Muscle adenine nucleotide metabolism during and in recovery from maximal exercise in humans

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Zhao, S., R. J. Snow, C. G. Stathis, M. A. Febbraio, and M. F. Carey. Muscle adenine nucleotide metabolism during and in recovery from maximal exercise in humans. J Appl Physiol 88: 1513–1519, 2000.—The relationship between changes in the muscle total adenine nucleotide pool (TAN = [ATP] + [ADP] + [AMP], where brackets denote concentration) and an increase in IMP concentration (29, 33) and an increase in IMP content. Skeletal muscle samples were obtained from the vastus lateralis muscle of seven untrained men (23.9 ± 2.3 yr, 74.4 ± 3.6 kg, and 55.0 ± 2.9 ml·kg⁻¹·min⁻¹ peak oxygen consumption) before and immediately after exercise and after 5 and 10 min of passive recovery. The exercise-induced increase in muscle IMP was linearly related to the decrease in muscle TAN (r = −0.97, P < 0.01), and the slope of this relationship (−0.83) was not different from 1.0 (P > 0.05), indicating a 1:1 stoichiometric relationship. This interpretation must be treated cautiously, because all subjects displayed a greater decrease in TAN compared with the increase in IMP content, and the TAN + IMP + inosine + hypoxanthine content was lower (P < 0.05) immediately after exercise compared with during rest. During the first 5 min of recovery, the increase in TAN was not correlated with the decrease in IMP (r = −0.18, P > 0.05). In all subjects, the magnitude of TAN increase was higher than the magnitude of IMP decrease over this recovery period. In contrast, the increase in TAN was correlated with the decrease in IMP throughout the second 5 min of recovery (r = −0.80, P < 0.05), and it was a 1:1 stoichiometric relationship (slope = −1.12). These data indicate that a small proportion of the TAN pool was temporarily lost from the muscle purine stores during sprinting but was rapidly recovered after exercise.

nucleotides; purine metabolism; adenosine 5′-triphosphate; skeletal muscle; ammonia

IT IS WELL KNOWN THAT high-intensity sprint exercise results in a reduction in the muscle total adenine nucleotide pool (TAN = [ATP] + [ADP] + [AMP], where brackets denote concentration) and an increase in IMP concentration (29, 33). It is believed that the recovery of the adenine nucleotide pool after intense exercise is achieved primarily by the reamination of IMP to AMP with the use of the reactions of the purine nucleotide cycle (21, 22). In support of this contention, Meyer and Terjung (21) reported that the removal of IMP matched the resynthesis of TAN in gastrocnemius muscles of rats during recovery from tetanic in situ stimulation. In addition, Sahlin et al. (26) demonstrated that the mean level of TAN restoration and IMP disappearance was similar in human muscle during 7 min of recovery from cycling exercise at an intensity that elicited exhaustion between 2 and 13 min. Interestingly, Sahlin and Ren (27) reported an apparent twofold increase in ATP restoration compared with IMP removal during the first 2 min of recovery from intense isometric contractions; however, after 4 min of recovery, the change in ATP and IMP content over this period appeared to be of similar magnitude. The greater ATP restoration compared with IMP disappearance during the initial phase of recovery in this study was probably explained by an increased production of ATP from the markedly elevated ADP pool observed at the end of exercise. The data from the studies by Graham et al. (10) and Bangsbo et al. (1) also indicate that the mean increase in human skeletal muscle ATP content was about twofold higher than the decrease in IMP during the first 10 min of passive recovery from high-intensity exercise. Unfortunately, these two studies did not provide statistical evidence to confirm that a mismatch in the recovery of these metabolites actually occurred. At least in the Graham et al. (10) study, a mismatch, if it occurred, could not be explained by an increased ATP synthesis from the ADP pool, as this pool size was unaltered during exercise and recovery. Moreover, the possibility that the recovery of ATP may be greater in magnitude than the removal of IMP raises the possibility that the ATP pool is being restored from a source other than the other adenine nucleotides or IMP during the early stages of recovery.

Apart from a rat study (21) that demonstrated a 1:1 stoichiometric relationship between muscle TAN and IMP, all of the human studies that have examined this relationship have only reported the mean muscle TAN and IMP content. This, however, does not indicate the relationship between TAN restoration and IMP disappearance for each individual subject over the recovery period. Consequently, the mean change in TAN and IMP may be of a similar magnitude, but the stoichiometric relationship between the two variables may not be 1:1. The aim of the present study, therefore, was to establish the relationship between the restoration of

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the TAN pool and removal of IMP during the early stages of recovery from high-intensity exercise in human skeletal muscle. We hypothesize that the removal of muscle IMP will be matched 1:1 with the increase in TAN content during the early phase of recovery from 30 s of sprint exercise.

