Muscle IMP accumulation during fatiguing submaximal exercise in endurance trained and untrained men

Jacinta Baldwin, Rodney J. Snow, Michael F. Carey, and Mark A. Febbraio. Muscle IMP accumulation during fatiguing submaximal exercise in endurance trained and untrained men. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R295–R300, 1999.—To examine the effect of training status on muscle metabolism during exercise, seven endurance-trained [peak oxygen uptake (VO_{2peak}) = 65.8 ± 2.4 ml·kg\(^{-1}\)·min\(^{-1}\)] and six untrained [VO_{2peak} = 46.2 ± 1.9 ml·kg\(^{-1}\)·min\(^{-1}\)] men cycled to fatigue at a work rate calculated to require 70% VO_{2peak}. Time to exhaustion was 36% longer (P < 0.01) in trained (TR) compared with untrained (UT) men (148 ± 11 vs. 95 ± 8 min). Although intramuscular glycogen content was reduced (P < 0.05) in both TR and UT at fatigue, IMP, a marker of a mismatch between ATP supply and demand, was only elevated (P < 0.01) in UT muscle at fatigue and was approximately fourfold higher at this point in UT compared with TR. These data demonstrate that fatiguing submaximal exercise was associated with a similar low level of intramuscular glycogen in both TR and UT men, but a mismatch between ATP supply and demand only occurred in UT individuals.

Endurance training results in an attenuation in IMP accumulation during steady-state submaximal exercise in humans (12, 13) and rats (8), a reduction in phosphocreatine (PCr) degradation (12–14), and an attenuation in lactate (12–14) and ammonia accumulation (3). These findings demonstrate that training improves the match between ATP synthesis and degradation during exercise at submaximal work rates. Few studies, however, have examined IMP accumulation at fatigue from prolonged exercise in endurance-trained individuals. It is difficult to make comparisons between the studies that do exist because of differences in experimental procedures, training status of the volunteers, and variable levels of glycogen within the fatigued muscles. For instance, Sahlin et al. (23) observed no IMP accumulation in skeletal muscle after prolonged exercise to fatigue in men who they described as training regularly (maximal oxygen uptake (VO_{2max}) = 3.26–5.24 l/min). However, postexercise IMP and glycogen were inversely correlated in this study.

The present study was undertaken to directly examine muscle energy metabolism during submaximal exercise concurrently with muscle glycogen depletion (2, 5) and can be delayed by providing exogenous carbohydrate during exercise (2, 5). There are several theories attempting to explain the requirement for carbohydrate to delay fatigue (6, 11). It has been suggested that muscle glycogen stores are progressively compromised during exercise, flux through glycolysis is reduced, leading to a fall in pyruvate formation (22) and a reduction in tricarboxylic acid cycle intermediates (10, 22), resulting in an impairment in ATP provision. The subsequent decrease in ATP provision from carbohydrate sources may lead to a transient increase in ADP concentration, stimulating the myokinase reaction. This reaction results in the formation of AMP, which is rapidly deaminated to IMP and ammonia via the activity of AMP deaminase (27). If this scenario is correct, an increase in the intramuscular concentration of IMP during prolonged, submaximal exercise may therefore serve as a marker of impaired ATP synthesis. Although this sequence of events is probable, it is important to note that an accumulation of muscle IMP only reflects an imbalance between rates of ATP synthesis and degradation. Consequently, it is possible that an increase in IMP content during prolonged exercise may result from an increased rate of ATP degradation and/or a decreased synthesis rate. Accordingly, many studies have noted the accumulation of IMP at fatigue after prolonged exercise in the presence of low intramuscular glycogen stores (1, 19, 20, 22) but not earlier when glycogen stores were adequate (20, 22). These findings demonstrate therefore that an imbalance between ATP synthesis and degradation rates occurred at some point during this type of exercise. Of note, however, these studies have been conducted in untrained individuals.

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exercise to fatigue in untrained and endurance-trained individuals. It was hypothesized that IMP accumulation at fatigue would be attenuated in endurance-trained, compared with untrained, men in the presence of similarly low levels of muscle glycogen.

