Effect of ribose supplementation on resynthesis of adenine nucleotides after intense intermittent training in humans

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Hellsten, Y., L. Skadhauge, and J. Bangsbo. Effect of ribose supplementation on resynthesis of adenine nucleotides after intense intermittent training in humans. Am J Physiol Regul Integr Comp Physiol 286: R182–R188, 2004; 10.1152/ajpregu.00286.2003.—The effect of oral ribose supplementation on the resynthesis of adenine nucleotides and performance after 1 wk of intense intermittent exercise was examined. Eight subjects performed a random double-blind crossover design. The subjects performed cycle training consisting of 15 × 10 s of all-out sprinting twice per day for 7 days. After training the subjects received either ribose (200 mg/kg body wt; Rib) or placebo (Pla) three times per day for 3 days. An exercise test was performed at 72 h after the last training session. Immediately after the last training session, muscle ATP was lowered (P < 0.05) by 25 ± 2 and 22 ± 3% in Pla and Rib, respectively. In both Pla and Rib, muscle ATP levels at 5 and 24 h after the exercise were still lower (P < 0.05) than pretraining. After 72 h, muscle ATP was similar (P > 0.05) to pretraining in Rib (24.6 ± 0.6 vs. 26.2 ± 0.2 mmol/kg dry wt) but still lower (P < 0.05) in Pla (21.1 ± 0.5 vs. 26.0 ± 0.2 mmol/kg dry wt) and higher (P < 0.05) in Rib than in Pla. Plasma hypoxanthine levels after the test performed at 72 h were higher (P < 0.05) in Rib compared with Pla. Mean and peak power outputs during the test performed at 72 h were similar (P > 0.05) in Pla and Rib. The results support the hypothesis that the availability of ribose in the muscle is a limiting factor for the rate of resynthesis of ATP. Furthermore, the reduction in muscle ATP observed after intense training does not appear to be limiting for high-intensity exercise performance.

Adenosine 5’-triphosphate; purines; skeletal muscle

DURING INTENSE EXERCISE a fraction of the ATP pool in human skeletal muscle is degraded to inosine 5’-monophosphate (IMP) (12, 13, 19). The majority of IMP formed remains within the muscle cell as this compound is not membrane permeable, and on termination of exercise IMP is reaminated and rephosphorylated to ATP. Nevertheless, a fraction of IMP is dephosphorylated to inosine that can leave the cell and enter the bloodstream as inosine or the degradation product hypoxanthine (8). From previous studies it is evident that the magnitude of release of inosine and hypoxanthine from the muscle depends on the intensity and duration of exercise (7, 8, 10). When intense exercise is repeated frequently, as in training, there is an accumulated loss of nucleotides from the muscle (6, 10, 11) that, at least in part, can be explained by a repeated release of purines (7). Lost nucleotides have to be restored either via the purine salvage pathway, involving resynthesis of AMP from inosine or hypoxanthine, or via de novo synthesis. It has been shown that the human muscle does not recover hypoxanthine or inosine from plasma at rest, during, or after exercise (7, 8). Thus restoration of the nucleotide pool appears to occur mainly via salvage of intracellular purines or via de novo synthesis. The rate of de novo synthesis of adenine nucleotides has in rats been determined to be 35 μmol-kg dry wt⁻¹h⁻¹ (23), and a similar relatively slow rate of resynthesis of adenine nucleotides in humans could explain the lowered resting ATP after a period of intense exercise training (11, 21).

The limiting step in nucleotide synthesis de novo has been debated, but the availability of phosphoribosyl pyrophosphate (PRPP) has been proposed to be critical. PRPP is synthesized from ribose 5’-phosphate, which, in turn, is formed from the phosphorylation of ribose. The level of ribose in the muscle is limited; thus an increased availability of ribose may enhance the formation of PRPP and the rate of synthesis of adenine nucleotides. In accordance with that notion, perfusion of rat skeletal muscle with ribose has been shown to markedly enhance the rate of nucleotide synthesis (4). However, to what extent ribose is a limiting factor for muscle ATP resynthesis in human skeletal muscle remains unclear. Such information will not only be useful for people performing regular physical exercise but may also be important for different groups of patients who have impaired skeletal muscle metabolism, such as patients with congestive heart failure (14, 25) and peripheral arterial disease (3).

In the earlier studies showing a lowered resting level of ATP after training (6, 11, 21), it was not examined to what extent the reduced ATP level affected high-intensity exercise performance. In a recent study by Op't Eijnde et al. (17), ribose supplementation did not result in improved intense exercise performance. However, as the study did not document an effect of ribose on ATP resynthesis, the role of reduced ATP levels for performance could not be elucidated.