METHODS

Subjects. Seven healthy, active, but nonspecifically trained, male subjects volunteered to participate in this study. Their age, height, weight, and peak oxygen consumption were 23.9 ± 2.3 yr, 180.5 ± 3.5 cm, 74.4 ± 3.6 kg, and 55.0 ± 2.9 ml·kg⁻¹·min⁻¹, respectively. Subjects were informed of the experimental procedures and possible risks before giving their informed consent to participate. All studies were approved by the Human Research Ethics Committee of Victoria University of Technology. Subjects were instructed to refrain from intense exercise, tobacco, alcohol, and caffeine for the 24 h before all testing. All subjects were asked to report to the laboratory before each testing session after an overnight fast.

Exercise protocol. The subjects performed a 30-s “all-out” sprint cycling bout on an air-braked cycle ergometer (Series A, Repco, Melbourne, Australia). The air-braked ergometer enabled computerized determination of peak power, mean power, total work, and fatigue index. The power output of the air-braked cycle ergometer is proportional to the cube of the wheel velocity, which was measured with the use of a tachometer (Hall-effect device and a cog at the wheel hub). The fatigue index was calculated as [peak power - end of the sprint power/peak power] × 100. Subjects were instructed to remain seated and pedal as fast as possible for the duration of the test. After 30 s of sprint cycling, subjects recovered lying supine on a bed for 10 min.

Muscle and blood sampling. Muscle samples were obtained from vastus lateralis muscle using the percutaneous needle biopsy technique modified for suction. These samples were obtained from the same leg from separate incisions at rest, immediately after exercise, and after 5 and 10 min of recovery. Leg selection was random. Muscle samples were frozen immediately in liquid nitrogen for later biochemical analysis. Blood samples from a forearm vein were obtained, via an indwelling catheter (20 gauge), at 2, 5, and 10 min of recovery. The catheter was kept patent by periodic flushing with 1 ml of 0.09% sodium chloride/5 IU heparin. Blood samples were drawn into a syringe and rapidly transferred to ice-cold tubes containing lithium heparin. Plasma was obtained via centrifugation (Centrifuge 5415 C, Eppendorf). Approximately 0.5 ml of plasma was immediately deproteinized in 1 ml of 3 mol/l ice-cold perchloric acid and spun again, and the supernatant was stored at −80°C before lactate analysis. Approximately 1 ml of plasma was frozen and stored in liquid nitrogen for later ammonia analysis. The remainder of the plasma was stored at −80°C for analysis of hypoxanthine and inosine.

Muscle analysis. All muscle samples were divided and weighed at −30°C. The muscle portion used for measuring ammonia/ammonium (NH₃) was dissected into small pieces and extracted in 0.6 mol/l perchloric acid-30% methanol at −20°C and then neutralized with 1.8 mol/l potassium hydroxide on ice. The extracts were analyzed within 1 h after extraction by using flow injection analysis (31) as described by Katz et al. (15). The muscle portion used for measuring all other metabolites was freeze dried, weighed, dissected free of connective tissue, and powdered. The powder was thoroughly mixed and divided into two parts. One part was extracted following the procedures outlined by Harris et al. (11) and assayed for phosphocreatine (PCr), creatine (Cr), and lactate by using enzymatic fluorometric techniques as described by Lowry and Passonneau (19). These muscle extracts were also analyzed for purine nucleotides (ATP, ADP, AMP, IMP), hypoxanthine, and inosine by using reverse-phase HPLC. This technique involved a modification of the method described by Wynants and Van Belle (37). An ICI (Australia) HPLC instrument fitted with a Hibar Lichrosphere 100 CH-18/2 (Merck; 240 × 4 mm) analytic column was used to perform the analysis. The other muscle powder portion was hydrolyzed in 2 mol/l hydrochloric acid at 100°C for 2 h with periodic agitation. Subsequently, this extract was neutralized with 0.667 mol/l sodium hydroxide and stored in liquid nitrogen for later muscle glycogen analysis. This extract was also analyzed for glycogen (as glucose units) according to the procedure of Lowry and Passonneau (19). Muscle metabolites, except for glycogen, lactate, hypoxanthine, inosine, and NH₃, were corrected to their highest individual total Cr content (PCr + Cr). All muscle metabolite concentrations are expressed per kilogram of dry mass (dm). The measurement of muscle NH₃ concentration was conducted on wet tissue but expressed as dm by using the wet mass-to-dm ratio determined for each muscle sample.

