Purine salvage to adenine nucleotides in different skeletal muscle fiber types

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Received 12 December 2000; accepted in final form 16 February 2001.

Brault, Jeffrey J., and Ronald L. Terjung. Purine salvage to adenine nucleotides in different skeletal muscle fiber types. J Appl Physiol 91: 231–238, 2001.—Rates of purine salvage of adenine and hypoxanthine into the adenine nucleotide (AdN) pool of the different skeletal muscle phenotype sections of the rat were measured using an isolated perfused hindlimb preparation. Tissue adenine and hypoxanthine concentrations and specific activities were controlled over a broad range of purine concentrations, ranging from 3 to 100 times normal, by employing an isolated rat hind limb preparation perfused at a high flow rate. Incorporation of \(^{3}H\)adenine or \(^{3}H\)hypoxanthine into the AdN pool was not meaningfully influenced by tissue purine concentration over the range evaluated (\(\sim 0.10–1.6 \ \text{m mol/g}\)). Purine salvage rates were greater (\(P < 0.05\)) for adenine than for hypoxanthine (35–55 and 20–30 nmol \(\text{h}^{-1}\) \(\text{g}^{-1}\), respectively) and moderately different (\(P < 0.05\)) among fiber types. The low-oxidative fast-twitch white muscle section exhibited relatively low rates of purine salvage that were \(\sim 65\%\) of rates in the high-oxidative fast-twitch red section of the gastrocnemius. The soleus muscle, characterized by slow-twitch red fibers, exhibited a high rate of adenine salvage but a low rate of hypoxanthine salvage. Addition of ribose to the perfusion medium increased salvage of adenine (up to 3–to 6-fold, \(P < 0.001\)) and hypoxanthine (up to 6–to 8-fold, \(P < 0.001\)), depending on fiber type, over a range of concentrations up to 10 mM. This is consistent with tissue 5-phosphoribosyl-1-pyrophosphate being rate limiting for purine salvage. Purine salvage is favored over de novo synthesis inasmuch as delivery of adenine to the muscle decreased (\(P < 0.005\)) de novo synthesis of AdN. Providing ribose did not alter this preference of purine salvage pathway over de novo synthesis of AdN. In the absence of ribose supplementation, purine salvage rates are relatively low, especially compared with the AdN pool size in skeletal muscle.

adenine; hypoxanthine; ribose

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5-phosphoribosyl-1-pyrophosphate (PRPP) transferase (APRT) and hypoxanthine/guanine PRPP transferase (HPRT), respectively. Absolute rates of purine salvage were measured using isolated muscle in vitro, where the incubation medium could be controlled (38). Purine salvage appears to be favored over de novo synthesis (30, 41, 43) and is increased significantly in muscle with ribose supplementation (7, 14, 24, 42) likely because of increased availability of PRPP. Thus, purine salvage can be expected to contribute significantly to AdN synthesis. However, the rates of purine salvage have not been determined, especially in the fast-twitch low-oxidative fiber type, which is most likely to produce IMP during intense muscle contractions (22).

The purpose of the present study was to directly measure purine salvage rates among skeletal muscle fiber types of the rat. Although hypoxanthine is the primary, if not sole, AdN degradation product within muscle to be salvaged, we also evaluated the rates of adenine salvage, since there is a robust activity of APRT, relative to HPRT, in skeletal muscle (29). This raises the potential for extracellularly derived adenine to be incorporated into the AdN pool (5). Thus, for completeness, we included an evaluation of both adenine and hypoxanthine as substrates for salvage. Because the fractional turnover rate of AdN is potentially threefold higher in the high-oxidative soleus muscle than in fast-twitch low-oxidative white sections of muscle (34), we hypothesized that significant differences in purine salvage rates would be found among fiber types. Furthermore, we hypothesized that purine salvage rates would be favored over de novo synthesis and elevated by ribose supplementation. The outcome of this study may help explain the physiological responses of muscle after repeated days of intense exercise where AdN contents are decreased.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY and Harlan, Indianapolis, IN) weighing 375–400 g were housed two or three animals per cage with unrestricted food (Purina rat chow) and water. Living quarters were maintained at 20°C with a 12:12-h light-dark cycle. This study was approved by the University of Missouri-Columbia Animal Care and Use Committee.