**METHODS**

Subjects. Seven endurance-trained (TR) and six untrained (UT) males (Table 1), volunteered to participate in the present study after being fully informed of the experimental procedures and giving informed consent. Subjects were designated as TR if they had a peak oxygen consumption (VO\textsubscript{2peak}) >60 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} and UT if they had a VO\textsubscript{2peak} <50 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}. In addition, average weekly training for the TR group consisted of 11 h of endurance exercise, which included ~175–200 km of cycling. In contrast, UT were sedentary. This experiment was approved by the Human Ethics Committees at the Royal Melbourne Institute of Technology and Victoria University of Technology. VO\textsubscript{2peak}. VO\textsubscript{2peak} was determined during incremental cycling exercise at 80–90 rpm, to volitional exhaustion on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands). During this test, expired air was directed by a Hans Rudolf valve through a ventilimeter into a mixing chamber, and was analyzed for oxygen and carbon dioxide by gas analyzers that were calibrated before each test using a commercially prepared gas mixture (Sensormedics MMC Horizon System; Medical Graphics, St. Paul, MN).

**Exercise trial.** A work rate was selected that would elicit ~70% VO\textsubscript{2peak}. Each subject performed the experimental trial on the same cycle ergometer used during the VO\textsubscript{2peak} test. Subjects were instructed to cycle at the predetermined work rate, at a pedal frequency of 80–90 rpm until fatigue. Fatigue was defined as the inability to complete one pedal revolution, since the work rate on the electrically braked ergometer is fixed and not influenced by pedal revolution rate. All subjects were given verbal encouragement to continue cycling from the same investigator. Before the trial, subjects arrived at the laboratory after an overnight fast, having refrained from exercise, alcohol, tobacco, and caffeine for 24 h.

Heart rate and pulmonary measurements. Heart rate (HR) was recorded via telemetry (Sport Tester, Polar, Finland) at rest, at 5 and 20 min, and at 20-min intervals until fatigue. Expired pulmonary gases were collected and analyzed, as described above, at 5 and 30 min and at 30-min intervals until fatigue. Oxygen uptake (VO\textsubscript{2}) and respiratory exchange ratio (RER) were calculated from these measurements. As the subjects neared fatigue, pulmonary gases were not collected, as it may have interfered with the performance of the subjects.

Tissue sampling and analysis. Muscle samples were obtained from the vastus lateralis, at rest and immediately after exercise, using the percutaneous needle biopsy technique modified for suction. Muscle samples were frozen in liquid nitrogen 25.7 ± 3.0 s after the cessation of exercise, and the time to freeze was not different (P > 0.05) when comparing TR with UT. Samples were analyzed for PCr, creatine, lactate, ATP, ADP, AMP, IMP, hypoxanthine, and glycogen as described previously (9, 26).

**RESULTS**

Performance and pulmonary variables. TTE was 36% longer (P < 0.01) in TR compared with UT (Table 2). Mean exercise VO\textsubscript{2} was higher (P < 0.05), and mean RER and HR were lower (P < 0.05) in TR compared with UT (Table 2).

Muscle metabolite concentrations. The muscle metabolite concentrations at rest and fatigue are summarized in Table 3. Although muscle glycogen and PCr concentrations decreased (P < 0.01) to similar levels at fatigue in TR and UT, an exercise-induced increase (P < 0.01) in muscle IMP and hypoxanthine was only observed in UT. In addition, although the IMP concentrations in TR were 0.05 ± 0.02 and 0.44 ± 0.15 mmol/kg dry wt at rest and fatigue, respectively, these values were not significantly different (P = 0.26). Furthermore, concentrations of muscle IMP and hypoxanthine were higher (P < 0.05) at fatigue when comparing UT with TR. The exercise-induced change in TAN tended (P = 0.06) to be greater in UT compared with TR (Fig. 1).

Plasma metabolite concentrations. The resting concentrations of all the measured plasma metabolites were
Plasma hypoxanthine was greater \( (P < 0.01) \) than rest at 20 and 60 min of exercise and at fatigue in UT, whereas in TR plasma hypoxanthine was elevated \( (P < 0.05) \) only at fatigue. Furthermore, the concentration of this metabolite was greater \( (P < 0.05) \) at fatigue in UT compared with TR (Fig. 2).