Plasma analysis. Plasma lactate was determined by using an enzymatic technique with spectrophotometric detection (19). Plasma ammonia samples were analyzed in duplicate by flow injection analysis within 72 h of collection (31). The determination of hypoxanthine, inosine, and uric acid was performed by HPLC by using a modified method of Wynants and Van Belle (37). The separation was achieved by using the same HPLC techniques as described for muscle. Before the HPLC analysis, the plasma samples were deproteinized with 1.5 mol/l perchloric acid and neutralized with 2.1 mol/l potassium bicarbonate.

Statistical analysis. ANOVA with repeated measures was employed to compare data over time. When the ANOVA was significant, Newman-Keuls post hoc tests were used to localize the difference. Biomedical Data Processing statistical software was used to compute the ANOVAs. Linear regression analysis was performed between the change in muscle TAN and the change in muscle IMP content. When a significant F value was achieved, the slope of the regression line was then tested to reveal whether the slope was significantly different from 1.0. If the slope was not different from 1.0, this indicated that there was a 1:1 stoichiometric relationship between the changes in muscle TAN and IMP content. Linear regression analyses were computed by using Microsoft Excel software. The level of probability to reject the null hypothesis was set at P < 0.05. All values are reported as means ± SE.

RESULTS

Sprint performance. The peak power and mean power output during the 30-s sprint were 744 ± 53 and 533 ± 35 W, respectively. Total work performed during exercise was 16.0 ± 1.1 kJ. The fatigue index was 40 ± 3%.

Muscle metabolites. The muscle metabolite concentrations at rest, immediately after the 30-s sprint, and at 5 and 10 min of recovery are shown in Table 1. The PCr content of the muscle decreased (P < 0.05) by 67% during the sprint bout but had returned to basal levels after 5 min of recovery. After 10 min of recovery, the PCr level was higher (P < 0.05) than at rest. As expected, the magnitude of change in muscle PCr was accompanied by an equal, but opposite, change in muscle Cr content.
Table 1. Metabolite concentrations in vastus lateralis at rest, immediately after 30-s sprint cycling, and at 5 min and 10 min of recovery

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rest</th>
<th>Postexercise</th>
<th>5 Min of Recovery</th>
<th>10 Min of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>25.9 ± 1.0</td>
<td>16.7 ± 1.3*</td>
<td>19.7 ± 1.5†</td>
<td>23.4 ± 1.3‡‡</td>
</tr>
<tr>
<td>ADP</td>
<td>2.20 ± 0.20</td>
<td>2.00 ± 0.15</td>
<td>2.08 ± 0.16</td>
<td>2.01 ± 0.15</td>
</tr>
<tr>
<td>AMP</td>
<td>0.07 ± 0.004</td>
<td>0.06 ± 0.007</td>
<td>0.06 ± 0.004</td>
<td>0.06 ± 0.006</td>
</tr>
<tr>
<td>IMP</td>
<td>0.10 ± 0.02</td>
<td>6.40 ± 1.28*</td>
<td>4.92 ± 1.08†</td>
<td>1.73 ± 0.66‡‡</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.98 ± 0.10</td>
<td>6.24 ± 1.08*</td>
<td>4.36 ± 0.76†‡</td>
<td>2.52 ± 0.48‡‡</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.02 ± 0.004</td>
<td>0.18 ± 0.10</td>
<td>0.43 ± 0.07*</td>
<td>0.39 ± 0.14*</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>PCr</td>
<td>84.3 ± 2.5</td>
<td>28.0 ± 1.2*</td>
<td>80.0 ± 0.7†</td>
<td>89.5 ± 2.0‡‡</td>
</tr>
<tr>
<td>Cr</td>
<td>45.3 ± 3.4</td>
<td>101.7 ± 5.2*</td>
<td>49.2 ± 4.1†</td>
<td>40.0 ± 3.3‡‡</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.5 ± 0.7</td>
<td>68.2 ± 5.7*</td>
<td>42.7 ± 3.2†</td>
<td>21.4 ± 1.1‡‡</td>
</tr>
<tr>
<td>TAN</td>
<td>28.2 ± 1.1</td>
<td>18.7 ± 1.3*</td>
<td>21.9 ± 1.5†‡</td>
<td>25.4 ± 1.3‡‡</td>
</tr>
<tr>
<td>TAN + IMP</td>
<td>283.3 ± 1.1</td>
<td>25.1 ± 0.7*</td>
<td>26.8 ± 1.1†‡</td>
<td>27.2 ± 0.9#</td>
</tr>
<tr>
<td>TAN + IMP + DE</td>
<td>283.3 ± 1.1</td>
<td>25.3 ± 0.7*</td>
<td>27.3 ± 1.1†‡</td>
<td>27.6 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. Metabolite concentrations are in mmol/kg dry mass. PCr, phosphocreatine; Cr, creatine; TAN, total adenine nucleotide pool (ATP + ADP + AMP); DE, inosine + hypoxanthine. *P < 0.05, significantly different from rest; †P < 0.05, significantly different from postexercise; ‡P < 0.05, significantly different from 5 min of recovery.