Hindquarter Perfusion

Perfusion medium. The perfusion medium, prepared on the morning of the experiment, consisted of 5% bovine albumin in Krebs-Henseleit buffer, 5 mM glucose, 100 µU/ml insulin, and typical plasma concentrations of amino acids (4). Immediately before use, this solution was filtered and warmed to 37°C. A portion was used to prime the perfusion apparatus, which included, in series, a peristaltic pump, a filter, a gas-exchange chamber supplied with 95% O₂-5% CO₂ for oxygenation and pH control to 7.4, and a bubble trap. The entire apparatus was located inside a Plexiglas cabinet maintained at 37°C. Perfusion pressure and temperature were monitored continuously throughout the experiment.

Surgical preparation. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip), and 100% O₂ was administered during preparation for hindquarter perfusion as developed previously (13). After the right carotid artery was catheterized, the testes, bladder, seminal vesicles, prostate, large intestine, and small intestine were removed, exposing the descending aorta and inferior vena cava. The hindfeet and tail were tied tightly with umbilical tape, and the animal was placed in the warmed clamp. With the pump circulating perfusate at an initial slow flow rate (~3 ml/min), an inflow catheter (16 gauge) was secured in the descending aorta and an outflow catheter (14 gauge) was secured in the vena cava. The rat was then killed with an overdose of pentobarbital sodium into the carotid artery. To clear most of the rat red blood cells, the initial 150 ml of venous effluent were discarded; then the perfusate was recirculated. This yielded a hematocrit of <1%, thereby avoiding significant purine salvage by the red blood cells (26).

Perfusion protocol. The perfusion flow rate was increased over 20–25 min to 50 ml/min. Aortic artery perfusion pressure was ~45 mmHg. This establishes a flow rate of ~0.6 ml/min/100 g perfused tissue mass (~13). During this time, a fresh 300-ml volume of perfusate was prepared with either adenine (0.3–3.0 mM) or hypoxanthine (0.4–4.2 mM) and the respective [2,8-³H]purine base (Moravek Biochemicals) at a specific activity of 600–2,900 dpm/nmol. When appropriate to the design of the experiment, D-ribose was included in the perfusion medium. The experiments that measured purine de novo synthesis rates also included 1 µCi/ml perfusate [U-¹⁴C]glycine (Moravek Biochemicals) at a glycine concentration of 0.38 mM, which is typical for rat plasma (4). The perfusate was switched to that containing labeled substrate after the perfusion pressure was stabilized. This defined time 0 for the experiment.

After 30 min of perfusion with the labeled purine base, the right lower hindlimb muscle sections were quick-frozen using aluminum tongs cooled in liquid nitrogen. Sections included the soleus (predominantly slow-twitch red fibers), plantaris (mixed fast-twitch fibers), superficial medial white gastrocnemius (predominantly fast-twitch white fibers), deep lateral red gastrocnemius (predominantly fast-twitch red fibers), and the remainder of the gastrocnemius (mixed fast-twitch fibers) (3). The right iliac artery was immediately ligated, and the musculature above the knee was tightly tied with umbilical tape to close the vascular system. The flow was then reduced to ~40 ml/min to yield the identical perfusion pressure and establish the same flow per gram of muscle as that before muscle collection. At 60 min, the perfusate was switched to identical medium without either purine base or radioactivity to evacuate the precursor from the vascular space. Timed samples of the venous effluent were initially collected to verify the effective removal of perfusate radioactivity. After 5 min without recirculation, the left lower hindlimb muscle sections were collected in a manner identical to that used to collect sections from the right side. Frozen tissue samples were stored at ~80°C until analyzed.

