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Sprint Training Reduces Urinary Purine Loss Following Intense Exercise in Humans

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Intermittent exercise, inosine, hypoxanthine, uric acid, purine excretion.

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Abstract

The influence of sprint training on endogenous urinary purine loss was examined in seven active male subjects (age: 23.1 ± 1.8 years, weight: 76.1 ± 3.1 kg, VO_2peak: 56.3 ± 4.0 $ml.kg^{-1}.min^{-1}$). Each subject performed a 30s sprint performance test (PT), before and after 7 days of sprint training. Training consisted of fifteen 10s sprints on an air-braked cycle ergometer performed twice per day. A rest period of 50s separated each sprint during training. Sprint training resulted in a 20% higher muscle ATP immediately after PT, a lower IMP (57% and 89%, immediately following and after 10 min recovery from PT, respectively), and inosine accumulation (53% and 56%, immediately following and 10 min after the PT, respectively). Sprint training also attenuated the exercise-induced increases in plasma inosine, hypoxanthine (Hx) and uric acid during the first 120 min of recovery and reduced the total urinary excretion of purines (inosine + Hx + uric acid) in the 24 hours recovery following intense exercise. These results show that intermittent sprint training reduces the total urinary purine excretion after a 30s sprint bout.

Key words: sprint training, inosine, hypoxanthine, uric acid, metabolism.

Introduction

The ATP content of untrained human skeletal muscle is about 20-25 mmol.kg^{-1} dry mass (d.m.), and several reports have demonstrated that intense intermittent sprint training can lower the resting content by up to 20% (Hellsten-Westing et al. 1993a; Stathis et al. 1994; Hellsten et al. 2004). The factors responsible for the set point of resting muscle adenine nucleotide (AdN=ATP+ADP+AMP) content are unknown, however, it is influenced by the balance between loss of purine base from the muscle and its production via *de novo* purine synthesis. Intense exercise results in a transient reduction of ATP and concomitant accumulation of IMP (Stathis et al. 1994). Most of this IMP is rapidly resynthesised to ATP during recovery, although a small portion is further degraded and results in the production of the purine bases inosine and hypoxanthine (Hx; Stathis et al. 1994). Purine bases, not recovered intramuscularly via purine salvage, efflux the muscle (Hellsten et al. 1999) and accumulate in the plasma (Bangsbo et al. 1992; Tullson et al. 1995; Stathis et al. 1999). Once in the plasma purine bases are not recovered by skeletal muscle (Hellsten et al. 1999). The magnitude of purine loss can be between 5% and 9% of the resting muscle ATP content (Bangsbo et al. 1992; Hellsten et al. 1999). It has been reported that the plasma purines are excreted in the urine (Nasrallah and Al Khalidi 1964; Sutton et al. 1980; Stathis et al. 1999), or converted to uric acid (Hellsten et al. 1994) and subsequently excreted (Nasrallah and Al Khalidi 1964; Sorensen and Levinson 1975; Stathis et al. 1999). Sorensen and Levison (1975) demonstrated that, in a resting individual, the uric acid loss via the gut is one third, whilst via the urine is two thirds of the total uric acid loss in the untrained individual.

Exercise intensity and duration can influence the plasma purine accumulation in recovery (Sjödin and Hellsten-Westing 1990; Hellsten et al. 1999), as does the

frequency and number of intermittent sprint bouts (Balsom et al. 1992a; Balsom et al. 1992b; Stathis et al. 1999). The number of repetitions in a sprint challenge also influences the excretion of urinary purines during recovery (Stathis et al. 1999).

Sprint training reduces the accumulation of IMP (Stathis et al. 1994; Harmer et al. 2000; Parra et al. 2000) and inosine (Stathis et al. 1994) in the muscle following an intense sprint. Furthermore, sprint training attenuates the plasma inosine, Hx and uric acid accumulation during recovery from an intense exercise bout (Stathis et al. 1994). However, the effect of sprint training on urinary purine excretion following a maximal sprint bout is unknown.

The aim of this study was to investigate the effects of an intense seven-day sprint training program on the loss of endogenous purine metabolites in the urine during recovery from an intense sprint bout. It was hypothesised that sprint training will reduce the accumulation of muscle IMP and inosine during a 30 s print bout and attenuate the concentration of plasma purine metabolites, inosine, Hx and uric acid, during recovery from a 30 s sprint bout, consistent with previous studies. This will lead to a reduction in urinary excretion of purine metabolites during recovery from the sprint.

Methods

Subjects. Seven active, non-specifically trained males (age: 23.1 ± 1.8 years, weight: 76.1 ± 3.1 kg, VO_2peak: 56.3 ± 4.0 $ml.kg^{-1}.min^{-1}$), volunteered for the study, which was approved by the Victoria University Human Research Ethics Committee. All subjects were fully informed of the experimental procedures and voluntarily consented to take part in the study.