Thus the main aim of the present study was to assess the effect of oral intake of ribose after frequent, high-intensity training on adenine nucleotide resynthesis. A secondary aim was to assess whether a decrease in resting ATP levels in the muscle is of importance for intense intermittent exercise performance.

METHODS

Subjects

Eight healthy male subjects with an average age of 25 ± 1.8 yr, a height of 183.9 ± 2.1 cm, and body mass of 86.2 ± 4.8 kg...
volunteered to participate in the study. They had a maximal oxygen uptake (\(\dot{V}O_2\) max) of 4.1 ± 0.2 l/min. All subjects were nonsmokers, and none were vegetarians. They participated regularly in physical activity, but none of the subjects were athletes. All subjects were fully informed of the nature of the study and the possible risk and discomforts associated with the experimental procedures before they gave their written consent to participate. The study was approved by the Frederiksberg and Copenhagen Ethics committee.

**Procedures**

Subjects performed two-legged cycling exercise in the upright position on a Monark cycle ergometer (829E). All subjects completed several cycle tests before the experiments. In one test \(\dot{V}O_2\) max was determined using a standard protocol. Before the start of the training, the subjects had been familiarized with the experimental procedures and had conducted 10–15 maximal 10-s cycle sprints separated by 50 s of rest, on at least one occasion.

**Protocol**

The study was of a random double-blind crossover design. The subjects participated in two identical 7-day training protocols on two occasions, 5–9 wk apart. The subjects performed two training sessions, separated by 5–7 h, per day and 14 training sessions in total. Each training consisted of 10 min of warming up at an intensity of 65% followed by a 3-min rest period and then 15 10-s maximal exercise bouts separated by a 50-s rest period. The subjects repeated the exercise protocol 72 h after the last training session (72-h test).

On one occasion the last training session was followed by oral intake of sucrose [ribose group (Rib)] and on the other occasion by sucrose [placebo group (Pla)]. The first supplementation was ingested 10 min after the last training session and thereafter at each main meal (breakfast, lunch, and dinner) for 3 days, with the last intake being at ~60 h after the last training session.

A muscle biopsy was taken from the vastus lateralis muscle of each leg at rest, 6–7 days before the first training session. Moreover, muscle biopsies were taken immediately after the last training session as well as 5, 24, and 72 h after the training. To facilitate immediate sampling on cessation of exercise, a small incision in the skin and fascia was made before the experiment. The biopsies obtained after exercise were frozen in liquid nitrogen within 10 s of cessation of exercise, and in general, the muscle samples were frozen within 5 s of collection. The biopsies were stored at −80°C until analyzed.

Before the last training session as well as before the test 72 h after the last training session, a venflon was inserted in an antecubital vein for collection of blood samples. In each test, blood (2–5 ml) was drawn in heparinized syringes, before the first sprint, before the last exercise bout (~10 s), and immediately after the last exercise bout as well as 5, 10, 20, 30, 45, 60, and 120 min in recovery after exercise.

A part of the blood sample was immediately centrifuged for 30 s, and plasma was collected and stored at −80°C. Another part was hemolized with Triton X-100 for analysis of blood lactate. The remaining part of the blood sample was stored on ice until analyzed.

**Analysis**

**Muscle biopsies.** Muscle samples were analyzed for total water content by weighing the samples before and after freeze drying. They were subsequently analyzed for creatine phosphate (CP), creatine, lactate, and glycogen by enzymatic fluorometry (16). Adenine nucleotides and IMP were determined on neutralized muscle extracts with a reverse-phase HPLC method (24).

**Plasma analysis.** Plasma hypoxanthine and urate were determined on neutralized plasma perchloric acid extracts with a reverse-phase HPLC method (24). Glucose and lactate were determined using a lactate/glucose analyzer (model 2300, Yellow Springs Instruments). Lactate was analyzed on hemolyzed fresh blood (5). Plasma concentrations of insulin and catecholamines were analyzed using RIA kits (Pharmacia).

**Power output.** The number of revolutions per second was recorded throughout the test, and based on the work load, which was 7.5% of the body mass, the power output expressed per second was calculated taking into account the work performed to accelerate the fly wheel. Subsequently, the peak power output and total work performed in each exercise bout was calculated.

**Statistics.** Differences between rest and exercise, between Rib and Pla, and between the 14th training session and the 72-h test were examined by two-way repeated-measures ANOVA with Tukey’s test for post hoc comparisons between groups. A significance level of 0.05 was chosen. SE is only given in the text where this value cannot be obtained from a figure.