During 30 s of sprint cycling, the muscle TAN content decreased (P < 0.05) by 9.5 mmol/kg dm, and muscle IMP increased (P < 0.05) from 0.10 to 6.40 mmol/kg dm. The increase in muscle IMP was linearly related to the decrease in muscle TAN (r = -0.97, P < 0.01; Fig. 1). Although the slope of the line was -0.83, it was not significantly different from 1.0, indicating that there was a 1:1 stoichiometric relationship between TAN depletion and accumulation of IMP during the sprint (Fig. 1). The TAN + IMP content was lower (P < 0.05) immediately after exercise compared with rest.

The magnitudes of the TAN increase during the first and second 5 min of recovery were similar (P > 0.05; Table 2). The decrease in IMP was significantly lower (P < 0.05) in the first compared with second 5 min of recovery (Table 2). During the first 5 min of recovery, the increase in TAN was not correlated with the decrease in IMP (Fig. 2A). In all subjects, the magnitude of the TAN increase was higher than the magnitude of the IMP decrease over this recovery period. In contrast, the increase in TAN was correlated with the decrease in IMP throughout the second 5 min of recovery (r = -0.80, P < 0.05), and there was a 1:1 stoichiometric relationship between the variables (slope = -1.12, Fig. 2B). After 10 min of recovery, muscle TAN levels were still 10% below (P < 0.05) resting values, and muscle IMP was not significantly different from preexercise levels. The sum of total adenine nucleotide content and their degradation products (TAN + IMP + hypoxanthine + inosine) after 30 s of sprint cycling were lower (P < 0.05) than at rest and after 5 and 10 min of recovery (Table 1).

Muscle inosine content was elevated at the end of the sprint and continued to increase (P < 0.05) during the first 5 min but remained unchanged during the second 5 min of recovery. No hypoxanthine was detected in muscle samples taken at rest and immediately after the sprint. At the end of 5 min of recovery, hypoxanthine increased to 0.06 ± 0.02 mmol/kg dm and then remained unchanged for a further 5 min of recovery.

Table 2. Changes in muscle metabolite concentrations at the completion of 30-s sprint cycling and during 1st and 2nd 5 min of recovery

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Exercise</th>
<th>1st 5 Min of Recovery</th>
<th>2nd 5 Min of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>-9.2 ± 1.4</td>
<td>3.1 ± 0.6</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>ADP</td>
<td>-0.21 ± 0.09</td>
<td>0.09 ± 0.05</td>
<td>-0.07 ± 0.16</td>
</tr>
<tr>
<td>AMP</td>
<td>-0.01 ± 0.005</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>TAN</td>
<td>-9.4 ± 1.5</td>
<td>3.1 ± 0.6</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>IMP</td>
<td>6.30 ± 1.27</td>
<td>-1.49 ± 0.22</td>
<td>-3.18 ± 0.64*</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5.27 ± 1.07</td>
<td>-1.88 ± 0.48</td>
<td>-1.74 ± 0.48</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.16 ± 0.09</td>
<td>0.26 ± 0.06</td>
<td>-0.04 ± 0.13</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.006 ± 0.005</td>
<td>0.05 ± 0.03</td>
<td>-0.003 ± 0.02</td>
</tr>
<tr>
<td>PCr</td>
<td>56.3 ± 3.2</td>
<td>52.0 ± 1.1</td>
<td>9.5 ± 2.2</td>
</tr>
<tr>
<td>Cr</td>
<td>56.4 ± 3.2</td>
<td>52.5 ± 1.2</td>
<td>-9.1 ± 2.2</td>
</tr>
<tr>
<td>Lactate</td>
<td>63.7 ± 5.3</td>
<td>25.4 ± 4.4</td>
<td>-21.3 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. Metabolite concentrations are in mmol/kg dry mass. Exercise, postexercise concentration – resting concentration; 1st 5 min of recovery, 5-min recovery concentration – postexercise concentration; 2nd 5 min of recovery, 10-min recovery concentration – 5-min recovery concentration. *P < 0.05, significantly different from 1st 5 min of recovery.
Muscle ammonia and lactate increased \((P < 0.05)\) during the sprint and then declined \((P < 0.05)\) throughout the 10-min recovery period.