Analyses

Metabolites from muscle sections were extracted in cold 3.5% (wt/vol) ethanolic (20% vol/vol) perchloric acid and neutralized with tri-n-octylamine and 1,1,2-trichlorotrifluoroethane (8). Perfusate samples were similarly extracted using perchloric acid. Extracts were stored at ~80°C until analyzed.

Purine nucleotides, nucleosides, and bases were separated using reverse-phase high-performance liquid chromatography (HPLC) and quantified by comparing peak area at 254 nm with known standards (37). ATP, ADP, IMP, hypoxan-
thine, and adenine fractions were collected, and radioactivity was counted by dual-channel liquid scintillation counting (quench corrected to disintegrations per minute). The specific activity of the purine base was calculated for every tissue and perfusate sample as disintegrations per minute per nanomole. Phosphocreatine was measured using anion-exchange HPLC as described by Wiseman et al. (40). Glycine was separated using reverse-phase HPLC after phenylisothiocyanate derivatization of perfusate or muscle extract (34). The glycine fraction was collected and counted by liquid scintillation counting.

To determine the muscle water content, a 150- to 250-mg portion of gastrocnemius mixed-fiber section was dried at 60°C to a stable weight. Metabolite contents and salvage rates are expressed at a water content of 76% (see RESULTS), which is typical for rested rat skeletal muscle (20).

Calculations
Salvage rates (nmol·h−1·g−1) were calculated from the [3H] incorporated into the ATP pool (dpm/g) divided by the specific activity of the tissue adenine or hypoxanthine (dpm/nmol) and corrected for the time of sampling. The net amount of label measured in the muscle AdN pool in this study likely represents the actual rate of purine salvage, since loss of label once incorporated into ATP should be inconsequential (34). The turnover of the adenine ring of ATP is relatively slow, and the specific activity of any lost adenine ring from ATP would have a much lower specific activity (0.3–0.4%) than the incoming precursor during salvage.

De novo synthesis rates were calculated similarly by dividing the [14C] radioactivity incorporated into the ATP pool by the specific activity of glycine from the muscle extracts (34).

Statistics
Analyses of variance followed by Tukey’s test were used to detect significant differences (P < 0.05) among means. Values are means ± SE.

RESULTS
Precursor Pool for Purine Salvage
Intracellular specific activity was measured, free of the confounding influence of the perfusate radioactivity, by washing out the vascular space with perfusate free of radioactivity and purine base. After delay for the void volume, the [3H] adenine content (dpm) of the venous effluent declined rapidly (Fig. 1) in a manner that could be characterized by a double exponential (Fig. 1, inset). The initial rate of efflux exhibited a half time of 0.5 min, which is taken to represent washout of the extracellular compartment (20). The second, slower exponential (half time = 2.2 min) likely represents [3H] adenine efflux from the intracellular space. Therefore, after a 5-min washout, we expect that essentially all the radioactivity and purine base measured from the muscle samples is from the intracellular space.

Accurate measurement of purine salvage rates requires knowledge and control of the precursor specific activity. We controlled intracellular precursor specific activity by loading the muscle with extracellular precursor. High uptake of purine base by the muscle was established by increasing perfusate concentrations over a 15- or 25-fold range (0.14–2.13 mM for adenine and 0.09–2.52 mM for hypoxanthine). Muscle concentrations of adenine and hypoxanthine, measured after perfusate washout, increased linearly (r = 0.99) with extracellular adenine and hypoxanthine concentrations. This resulted in plantaris concentrations of 0.16–1.48 μmol/g for adenine and 0.07–1.66 μmol/g for hypoxanthine, 3- to 100-fold higher than normal resting muscle values. More importantly, tissue adenine and hypoxanthine specific activities increased proportionally (r = 0.99) with extracellular specific activities, except for the lowest extracellular concentration of adenine used (0.14 mM). Furthermore, specific activities were not different among fiber type sections. This suggests that extracellular adenine or hypoxanthine is the primary source for the intracellular pool in our experiments. Therefore, we used tissue adenine and hypoxanthine specific activities for calculation of salvage rates. Additionally, muscle samples taken at 30 min, with labeled precursor in the vasculature, yielded specific activities similar to those at the later time point after washout of the labeled perfusate. This suggests that extracellular and intracellular specific activities were essentially the same by 30 min. Therefore, the specific activities measured in the 30-min muscle samples should be valid for calculating purine salvage rates. Furthermore, any error would overestimate the intracellular specific activity and, therefore, underestimate the salvage rates. This is not evident in our time-course experiments, where the first 0.5-h salvage rates were similar to or faster than the second 0.5-h rates (see below).