**RESULTS**

**Muscle Nucleotides and IMP**

Pretraining skeletal muscle ATP levels were 26.0 ± 0.2 mmol/kg dry wt in Pla and 26.2 ± 0.5 mmol/kg dry wt in Rib. Immediately after the last training session, muscle ATP was lowered (\(P < 0.05\)) by 25 ± 2 and by 22 ± 3% in Pla and Rib, respectively (Fig. 1A). In both Pla and Rib, muscle ATP levels...
at 5 and 24 h after training were still lower (P < 0.05) than pretraining levels. At 72 h after training, the ATP level (24.6 ± 0.6 mmol/kg dry wt) was not different (P > 0.05) from that at pretraining in Rib, whereas in Pla the ATP level (21.1 ± 0.5 mmol/kg dry wt) was still lower (P < 0.05) than pretraining levels. The ATP level at 72 h was also higher (P < 0.05) in Rib than in Pla. There was no order effect; thus the ATP level was maintained lower (P < 0.05) than immediately after exercise and was significantly (P < 0.05) lower than both pretraining and the same (P < 0.05) as after 72 h of recovery. There were no differences (P > 0.05) in muscle glycogen levels between Pla and Rib (Table 1).

**Plasma Hypoxanthine and Urate**

Plasma hypoxanthine concentrations at 10 and 20 min after the last training session were higher (P < 0.05) than before exercise in both Pla and Rib, and thereafter it was similar (P > 0.05) to preexercise levels (Fig. 2). In the 72-h test the plasma hypoxanthine level was higher (P < 0.05) than at rest during the first 60 min after exercise in Rib and between 10–30 min in Pla. Furthermore, in Rib, plasma hypoxanthine levels during the whole recovery period after the 72-h test were higher (P < 0.05) than after the last training session and higher (P < 0.05) in Rib compared with Pla during the first 45 min after exercise.

Plasma urate levels after the last training session were similar (P > 0.05) in Pla and Rib (Fig. 3). In the 72-h test, the plasma urate levels in Rib were higher (P < 0.05) than after the last training session and higher (P < 0.05) than in Pla from 10 min after exercise. In Pla, plasma urate in the 72-h test was not different (P > 0.05) from either preexercise or the first 72 h after exercise.

**Muscle CP, Glycerogen, and Lactate**

Muscle CP levels were 86.0 and 84.1 mmol/kg dry wt before training in Pla and Rib, respectively, and immediately after the last training session the levels were lowered (P < 0.05) by 70 ± 3 and 61 ± 4%, respectively (Table 1). After 5 h of recovery, muscle CP was similar (P > 0.05) to pretraining levels in both Pla and Rib, but the level was lower (P < 0.05) in Rib compared with in Pla.

The muscle lactate concentrations immediately after the last training session were similar (P > 0.05) in Pla and Rib (89.6 and 79.9 mmol/kg dry wt, respectively), and the levels at 5, 24, and 72 h after exercise were not different (P > 0.05) from pretraining concentrations (Table 1). Immediately after the last training session, the muscle glycogen concentration in Pla (418 mmol/kg dry wt) was not different (P > 0.05) from the pretraining level (474 mmol/kg dry wt; Table 1). After 24 h of recovery from exercise the muscle glycogen concentration in Pla (647 mmol/kg dry wt) was higher (P < 0.05) than both before training and immediately after the last training session and the same (P > 0.05) as after 72 h of recovery. There were no differences (P > 0.05) in muscle glycogen levels between Pla and Rib (Table 1).

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**Table 1. Muscle metabolite concentrations before and after a 7-day period of intense training in the placebo and the ribose trial**

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>5 h Posttraining</th>
<th>24 h Posttraining</th>
<th>72 h Posttraining</th>
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</thead>
<tbody>
<tr>
<td><strong>Muscle CP, mmol/kg dry wt</strong></td>
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</tr>
<tr>
<td>Pla</td>
<td>86.0±3.0</td>
<td>25.4±2.1*</td>
<td>90.1±2.9</td>
<td>91.0±3.7</td>
<td>79.4±5.2*</td>
</tr>
<tr>
<td>Rib</td>
<td>84.1±2.4</td>
<td>32.9±3.0†</td>
<td>76.9±4.8†</td>
<td>83.3±2.3</td>
<td>84.5±4.0</td>
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<tr>
<td><strong>IMP, mmol/kg dry wt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pla</td>
<td>0.0±0.0</td>
<td>1.4±0.3*</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
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<tr>
<td>Rib</td>
<td>0.0±0.0</td>
<td>1.2±0.4*</td>
<td>0.1±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
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<tr>
<td><strong>Glycogen, mmol/kg dry wt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pla</td>
<td>474±19</td>
<td>418±13</td>
<td>455±44</td>
<td>647±21*</td>
<td>639±59*</td>
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<tr>
<td>Rib</td>
<td>475±20</td>
<td>428±27</td>
<td>466±28</td>
<td>655±26*</td>
<td>711±51*</td>
</tr>
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<td><strong>Lactate, mmol/kg dry wt</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pla</td>
<td>10.7±1.8</td>
<td>89.6±5.9*</td>
<td>5.7±1.4</td>
<td>6.3±1.3</td>
<td>8.8±1.9*</td>
</tr>
<tr>
<td>Rib</td>
<td>7.5±1.4</td>
<td>79.9±6.6*</td>
<td>7.1±0.8</td>
<td>6.6±0.8</td>
<td>7.8±1.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. CP, creatine phosphate; IMP, inosine 5’-monophosphate. *Denotes significant difference from pretraining, †denotes significant difference from Pla.