Values are means \( \pm \) SE given in mmol·kg dry wt; \( n = 7 \) trained men and \( n = 6 \) untrained men. TAN, total adenine nucleotide pool \( (\text{ATP} + \text{ADP} + \text{AMP}) \); PCR, phosphocreatine. Data for ATP, ADP, and lactate indicate a time effect \( (P < 0.05) \). Data for lactate also are a main effect for training status \( (P < 0.05) \); *different from untrained at rest \( (P < 0.05) \); †different from untrained at fatigue \( (P < 0.05) \); ‡different from rest \( (P < 0.05) \).

not different between TR and UT. Plasma hypoxanthine was greater \( (P < 0.01) \) than rest at 20 and 60 min of exercise and at fatigue in UT, whereas in TR plasma hypoxanthine was elevated \( (P < 0.05) \) only at fatigue. Furthermore, the concentration of this metabolite was greater \( (P < 0.05) \) at fatigue in UT compared with TR (Fig. 2). Plasma lactate increased \( (P < 0.05) \) during exercise in both groups. Concentrations of this metabolite were higher in UT at 20 min \( (P < 0.01) \) and at 40 min \( (P < 0.05) \) compared with TR (Fig. 3). Plasma glucose was not different at any time during exercise between the two groups (Fig. 3). There was a main effect \( (P < 0.01) \) for time for glucose, and it was greater \( (P < 0.01) \) than rest at 20 and 40 min. Although the concentration of this metabolite at fatigue was lower than at 20 min \( (P < 0.01) \) and 40 min \( (P < 0.05) \) of exercise, it was not different from rest (Fig. 3).
**DISCUSSION**

The results from the present study demonstrate that prolonged submaximal cycling to fatigue results in reduced intramuscular glycogen stores in both TR and UT men. There was, however, an accumulation of IMP in UT but not TR skeletal muscle at fatigue. Although the TAN pool was not statistically different between TR and UT, the exercise-induced change in TAN tended (P = 0.06) to be greater in UT compared with TR. Furthermore, a t-test performed on the TR data alone indicated that there was no change in TAN when comparing values at rest with those at fatigue. These findings demonstrate that the mismatch between ATP synthesis and degradation that occurs during prolonged exercise in UT muscle is not evident in endurance TR muscle.

The present study is the first to simultaneously compare muscle IMP accumulation in endurance TR and UT individuals during prolonged exercise to fatigue. The findings of this study are consistent with previous research in which an accumulation of IMP in UT muscle has been observed after submaximal fatiguing exercise (1, 19, 20, 22). These authors speculated that IMP accumulates during this type of exercise because of a reduced ATP supply, via oxidative metabolism. Although this is possible, it cannot be excluded that IMP accumulates in UT subjects as a result of an increased ATP demand or a combination of an increased ATP demand and reduced ATP supply. For example, an increase in contracting muscle ATP demand may occur during prolonged submaximal exercise when more type II muscle fibers are recruited to maintain the work rate (24). As previously discussed, it is difficult to interpret the existing literature on the influence of endurance training on muscle IMP accumulation at fatigue. It is interesting to note, however, that Spencer et al. (25) report that the IMP concentration in TR muscle at fatigue from prolonged exercise was eightfold greater than at rest. This value is very similar to the magnitude of change observed for TR in the present study. Furthermore, we found this change not to be different (P = 0.26); in fact, three of the seven TR subjects in the present study had IMP concentrations at fatigue similar to their resting levels.

Hypoxanthine is a degradation product of IMP, and it is able to diffuse across the sarclemma (27). Plasma hypoxanthine, therefore, may serve as an extracellular marker of IMP accumulation. The marked accumulation of IMP in contracting UT muscle in this study was consistent with the increased plasma hypoxanthine at fatigue (Fig. 2). However, given that the rise in plasma hypoxanthine was significant at 20 min of exercise and continued to accumulate at 60 min and at fatigue, it is likely that IMP accumulated in the contracting UT muscle throughout exercise and may not simply be related to glycogen availability. There was a small increase in plasma hypoxanthine at fatigue in TR men. However, the concentration (~6 µM) was low and provides further evidence to suggest that there was no significant change in the adenine nucleotide pool in contracting TR muscle at fatigue.

The lower IMP concentration observed in TR muscle in the present study extends the findings of previous research, which has observed that exercise of the same relative intensity results in a smaller disturbance in homeostasis in endurance TR compared with UT muscle before fatigue (12–14). Although the attenuated accumulation of IMP observed in endurance TR individuals during nonfatiguing prolonged exercise is most likely due to an enhanced oxidative capacity (8), Green et al. (12) have suggested that a reduction in IMP accumulation may occur independent of improved mitochondrial capacity. It is possible that the reduction in muscle IMP accumulation after endurance training may result from alterations in the neuromuscular recruitment pattern (4). Such an adaptation may lead to an increase in mechanical efficiency, resulting in less metabolic strain and more uniform glycogen utilization in any one group of muscles or muscle fibers. As a consequence, an improved match between ATP synthesis and degradation would be maintained, and no IMP accumulation would occur within the contracting fibers.