Water contents of all muscles were 76.1 ± 0.17 for the adenine animals (n = 25) and 76.1 ± 0.18 for the hypoxanthine animals (n = 16).
Effect of Time

As shown in Fig. 2, hypoxanthine salvage (nmol/g) increased in proportion to time despite the nearly two-fold difference in rates (nmol·h\(^{-1}\)·g\(^{-1}\)) among fiber types. Because the rates did not change over time, we have averaged rates obtained from the muscles at the 30- and 65-min time points for analysis of hypoxanthine salvage rates. On the other hand, the rates of adenine salvage decline appreciably in all fiber types during the second 0.5 h of the experiment (Fig. 2). We suspect that this process is specific to adenine and not due to a general decline in tissue viability. ATP (7.61 ± 0.10 μmol/g, n = 27) contents remained high for the length of the experiment. Furthermore, identical perfusion conditions in the hypoxanthine experiments did not show this decrease in salvage rate. Finally, as illustrated in Fig. 3, adenine salvage remained linear (r = 0.99) over time through the entire experiment with the addition of ribose. Consequently, adenine salvage is calculated using the 30-min value.

Effect of Precursor Concentration

Figure 4 illustrates the absence of marked influence of purine base concentration on salvage rates in the mixed-fiber plantaris. The modest increase over adenine concentration (~25%) is relatively inconsequential, considering the large increase (~10-fold) in perfusate concentration. Thus neither adenine nor hypoxanthine concentration appreciably influenced salvage rates in skeletal muscle. One exception was with the soleus muscle, where a relatively high rate was observed at the lowest concentration of hypoxanthine. This may be a spurious result reflecting a greater error in determining precursor specific activity because of the small tissue hypoxanthine content. This difference in the soleus appears to be anomalous, since differences were not apparent in the other fiber type sections for hypoxanthine, nor were there any differences for any of the fiber types for adenine salvage. As a result, we have pooled the data from the four concentrations to calculate the average salvage rate for each fiber type for adenine and hypoxanthine.
Muscle Fiber Type

Purine salvage rates differ modestly, but significantly, among skeletal muscle fiber types (Table 1). The low-oxidative white gastrocnemius generally has the lowest salvage rate, whereas the high-oxidative red gastrocnemius exhibits the fastest. Interestingly, hypoxanthine salvage rates in the high-oxidative slow-twitch soleus were similar to those in the low-oxidative fast-twitch white gastrocnemius. Salvage rates of adenine are nearly twice as high as those of hypoxanthine. Moreover, the addition of 4.0 mM ribose increased adenine salvage three- to sixfold and hypoxanthine salvage six- to eightfold among fiber types. As illustrated for the plantaris muscle in Fig. 5, this influence of ribose is concentration dependent.

Relationship Between Purine Salvage and De Novo Synthesis

After confirming \(n = 2\) the reproducibility of de novo synthesis rates that we measured previously \(n = 4\) (34, 35), we examined de novo synthesis in the presence of adenine. As illustrated in Fig. 6, de novo synthesis was significantly reduced \((P < 0.05)\) in all fiber types by the addition of adenine [0.2 mM \((n = 2)\), 0.5 mM \((n = 1)\), or 1.0 mM \((n = 2)\)]. Similarly, perfusion with adenine markedly reduced \((P < 0.05)\) the increase in de novo synthesis rates stimulated by 4.0 mM ribose supplementation \((n = 2)\).