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Fig. 2. Plasma hypoxanthine concentrations before and after the last (14th) training session (circles) and before and after a test performed at 72 h (triangles) after the last training session. Oral intake of either placebo (filled symbols) or ribose (open symbols) was commenced 10 min after the last training session (circles) and before and after a test performed at 72 h (triangles). *P < 0.05, significantly different from preexercise. †P < 0.05, significantly different from placebo (72 h test). ‡P < 0.05, significantly different from last (14th) training session.
enhanced resynthesis of ATP, suggesting that a limiting factor for the rate of resynthesis of ATP is the availability of ribose and thus PRPP in the muscle. The present data furthermore show that the magnitude of decrease in ATP observed after 1 wk of frequent high-intensity training is not limiting for intense exercise performance, as performance 72 h after the last training session was similar with and without ribose supplementation, despite a difference in resting ATP levels.

The present and previous (6, 11, 21) studies show that a period of high-intensity training can lead to a loss of ATP from the muscle, resulting in lowered resting ATP levels, lasting for over 72 h. This observation suggests that the repeated loss of ATP from the muscle during this type of frequent intense training is higher than the rate of ATP resynthesis via the purine salvage or the de novo pathways. The rate of purine salvage is probably low in humans as it has been observed that there is no uptake of purines by the muscle even when plasma levels are high in association with exercise (7, 8). Moreover, in humans intracellular muscle purine levels show a minor increase after exercise that can only account for a negligible fraction of the reduced ATP (7, 22). Therefore, in humans ATP resynthesis probably occurs mainly via de novo synthesis. The rate of de novo synthesis of nucleotides has in rat mixed muscle been determined to 35 μmol·kg dry wt⁻¹·h⁻¹ (23). In the present study, 1 wk of frequent training resulted in an

Fig. 3. Plasma urate before and after the last training session (circles) as well as before and after the test 72 h (triangles) after the last training session in placebo (filled symbols) or ribose trial group (open symbols). Data are means ± SE. *P < 0.05, significantly different from preexercise. #P < 0.05, significantly different from placebo (72-h test). $P < 0.05, significantly different from last (14th) training session.

Fig. 4. Plasma glucose (A) and insulin (B) before and after the last training session in placebo (filled circles) or ribose trial group (open circles). Data are means ± SE. *P < 0.05, significantly different from before supplementation (Suppl). #P < 0.05, significantly different from placebo.
Table 2. Plasma epinephrine and norepinephrine concentrations before and after the last (14th) intense training session and the test performed at 72 h in the placebo and the ribose trial

<table>
<thead>
<tr>
<th>Epinephrine, ng/ml</th>
<th>Norepinephrine, ng/ml</th>
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<tbody>
<tr>
<td>Last (14th) training session</td>
<td></td>
</tr>
<tr>
<td>Preexercise</td>
<td>Pla Rib</td>
</tr>
<tr>
<td>0 min</td>
<td>0.31±0.48*</td>
</tr>
<tr>
<td>10 min</td>
<td>5.31±0.38*</td>
</tr>
<tr>
<td>30 min</td>
<td>0.26±0.07</td>
</tr>
<tr>
<td>72 h test</td>
<td></td>
</tr>
<tr>
<td>Preexercise</td>
<td>0.29±0.08</td>
</tr>
<tr>
<td>Posttest</td>
<td>3.38±0.83*</td>
</tr>
<tr>
<td>0 min</td>
<td>0.48±0.14</td>
</tr>
<tr>
<td>10 min</td>
<td>0.37±0.07</td>
</tr>
<tr>
<td>30 min</td>
<td></td>
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</table>

Values are means ± SE; n = 8. Pla, placebo trial; Rib, ribose trial. *P < 0.05, significantly different from preexercise value (n = 6).