It is possible that the degree of metabolic disturbance observed in UT during exercise was a function of the work rate employed. Green et al. (13) observed that muscle IMP accumulation was attenuated after 30 min of submaximal exercise, at a given percentage of VO_{2\text{max}}, after 4 and 8 wk of training. The aerobic capacity of these subjects was greater after training (15) and, because the work rate was not adjusted accordingly, it follows that the exercise bouts were performed at a lower relative work rate at 4 and 8 wk. There was an ample supply of glycogen both before and after training, so it is possible that the work rate may have been a factor in IMP accumulation. In the present study, although UT were working at the same relative VO_{2\text{peak}} as TR, both RER and HR for the duration of the cycling bout were higher (Table 2). Furthermore, plasma hypoxanthine (Fig. 2) increased markedly early in exercise in UT when glycogen stores were probably adequate (12), and plasma lactate (Fig. 3) was twofold higher during the first 60 min of exercise in UT compared with TR. It appears from these data that UT, when exercising at 70% VO_{2\text{peak}}, were unable to adequately meet the exercise demand via aerobic processes. In contrast, the data from TR indicate that they appeared to maintain metabolic control throughout the exercise bout.

In addition to endurance training, other factors may account for at least some of the differences in muscle metabolism observed between the groups in the present study. Differences in muscle fiber-type composition of the subjects may be important. It is well established that endurance TR individuals have a greater proportion of slow-twitch (type I) fibers than the general population (21). Most of the fiber-type composition differences between individuals can probably be attributed to genetic factors (18); however, endurance training is known to cause a small shift in muscle fiber composition toward type I fibers (17). Previous research has shown that glycogen-depleted type I fibers accumu-
late less IMP during prolonged nonfatiguing exercise compared with glycogen-depleted type II fibers (19). Because endurance TR individuals would be expected to recruit fewer type II fibers at the exercise intensity used in the present study, it is possible that the reduction in muscle IMP content observed in TR compared with UT may, at least partly, be due to this factor.

The present study is unable to clarify whether fatigue in either group resulted from peripheral or central origins. Nevertheless, it is likely that a hypoglycemic-mediated dysfunction of the central nervous system did not occur in either endurance TR or UT men because plasma glucose values did not fall below 4.5 mM in either group (Fig. 3). This finding is consistent with some (25), but not other (2, 5), observations. It is possible, however, that performance may have been affected via changes in other central parameters, such as plasma levels of dopamine and/or prolactin, that occurred independent of plasma glucose concentration. It has been proposed for a number of years (28) that the serotonergic system may play a crucial role in the central control of fatigue during prolonged exercise. Prolactin has been proposed as a marker of central serotonergic activity, and increases in plasma prolactin concentration have been observed as exercise intensity increased (7). Aside from the plasma glucose data, the present paper is, unfortunately, unable to add to the central fatigue discussion. Further investigation into the role of the central nervous system during prolonged exercise to fatigue is needed.

In summary, although fatigue during prolonged submaximal exercise was associated with a similar low level of intramuscular glycogen in both TR and UT muscle, IMP accumulated in UT but not TR muscle. Consequently, these data indicate that there was a mismatch between ATP supply and demand during submaximal exercise to fatigue in UT that was not evident in TR muscle.

The present study was able to clearly outline the different muscle metabolic profiles of highly endurance TR and UT groups during submaximal exercise to fatigue. Importantly, the present study is the first to demonstrate, under the same experimental conditions, that UT have an imbalance in skeletal muscle ATP supply and demand at fatigue, but TR do not. Although speculative, our results indicate that the cause of fatigue in UT subjects may be related to muscle metabolic stress; however, this is unlikely to be the case in TR subjects. If this interpretation is correct, it raises the question of what is causing fatigue during submaximal exercise in TR. The findings from the present study also highlight several areas where further research is required. First, our results need to be confirmed using a longitudinal experimental design. Furthermore, the time course for the accumulation of IMP during fatiguing submaximal exercise and how this is affected by endurance training needs to be established. In these types of studies the mechanism(s) causing the accumulation of muscle IMP (i.e., decreased ATP synthesis and/or increased ATP degradation) requires examination. Finally, our knowledge of the influence of training on muscle metabolic stress would benefit from single fiber-type analysis as this would allow a more accurate examination of the link between glycogen depletion and IMP accumulation during fatiguing submaximal exercise.

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