Plasma metabolites. As expected, the concentration of plasma lactate and the extracellular markers of muscle adenine nucleotide catabolism (ammonia, inosine, and hypoxanthine) increased \((P < 0.05)\) above basal levels at various stages of the 10-min recovery period (Fig. 3, A-D).

**DISCUSSION**

TAN recovery during the first 5 min of recovery from sprint cycling. A major finding of this study was that the increase in TAN was not related to the decrease in IMP during the first 5 min of recovery from 30-s all-out sprint cycling. In fact, there was no significant decrease in muscle IMP content, but a small, yet significant, increase in TAN occurred during the early stage of recovery.

The increase in TAN during the first 5 min of recovery, in the absence of any change in IMP, indicates that skeletal muscle IMP reamination was not the major source for the restoration of the TAN pool in this time period. This finding indicates that a source, other than IMP reamination, contributed to muscle TAN resynthesis during the early stages of recovery. The present experiment cannot identify this source; however, research conducted on rat heart (4, 13, 14, 17, 23) and skeletal muscle (34, 36) indicates that the unknown source may be an oligomeric adenosine tetraphosphate derivative named oligophosphoglyceroyl-ATP (OPG-ATP). This compound is composed of ATP and 3-phosphoglycerate and is a polymer of alternating units of the two components (14, 17). The oligomer is readily reconverted to ATP and 3-phosphoglycerate. This OPG-ATP is an acid-insoluble compound (4), and, consequently, normal procedures for muscle adenine nucleotide analysis involving acid extraction (as used in the present study) would not detect the OPG-ATP.

To argue that OPG-ATP contributed to TAN resynthesis during the early stages of recovery, it follows that

![Fig. 2. TAN resynthesis and IMP disappearance during the first 5 min (A) and the second 5 min (B) of recovery from 30 s of maximal sprint cycling. Dotted line indicates line of identity. \(r = 0.18\)](image)