Peak oxygen consumption (VO_2peak). The VO_2peak of each subject was determined approximately one week prior to beginning the experimental trials. The exercise protocol involved riding on a cycle ergometer (Lode, Groningen) for three min at three submaximal work rates, after which the work rate was increased every min thereafter until volitional exhaustion. Expired air was directed by a Hans Rudolph valve through a ventilometer (Pneumoscan S30) into a mixing chamber and analysed for oxygen and carbon dioxide content by gas analysers (Applied Electrochemistry S-3A O_2 and CD-3A CO_2). These analysers were calibrated before each test using commercially prepared gas mixtures. Oxygen consumption was calculated by a microprocessor using standard equations.

Sprint testing protocol. Performance tests (PT) were 30s maximal sprints completed before and after a training regime. These were performed on an air-braked cycle ergometer, modified to enable computerized determination of peak power and mean power. Fatigue index (FI) was calculated using the equation FI = (peak power – power at the end of 30 s exercise)/ peak power x 100. The power output of the air-braked cycle ergometer is approximately proportional to the cube of the wheel velocity, which was measured using a tachometer (Hall-effect device and a cog at the wheel hub). The subjects were instructed to remain seated and pedal as fast as possible for the 30s exercise periods. Subjects were familiarised with the sprint task at least one week prior to the PT. The final PT was performed at least 24 hours and less than 36 hours following the last training session. The subjects were also instructed to refrain from strenuous exercise, caffeine and alcohol consumption 24 hours prior to the PT. In addition, subjects recorded their diet for the 24 hours prior to the first PT and were asked to consume similar foods before the final PT. All PT were conducted in the morning after an overnight fast. During the first 15 min of recovery from each

PT the subjects ingested 500 ml of water. Subjects were also provided with food (snack bar, fruit juice and salad sandwich) after two hours of recovery in each trial and were restricted from any other food intake until after an 8 hour urine sample was obtained.

Training protocol. Sprint training was performed twice per day for a week and each session was separated by at least 6 hours. A training session involved 15 x 10s maximal cycling bouts on an air-braked cycle ergometer with 50-s of rest between bouts.

Blood and urine sampling, treatment and analysis. Blood was sampled from an antecubital vein, via an indwelling catheter, at rest, and following 0, 10, 15, 20, 30, 60 and 120 min of passive recovery after the PT. The blood was immediately placed into lithium heparin tubes and spun in a centrifuge. Subsequently, 100 µl of plasma were added to 200 µl of ice-cold 3 M perchloric acid, spun and the supernatant was stored at -80 ^{o}C before analysis for lactate. Plasma lactate was determined in duplicate, using an enzymatic spectrophotometric technique (Lowry and Passoneau 1972). The remaining plasma was stored in liquid nitrogen for analysis of inosine, Hx and uric acid. The plasma stored for these metabolites was deproteinised with 1.5 M perchloric acid and subsequently neutralised with 2.1 M potassium hydrogencarbonate immediately prior to analysis. Plasma Hx, inosine and uric acid were determined on neutralised perchloric acid extracts, using a modification of the reverse-phase high performance liquid chromatography (HPLC) technique described by Wynants and Van Belle (1985). Separation was achieved using a Merck Hibar Lichrosphere 100 CH-18/2 250 x 4 mm column with the use of a Waters chromatography workstation.

Urine was collected for 12 hrs prior to the PT and for the first 2, and the subsequent 6, and 16 hour periods following the PT. Urine volume was determined and an aliquot of

the samples were stored at –80°C before analysis. Samples were deproteinised, neutralised and analyzed for inosine, Hx and xanthine using the same sample treatment and HPLC procedures as described for plasma. Urinary uric acid concentration was determined by an enzymatic colorimetric method using a Beckman Synchron CX ® system. Exercise-induced endogenous urinary purine excretion was determined by calculating the difference in excretion rate during recovery from the PT, relative to the pre-PT basal excretion rate.

Muscle sampling treatment and analysis. Muscle biopsies were obtained from the vastus lateralis under local anasthesia (1% xylocaine) using the percutaneous needle biopsy technique. Muscle was sampled from the same leg from separate incisions at rest, immediately after exercise, and 10 min into recovery. Leg selection was random with the contralateral leg biopsied in the second PT . Muscle samples were frozen in liquid nitrogen within 5 s of obtaining biopsies. Samples were weighed at –30°C and freeze-dried, weighed, dissected free of any connective tissue, powdered, and extracted according to the method of Harris et al. (1974). The neutralized extract was assayed enzymatically for phosphocreatine (PCr), creatine, and lactate by fluorometric analyses (Lowry and Passoneau 1972). Reverse-phase HPLC was used to quantify ATP, ADP, AMP, IMP, inosine and hypoxanthine concentrations using the method of Wynants and Van Belle (1985) with the same apparatus as described for plasma analysis. Muscle ATP, ADP, AMP, IMP, Cr and PCr were adjusted to the peak total creatine content for each subject in each PT for pre and post-training trials.

