP level at this point (Fig. 4). This lack of a correlation between IMP and glycogen content when glycogen is compromised is in contrast to the data of Sahlin et al. (33). Of note, however, is the fact that in the previous study the exercise intensity at which the subjects exercised ranged from 67 to 86% of \( V_{O_2\text{max}} \). In addition, although the correlation was significant, three of seven subjects demonstrated no IMP accumulation at fatigue.

Another important finding in this study was that the small accumulation of IMP occurred progressively throughout exercise and did not occur late in exercise, when glycogen was compromised (Fig. 4). In fact, when a power analysis was performed on these data, the number of subjects needed to obtain a statistical difference was 102. Therefore, despite the facts that IMP rose slightly over time and our subject number was low, we are confident that our data demonstrate no biological relationship between glycogen content and IMP formation. In addition, the fact that the TAN pool was not altered at all suggests that the small and insignificant rise in IMP over time was physiologically unimportant.
A limitation of the present study is that analyses were conducted on whole, mixed-fiber muscle samples. It is possible that the accumulation of IMP may have been related to fiber-type activation. As discussed above, IMP accumulated progressively throughout exercise rather than at fatigue (Fig. 4). It has been demonstrated that type II fiber activity increases as submaximal-intensity exercise progresses (20). Furthermore, Norman et al. (28) demonstrated that glycogen-depleted type II fibers accumulate more IMP compared with type I fibers. It would have been desirable in the present study to perform pooled single-fiber analyses. However, given the small content of IMP in mixed muscle and the present analytic techniques, this was not possible. It is important to note that as intramuscular glycogen stores became compromised toward the end of exercise in the present study, circulating glucose did not fall (Fig. 3). In fact, two subjects were relatively hyperglycemic at fatigue when compared with rest, despite glycogen levels being <50 mmol/kg dw in all subjects. This is in agreement with some (2, 34, 37, 38), but not all (9, 13), previous studies. Although, there are no published data to our knowledge that examine glucose uptake during prolonged exercise to fatigue, we have recently demonstrated that when euglycemia is maintained during prolonged exercise, isotopic tracer-determined glucose uptake (rate of disappearance) neither falls nor indeed plateaus at the point of fatigue (1). This observation is important when the effect of carbohydrate availability on muscle energy metabolism is considered. If glucose availability, glucose rate of disappearance, and carbohydrate oxidation are not compromised, one would expect exercise to continue if fatigue is related to muscle energy metabolism because glucose moieties would be available for flux through glycolysis and the TCA cycle.

Although there was little evidence of metabolic stress within the muscle at fatigue, the relationship between glycogen content and exercise duration suggests that the maintenance of contractile force is dependent on glycogen availability. It has been previously suggested that glycogen may be required for contractile processes independent of energy metabolism (21). Studies in both animals (6, 8, 36) and humans (4) have suggested a link between sarcoplasmic reticulum Ca$^{2+}$ uptake and release and glycogen availability. In addition, topographical localization of glycogen within human skeletal muscle has been observed (19). Therefore, depletion of glycogen in the sarcoplasmic reticulum may possibly lead to a failure of contractile force, although further research in this area is warranted.

In summary, the data from this study indicate that fatigue during prolonged exercise may be related to carbohydrate availability. It is clear, however, that when untrained subjects exercise below their LT, there is little evidence of compromised high-energy metabolism within the contracting muscle at fatigue. It is possible, therefore, that in these circumstances insufficient carbohydrate availability may affect other cellular processes, which may cause a disturbance in contractile function.

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