DISCUSSION

The present results extend the findings of Tully and Sheehan (38), who used isolated muscle incubated in vitro, in showing that purine salvage to AdN proceeds at a modest rate that is not identical across skeletal muscle fiber types. As expected from the relative activities of HPRT and APRT (29), hypoxanthine is salvaged at a lower rate \((P, 0.05)\) for all fiber sections than is adenine; the greatest contrast in salvage rates between these two purines is apparent by the exceptionally low rate of hypoxanthine salvage in the slow-twitch red fibers of the soleus muscle compared with the high rate

### Table 1. Purine salvage rates in the different skeletal muscle fiber types of the rat

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Plantaris Mean(\pm SE)</th>
<th>Soleus Mean(\pm SE)</th>
<th>Gastrocnemius Mean(\pm SE)</th>
<th>Mean Difference Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>20</td>
<td>48.6 (\pm 3.7)</td>
<td>47.5 (\pm 2.9)</td>
<td>55.9 (\pm 4.2)</td>
</tr>
<tr>
<td>Hypoxanthine(^b)</td>
<td>12</td>
<td>29.3 (\pm 4.3)</td>
<td>19.2 (\pm 1.6)?</td>
<td>31.8 (\pm 2.2)</td>
</tr>
<tr>
<td>Adenine + 4.0 mM ribose(^c)</td>
<td>5</td>
<td>250 (\pm 20)</td>
<td>143 (\pm 13)</td>
<td>262 (\pm 25)</td>
</tr>
<tr>
<td>Hypoxanthine + 4.0 mM ribose(^c)</td>
<td>4</td>
<td>200 (\pm 30)</td>
<td>116 (\pm 27)?</td>
<td>244 (\pm 45)</td>
</tr>
</tbody>
</table>

Values are means \(\pm SE\). Concentrations of purine base were combined in experiments without ribose; 2.0 mM purine base was used when ribose was added. Mean difference needed to be significant, \(P < 0.05\) by Tukey's procedure. \(^b\)Hypoxanthine salvage rates lower than adenine salvage rates \((P < 0.05)\). \(^c\)Significantly lower than red gastrocnemius \((P < 0.05)\). \(^\dagger\)Significant ribose effect to increase purine salvage rates \((P < 0.001)\). \(\dagger\)Significantly less than all other fiber sections \((P < 0.05)\).

Fig. 5. Increase in adenine salvage in the plantaris muscle as a function of ribose concentration ([ribose]) in the perfusion medium \((n = 2–5/group)\). Inset: same results expressed on a logarithmic scale for ribose.

Fig. 6. Influence of adenine and ribose on de novo synthesis rates in soleus, red gastrocnemius, and white gastrocnemius muscle sections. Addition of adenine [0.2, 0.5, or 1.0 mM, \(n = 5\) combined] to the perfusion medium decreases de novo synthesis rates with \((P < 0.05)\) and without \((P < 0.005)\) ribose [4.0 mM ribose and 0.2 mM adenine, \(n = 2\) stimulation]. Inherent de novo synthesis rates were taken from our previous work \((34)\). Ribose-enhanced rates \((n = 6)\) include data from our previous work \((n = 4)\) (35).
of adenine salvage for this same fiber section (Table 1). Results of the present study were obtained using an isolated perfused hindlimb preparation, where the purine substrate was delivered through the vasculature at a high flow rate over a wide range of concentrations. Even though the uptake of purines by myocytes in culture can be achieved at low concentrations of purines [e.g., Michaelis-Menten constant of 1–10 μM (7)], we used relatively high purine concentrations to control the precursor pool specific activity and to assess the influence of supply concentration in the complex whole muscle tissue. After extensive washout of the extracellular compartment (Fig. 1), tissue specific activities of adenine and hypoxanthine were found to be similar to the extracellular perfusate specific activities over a broad range of plasma concentrations. This provides assurance that our measured rates of purine salvage have not been confounded by a mixture of intracellular- and extracellular-derived precursor. Thus we believe that our results represent valid rates of purine salvage.