![Fig. 3. Venous plasma lactate (A), ammonia (B), inosine (C) and hypoxanthine (D) at rest and during recovery from 30 s of maximal sprint cycling. Values are means ± SE; \(n = 7\) subjects. *P < 0.05, significantly different from rest.)
the oligomer must have been produced during the 30-s sprint bout and/or an OPG-ATP pool existed at rest that was used during the early phase of recovery. Tullison and Terjung (36) reported that the oligomer content in resting rat skeletal muscle was between 0.2 and 0.4 µmol/g wet wt. There are no data available on the influence of exercise, or recovery from intense exercise, on the content of the oligomeric pool in rodent skeletal muscle. Furthermore, no OPG-ATP measurements using human skeletal muscle have been reported. Unfortunately, the indirect evidence produced by the present study that OPG-ATP accumulated in contracting skeletal muscle is equivocal. For example, the TAN + IMP + inosine + hypoxanthine content was 3 mmol/kg dm lower (P < 0.05) after exercise compared with during rest, indicating that some of the purine pool had disappeared, possibly to produce OPG-ATP. Our plasma hypoxanthine and inosine data (Fig. 3, C and D) demonstrate that very little accumulation of these metabolites occurred in this fluid compartment during exercise. Consequently, it is very unlikely that the 3 mmol/kg dm decrease in the muscle purine pool content could have resulted from a large efflux of purines into the extracellular fluid during the 30-s sprint. The loss of muscle TAN content observed in the present study is consistent with the study by Tullison et al. (33), who reported that 18% of the fall in ATP was not accounted for by the accumulation of muscle purines after exhaustive exercise at 120–130% of maximum oxygen consumption. In contrast to the above argument, linear regression analysis revealed that there was a 1:1 stoichiometric relationship between the decrease in TAN and the increase in IMP during the 30-s sprint in the present study (Fig. 1), suggesting that no OPG-ATP was produced. It is worth pointing out that all seven subjects displayed a greater decrease in TAN compared with the increase in IMP content. This suggests that the relationship between the two variables may be different from 1:1. To examine this possibility, we combined the data from the present study with that of six subjects from a previous study by our laboratory (29) that employed an identical 30-s sprint cycling protocol using the same equipment and analytic procedures as the present study. The analysis of the combined results demonstrated that the decrease in TAN was correlated with the increase in IMP (r = −0.89, P < 0.05, n = 13), but the slope of relationship was different (P < 0.05) from 1.0 (Fig. 4). Such a finding supports the possibility that OPG-ATP may have accumulated in the contracting muscle. If the OPG-ATP reverts to ATP during early recovery, it may explain the present observation that significant restoration of TAN but no IMP reamination occurred during the first 5 min of recovery from 30-s sprint cycling. In support of this possibility, the magnitude of the restoration of the TAN pool (3 mmol/kg dm) during the first 5 min of recovery was equivalent to the fall in the sum of TAN + IMP + inosine + hypoxanthine pool during exercise. Clearly, OPG-ATP as a possible source contributing to the restoration of TAN during the first 5 min of recovery is speculative. Future studies need to investigate whether OPG-ATP exists in human skeletal muscle and, if so, what happens to its concentration during exercise and recovery.

The present study showed that the content of muscle IMP did not decrease (P > 0.05 during the first 5 min of recovery. During early recovery, IMP metabolism involves IMP reamination, IMP degradation, and the purine salvage pathway (Fig 5; Ref. 35). IMP reamination and degradation both result in a decrease in IMP levels. In contrast, the purine salvage pathway plays a role in the resynthesis of IMP from hypoxanthine (8, 20). In theory, alterations in muscle IMP content during recovery must involve a balance between the IMP removal pathways (reamination and degradation) and the IMP-producing pathway (purine salvage). The lack of a significant decrease in IMP during the early stages of recovery is best explained by an inhibition of the IMP reamination pathway. This explanation is most likely to be correct because the activities of the other pathways are small in contrast to the maximally activated IMP reamination pathway. For example, in the present experiment, the accumulation of the degradation products of IMP (e.g., inosine and hypoxanthine) within the muscle and plasma during the first 5 min of recovery

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**Fig. 4.** Relationship between TAN depletion and IMP accumulation during 30 s of maximal sprint cycling. •, Data obtained from the present study; △, data obtained with permission from previous study (29). Dotted line indicates line of identity.

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**Fig. 5.** Biochemical pathways involved in skeletal muscle IMP metabolism. 1, ATPase; 2, adenylate kinase; 3, AMP deaminase; 4, cytoplasmic 5'-nucleotidase; 5, purine nucleoside phosphorylase; 6, xanthine oxidase; 7, adenylosuccinate synthetase; 8, adenylosuccinate lyase; 9, hypoxanthine/guanine 5-phosphoribosyl 1-pyrophosphate (PRPP) transferase. sAMP, succinyl AMP; PNC, purine nucleotide cycle.
can account for <20% of the nonsignificant decrease in muscle IMP content (i.e., 1.49 mmol/kg dm). This is consistent with other research that demonstrates that the dephosphorylation of IMP is a minor fate for muscle IMP content during recovery from intense exercise (33). The activity of the pyruvate salvage pathway is likely to be even lower than the IMP degradation pathway because the human muscle enzyme catalyzing pyruvate salvage (pyruvate dehydrogenase) has an in vitro maximal activity (\(-0.1 \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g dm}^{-1}\)) 9–24 times lower than the enzyme activity involved with degrading IMP (e.g., purine nucleoside phosphorylase; Refs. 12, 28). Furthermore, the in vivo activity of pyruvate dehydrogenase in skeletal muscle is also likely to be attenuated by the limited availability of one of its substrates (5-phosphoribosyl 1-pyrophosphate; Ref. 16).