The present finding of an effect of ribose on the resynthesis of ATP after high-intensity training differs from that of Op’t Eijende et al. (17) who observed that ribose did not significantly affect nucleotide resynthesis after a period of intense training. However, the abovementioned (17) study design included only measurements up to 24 h after the final training session, which, according to the present study, was insufficient time allowed for resynthesis of ATP at the dose of ribose administered.

The plasma concentrations of hypoxanthine and urate in Rib were markedly lower after the 14th training session compared with after the 72-h test. It is likely that the reduced plasma purine level is related to lowered resting muscle ATP levels, which is supported by the observation that the plasma hypoxanthine concentration after the test at 72 h was approximately twofold higher in Rib than in Pla and the plasma urate was higher in Rib than in Pla. The finding that, in Pla, plasma hypoxanthine was higher in the initial phase of recovery from
the 72-h test compared with after the last training session despite that muscle ATP was the same, suggests also that factors other than ATP are influencing plasma hypoxanthine concentrations. Plasma catecholamines have been suggested to affect purine turnover (8), but they cannot explain the differences observed, because the catecholamine concentrations were the same in Rib and Pla both after the last training session and after the test performed 72 h after the last training. The findings in the present study are in agreement with the observation that the exercise-induced accumulation of IMP is markedly reduced when resting muscle ATP levels are lowered after a period of high-intensity exercise (21).

A lowering of resting ATP levels, induced by high-intensity training, could potentially affect high-intensity exercise performance. Previous studies showing a reduced resting level of muscle ATP after a period of high-intensity training did not deduce to what extent this reduction affected high-intensity performance (11, 21). In the present study there was no difference in performance between Pla and Rib for the 15×10-s all-out test performed at 72 h after the last training session despite the difference in resting ATP levels. Thus a lowering in resting ATP of 4–5 mmol/kg dry wt does not appear to affect high-intensity intermittent exercise performance.

The decrease in resting adenine nucleotides observed after training in the present study was 15 and 20%, which is similar in magnitude to the decreases that have been previously reported after high-intensity exercise training (6, 11, 21). The results of our study also show that the loss of approximately 4–5 mmol/kg dry wt in resting ATP after only 1 wk of high-intensity training is reproducible as a similar loss was observed after both of the present training periods and also of a similar magnitude as that previously observed with the same training protocol (11). Although the mechanism underlying the loss of ATP from the muscle during intense exercise has not been directly determined, the present and other studies provide evidence that at least some of the loss is accounted for by a considerable release of purines from muscle to plasma after high-intensity exercise (7, 8). Whether this release of purines from muscle serves a physiological purpose, such as in the generation of the antioxidant urate (9), remains speculative.

Insulin levels increased 20 min after exercise after intake of ribose in combination with sucrose as well as after intake of placebo in combination with sucrose, whereas blood glucose levels at 60 and 90 min after intake were lower than preintake levels only in the ribose group. The reasons for the lowered blood glucose may be the elevated insulin levels and muscle glycogen resynthesis, but it does not explain the difference between Pla and Rib. The ribose supplementation as well as the placebo contained 200 mg/kg body wt sucrose as ribose intake alone was observed in a pretrial to lower the blood glucose level from 4.3 to 2.2 mM. Thus at this dose of ribose and above it is clear that sugar has to be added to the supplement to prevent hypoglycemia. Another reason for the lowered blood glucose is that glucose is used for muscle glycogen synthesis. The muscle glycogen level immediately after exercise was lower but not significantly different from the pretraining level despite the work performed in the last training bout. This is probably an effect of training, because intense training leads to elevated resting muscle glycogen levels (15). Similarly, it can explain that the muscle glycogen level at 24 h after training was higher than before the training combined with a significant ingestion of carbohydrates in the recovery period. The lower levels of blood glucose in Rib cannot be explained by difference in insulin levels or glycogen synthesis; however, it has been suggested that ribose can inhibit the degradation of glycogen in the liver (2, 19).

In conclusion, the results of the present study support the previous observation that 1 wk of frequent intense training is sufficient to markedly lower the resting muscle adenine nucleotide level. The data moreover show that oral intake of ribose after training enhances the rate of adenine nucleotide resynthesis, probably by increasing the rate of PRPP synthesis. The finding, therefore, suggests that availability of PRPP is a rate-limiting factor for ATP resynthesis in human skeletal muscle. Finally, the reduction in resting ATP observed after intense training does not appear to affect intense intermittent exercise performance.

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