Another potential complication in the interpretation of our results stems from purine metabolism in non-skeletal muscle cells within the tissue. Because the salvage precursor obligatorily passes through the vasculature to reach the myocytes, considerable metabolism by the vascular endothelium could act as a barrier and impact salvage measurements. Indeed, cultured endothelial cells are capable of purine salvage and de novo synthesis (9). Although skeletal muscle endothelial cells have been implicated in transport and metabolism at micromolar concentrations of purines in perfusion studies (12), we suspect that purine metabolism by the endothelium would become quantitatively less important at the higher precursor concentrations used here. Purine base concentrations in the present study were 3- to 100-fold higher than normally found in whole muscle tissue. Thus it seems unreasonable to assert that uptake of adenine and hypoxanthine was limited to the endothelium, which represents an exceedingly small fraction (~0.5%) of the total tissue volume. Furthermore, salvage rates in the endothelial cells would have to be exceptionally high to account for a substantial fraction of the response of the whole muscle tissue. For example, capillary endothelial cells would have to exhibit a salvage rate ~20-fold greater than that of the myocytes to contribute ~10% of the purine incorporated into AdN in this study. This estimate is calculated on the measured capillary surface area of 25 mm²/mm³ fiber for mixed-fiber muscle (28), an in vivo cell height of 0.2 μm (31), and an extracellular volume of 15% (20). Additionally, the soleus muscle, which has an exceptionally high capillary density and, consequently, a high content of endothelium compared with the other fiber sections examined, has one of the lowest hypoxanthine salvage rates. If the endothelium or other component of the vasculature were a major site of purine salvage, the soleus would be expected to have a relatively high salvage rate. As a result, it is unlikely that the endothelium is a major contributor to the total purine salvage rates measured in our study.

The relatively stable rates of purine salvage observed over a ~15- to 25-fold range of precursor supply (Fig. 4) imply that a low purine supply, possibly characteristic of that found physiologically, is sufficient to sustain the modest rates of purine salvage to AdN normally found in skeletal muscle. This is similar to the findings of Brown et al. (7) for mature cardiac myocytes incubated in culture. Thus, although high concentrations of purine precursor were used experimentally in this study, they are not needed physiologically. It is well established that PRPP and its precursor ribose-5-phosphate are rate limiting in AdN synthesis for de novo synthesis (6) and purine salvage pathways (23). This is why AdN synthesis is markedly accelerated by provision of glucose (6) and ribose (42, 43). We have verified that ribose supply increases adenine and hypoxanthine salvage rates by three- to eightfold (Table 1). Furthermore, we have shown that the influence of ribose appears to be saturable at concentrations somewhat higher than the 10 mM maximum used. Interestingly, perfusing the muscle with 4.0 mM ribose not only increased the adenine salvage rate dramatically but also removed the nonlinearity in adenine salvage that was observed over time. The decline in adenine salvage during the second 0.5 h of perfusion observed in the absence of ribose (Fig. 2) was eliminated by 4.0 mM ribose. This suggests that, in the case of adenine, but not hypoxanthine, ribose-5-phosphate became limiting over time. The nonlinearity is not likely due to the characteristic impediment to purine salvage caused by the absence of glucose (6), since 5 mM glucose was maintained in the perfusion medium. On the other hand, the higher inherent rates of adenine salvage, compared with hypoxanthine (Table 1), are consistent with PRPP being limiting. However, direct measurements of muscle ribose-5-phosphate and PRPP contents will be needed to test this possibility.