The enzymes adenylosuccinate synthetase and adenylosuccinate lyase are involved in IMP reamination. The former is regarded as the rate-limiting enzyme in the reamination process (18). There are several factors that may inhibit the activity of adenylosuccinate synthetase during the early recovery period after high-intensity exercise. These include a high muscle IMP content (9, 30), a low pH (7, 24), and a reduced PCR content (32). In the present study, the muscle IMP content reached 6.4 mmol/kg dm after a 30-s sprint, which corresponds to an intramuscular concentration of \(-2.1 \mu \text{mol/l} \). This concentration is at a level at which marked inhibition of the synthetase is known to occur in vitro (9, 30). Therefore, it is possible that the adenylosuccinate synthetase is at least partially inhibited by the high IMP concentration during the early recovery period. It should be noted, however, that some researchers argue that IMP inhibition of adenylosuccinate synthetase does not occur (20). Another possible factor that may inhibit the rate of IMP reamination during recovery from high-intensity exercise is muscle acidosis. The pH optimum for adenylosuccinate synthetase from rat and rabbit skeletal muscle is between 6.8 and 7.2 (7, 24).

Brief, high-intensity exercise results in a decrease in human muscle pH to values around 6.6–6.8 (5, 6, 25) and remains within this range for a least 6 min after a 30-s sprint cycling bout (6). Therefore, it is possible that the exercise-induced muscle acidosis may inhibit adenylosuccinate synthetase activity during the early stages of recovery. The third factor that may affect the rate of IMP reamination is the level of muscle PCR. Tullson et al. (32) demonstrated in recovering rat skeletal muscle that IMP reamination occurred only when the level of PCR content reached at least 75% of resting levels. These authors argued that recovering skeletal muscle preferentially restores the PCR content before significant IMP reamination takes place. In the present study, PCR levels had returned to resting levels after 5 min of recovery, indicating that IMP reamination should not have been limited by inadequate PCR resynthesis, at least in the latter stages of the 5-min recovery period. It should be noted that the rate of PCR resynthesis after 30 s of maximal sprint exercise in the present study appears to be quicker than that reported by others (5, 6).

TAN recovery during the second 5 min of recovery from sprint cycling. We observed a 1:1 stoichiometric relationship between the increase in TAN and decrease in IMP during the second 5 min of recovery, suggesting that during this period the restoration of the TAN pool is achieved primarily by IMP reamination. The metabolic conditions that exist after the first 5 min of recovery are more likely to be favorable to the activation of adenylosuccinate synthetase because recovery of PCR stores is complete (Table 2) and muscle pH begins to return toward preexercise levels (2, 6). It is interesting that the restoration of TAN and removal of IMP is a 1:1 stoichiometric relationship during this period, because the muscle and plasma inosine and hypoxanthine data (Table 1 and Fig. 3, C and D) demonstrate that a proportion of the IMP is being degraded rather than reaminated. The fact that the TAN:IMP relationship remains a 1:1 stoichiometric relationship indicates that the extent of IMP degradation must be relatively small. This is consistent with the findings of other research (3).

In the present study, the estimated rate of IMP reamination was \(-0.06 \text{mmol} \cdot \text{kg} \cdot \text{dm}^{-1} \cdot \text{min}^{-1}\) during the second 5-min period of recovery. This estimate assumes that all the removal of IMP during this period was directed toward reamination. Clearly, a large proportion of the removal of IMP must have been by reamination because the increase in the TAN pool matched IMP removal during this period. It is difficult to compare the rate of IMP reamination reported by other studies because, as indicated previously, metabolic conditions may have a substantial influence on the rate of IMP reamination. In addition, the duration of recovery could also affect the calculation of the IMP reamination rate. For example, in the present study, the IMP reamination rate calculated directly from the reduction of IMP content between the period immediately postexercise and at 10 min of recovery would be different from that between 5 and 10 min of recovery.

In conclusion, the muscle TAN content increased significantly; however, the muscle IMP content did not significantly alter during the first 5 min of recovery from 30-s sprint cycling. Furthermore, there was no correlation between these variables. These data indicate that a source other than IMP reamination must have contributed to TAN resynthesis during this time. This study was unable to identify this source, but the observation that the fall in TAN during exercise could not be accounted for by changes in the purine pool suggests that a proportion of the TAN pool is involved in other reactions that may be rapidly reversed when contraction ceases. During the second 5 min of recovery, the magnitude of muscle TAN increase matched the muscle IMP decrease, suggesting that IMP reamination was the source for TAN restoration during this phase of recovery.

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REFERENCES