Statistical analysis. All values are reported as means ± SE. Where appropriate, the metabolite and performance data were analysed using analysis of variance (ANOVA) with repeated measures (BMDP statistical software). Simple main effects analyses and Newman-Kuels post hoc tests were used to locate differences when ANOVA

revealed a significant interaction. Comparison of performance variables, total creatine content and urinary purine loss during recovery were analysed using paired-sample T-Tests. The level of probability to reject the null hypothesis was set at $P \leq 0.05$.

Results

Performance variables. No change in the peak power (1085.1 ± 40.3 v 1081.7 ± 38; untrained vs trained), mean power (617.9 ± 20.4 v 630.4 ± 10.1) or fatigue index (58.9 ± 1.6% v 57.5 ± 1.4%) was observed with sprint training.

Muscle metabolites **.** The muscle metabolite content at rest, immediately post-exercise and 10 min into recovery are summarised in Table 1. No differences were observed with resting muscle metabolites before and after training. The resting ATP levels showed a tendency to be lower after training using different analytical methods ($p=0.06$ and 0.13, enzymatic and HPLC methods, respectively). Exercise decreased muscle ATP in both the pre- and post-training PT but the levels upon termination of exercise was significantly higher in the post-trained compared with the pre-trained state (Table 1). Concomitantly, sprint exercise resulted in an accumulation of IMP and inosine, however, the end exercise IMP and inosine were lower ($P<0.05$) with training (Table 1). The PCr, Cr and lactate levels after exercise were unaffected by the training.

The ATP content was partially restored 10 min into recovery from post-exercise values, but was still lower than pre-exercise amounts, irrespective of training status. Additionally, the levels of ATP at 10 min recovery were not different between trials. Similarly, the IMP content reduced during recovery but had not returned to resting levels in both trained and untrained states at 10 min. Muscle inosine continued to

increase during recovery in both trials, however the content was lower post-training compared with pre-training (Table 1). The PCr and Cr levels at 10 min recovery following training were not different to pre-exercise levels, whereas they were significantly lower and higher, respectively, than rest in the untrained state. Muscle lactate levels during recovery were not affected by training. Muscle ADP and AMP were not different following PT and 10 min recovery and were not different across training. The mean total creatine was higher in the post-trained state (123.1 ± 5.2 vs 131.5 ± 6.4; $P<0.05$, untrained vs. trained, respectively).

Plasma metabolite concentrations. The resting concentration of all measured metabolites were similar pre- and post-training (Fig 1). Training resulted in significantly lower levels of inosine in the 15-30 min (Fig 1A) and hypoxanthine in the 15-60 min (Fig 1B) period of recovery, whilst uric acid was lower with training from 60 min to the end of measurement (120 min; Fig 1C). Plasma lactate was not different at any time-point of recovery with training (data not shown).

Urinary excretion. Urinary excretion of inosine, Hx and uric acid was elevated during the first 2 hour period after exercise. It should be noted that there was a reduction in inosine excretion in the trained compared with the untrained state after 2 hours (Fig 2A) and a lower uric acid excretion in the 24 hour recovery period following PT with training (Fig 2B). The total endogenous purine metabolite (inosine + Hx + uric acid) excretion was not different in the first 2 hours (Fig 2A; 775±133 v 739±108 μmoles, untrained v trained, respectively). However, there was a tendency ($p=0.12$) for an attenuated excretion with training after 8 hours recovery, (not shown; 1378±213 v 981± 141 μmoles; respectively) and a reduced ($p<0.05$) excretion in the

24 hour recovery period (1603±267 v 1237±264 μmoles, untrained vs trained, respectively; Fig 2B).

Discussion

Sprint training reduced the muscle (Table 1) and plasma purine concentrations (Fig 1) and the excretion of urinary purines during recovery from a 30s sprint bout (Fig 2). This reduction likely represents a training-induced adaptation to minimise the loss of purines from skeletal muscle. Such an adaptation is advantageous in reducing the subsequent metabolic cost of AdN replacement by the purine *de novo* biosynthesis pathway.