Our results illustrate the reciprocity between the de novo and purine salvage pathways, likely through competition for PRPP (41). The large decline that was observed in the rates of de novo synthesis on the addition of adenine to the perfusion medium (Fig. 6) can be attributed to the greater activities and affinities of two key enzymes in the salvage pathway (APRT and HPRT) for PRPP than of amidotransferase, the rate-limiting step in the de novo pathway (27, 39). Interestingly, the higher the inherent rate of de novo synthesis among fiber sections, the greater was the reduction with adenine. This is consistent with competition for available PRPP. Thus the relatively low rate of de novo synthesis, characteristic of the low-oxidative white fibers, was reduced the least by adenine. We also have shown that the accelerated rate of de novo synthesis, established by ribose supplementation, could not be sustained during conditions of accelerated adenine salvage. De novo synthesis rates declined; however, the absolute rates of de novo synthesis in the presence of adenine + ribose were better preserved than in the absence of ribose (Fig. 5). This again places importance
on PRPP availability, while the relative capacity for de novo synthesis is secondary to the more efficient salvage pathway for AdN synthesis. This does not mean that the de novo pathway is inconsequential, as illustrated by knockout mice that lack the HPRT gene (1, 21) or lack the HPRT and APRT genes (11). Neither knockout mouse is an adequate behavioral model for their intended purpose to assess the pathogenesis of Lesch-Nyhan syndrome, but both appear healthy and have proven useful in the study of purine management. The elevated rates of de novo synthesis measured in some tissues of the HPRT mutant mice (1) likely contribute to the apparent health of the animal. It is likely that de novo synthesis is also elevated in the double-knockout animals. Even though these animals appear to function normally at rest, analysis of purine management during or after intense exercise, where AdN degradation is accelerated, may elicit additional differences in AdN metabolism.

The need for de novo synthesis and purine salvage of AdN is probably most acute after circumstances that cause exaggerated AdN degradation, such as ischemia and/or intense muscle contractions. De novo synthesis of AdN proceeds at a relatively low rate in skeletal muscle (34), is reduced during muscle contractions (35), and is tempered in deference to purine salvage (Fig. 5) (30, 41). Thus the process of purine salvage must be important in skeletal muscle, especially after intense exercise. AdN degradation in humans, as evident by release of hypoxanthine, continues for an extended time during recovery (18) in a manner related to exercise intensity (33). This degradation can be extensive, with as much as 40–50% of the muscle decline in ATP accounted for by efflux of hypoxanthine (15). Any net efflux of inosine and/or hypoxanthine from the muscle requires compensation, if the muscle’s AdN pool is to be maintained. Interestingly, a number of studies have shown that muscle ATP concentration is significantly lower (15–20%) after several days of intense cycle exercise (16, 19, 32). Either the muscle is remodeling to a phenotype exhibiting a lower ATP concentration or AdN production processes are inadequate to fully recover muscle ATP concentration. An increase in the enzymatic activity of HPRT in high-intensity trained individuals (17) implies that adaptations have occurred to enhance ATP recovery. Thus it may be that an insufficiently low rate of purine salvage is the cause for the reduced ATP concentration after repeated days of high-intensity cycle exercise. Results of the present study support this hypothesis in showing that the rates of hypoxanthine salvage are lowest in the low-oxidative fast-twitch white fibers. Furthermore, this fiber type exhibits the lowest rate of de novo synthesis (34). Although there is not a precise equivalent of this phenotype in human muscle, the fast-twitch fibers of humans are the most likely to produce copious amounts of IMP during intense exercise that can lead to AdN degradation (10, 22). If our findings in rat muscle are at all applicable to human muscle, then the decline in muscle ATP after repeated days of intense cycling could be due to insufficient resynthesis of AdN.

The influence of ribose may be particularly germane in this context. The large increases in purine salvage with ribose in the perfusion medium suggest that ribose supplementation could improve ATP recovery during repeated days of intense cycle exercise. Future experiments are needed to test this hypothesis.

In conclusion, we show that purine salvage rates to AdN are greater for adenine than for hypoxanthine and differ among the various skeletal muscle fiber types. The marked increase in purine salvage that occurs with ribose supply could be important during conditions where purine salvage is critical to the maintenance of the AdN pool within the muscle.

This study was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-21617.

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