As expected sprint training reduced the extent of ATP degradation (44.6%) and the IMP and inosine accumulation (56.7% and 66%, respectively) in the muscle during a 30 s sprint. A similar finding has been reported in other studies (Stathis et al. 1994; Harmer et al. 2000; Parra et al. 2000) and demonstrates an improved balance between the rates of ATP degradation and resynthesis with sprint training. Assuming any contribution of altered exercise efficiency *per se* is likely to be minimal, the rate of ATP degradation during the PT is likely to be the same as the peak and mean power values were not different pre and post-training. Thus, a more likely explanation is a training-induced improvement in the re-phosphorylation rate of ATP; either through anaerobic or aerobic sources.

The magnitude of the PCr degradation during the PT was not different following training indicating it does not contribute to an enhanced ATP resynthesis rate. Other contributing factors to ATP resynthesis include anaerobic glycolysis and oxidative phosphorylation. There is evidence of increased enzymatic activities of anaerobic glycolysis (phosphofructokinase and aldolase) with sprint training (Parra et al. 2000),

that could contribute to improved ADP re-phosphorylation. However, the present data indicate that anaerobic glycolysis was not likely to contribute to the improved ATP resynthesis rates following sprint training as the muscle lactate accumulation (Table 1) and the plasma lactate recovery profiles were not different with training (data not shown). An increasing body of evidence suggests there is an increase in the oxidative potential of skeletal muscle following sprint training (Parra et al. 2000; Burgomaster et al. 2005). Clearly no oxygen consumption or muscle aerobic enzyme activity were measured in the current study so we are unable to provide any evidence to support that notion that aerobic metabolism was enhanced in the sprint trained state. The tempered accumulation of IMP observed during the PT with sprint training (Table 1) must be due to a reduced flux through AMPd or an increased activity of pathways utilising IMP. Although it is not clear how the different modulators influence AMPd during intense exercise in vitro studies have demonstrated increases in AMPd activity with elevated substrate availability (Raggi and Ranieri-Raggi 1987) and increased concentrations of ADP, AMP and H^+ (Wheeler and Lowenstein 1979; Lowenstein 1990). Inhibition of AMPd occurs with elevated orthophosphate, ATP and guanosine 5'-triphosphate (GTP; Wheeler and Lowenstein 1979; Lowenstein 1990). Hence a reduced net production of AMP with the abovementioned improvement in the balance between ATP hydrolysis and ATP resynthesis rates best explains the reduced IMP accumulation following the PT with sprint training (Table 1). Other factors that influence AMPd activity, such as PCr content (Rush et al. 1998), are unlikely to play a role in the reduced AMPd activity in the trained state as the PCr content in mixed muscle was the same at the end point of exercise in the PT (Table 1). Furthermore, the attenuated accumulation of IMP observed following the PT during sprint training is not likely due to increased degradation of IMP to inosine or reamination to AMP via

the purine nucleotide activity (PNC). A small but significant sprint-trained reduction in inosine content following the PT (Table 1) indicates that an elevated degradation of IMP to inosine is unlikely. Evidence from rodent muscle demonstrates that the purine nucleotide cycle does not operate in muscles contracting during high intensity exercise (Meyer and Terjung 1980). Furthermore, sprint training attenuated plasma inosine, Hx and uric acid concentrations during the first two hours of recovery following the sprint (Fig 2). Lower plasma Hx following training has been reported in an earlier training study (Stathis et al. 1994) and is best explained by a reduction in the magnitude of purine efflux from the muscle, although no experimental evidence is available to confirm this possibility.

Urinary purine excretion in the 24 hours after the PT was reduced following sprint training (Fig 3) and is best explained by a decreased delivery of purine to the kidney due to lower plasma purine concentration. Attenuated plasma purine is a likely result of lower muscle purine base production and efflux into the plasma. The lower urinary purine excretion may also be influenced by a training-induced change in the loss of purines via the gut (Sorensen and Levinson 1975). Sorensen and Levinson (1975) demonstrated that in normal untrained individuals one third of basal uric acid excretion occurs via the gut and, as yet, no studies have investigated the influence of intense exercise and/or sprint training on this avenue of purine loss.

Previous studies have reported reduced resting muscle ATP after sprint training (Hellsten-Westing et al. 1993a; Stathis et al. 1994; Hellsten et al. 2004). A significant reduction in resting ATP following a similar training protocol to that used in this study was achieved with nine (Hellsten-Westing et al. 1993a) and eight (Hellsten et al. 2004) participants. The current study demonstrates a strong tendency for a lower resting ATP levels in the muscle after sprint training (Table 1; $p=0.06$ and 0.13,

enzymatic and HPLC analysis, respectively). A greater number of participants will be required to clarify the situation, as the statistical power of the test for detecting a significant decrease is low (i.e., 38%) in the present study indicating that a Type 2 error is likely. The imbalance between ATP loss and its resynthesis/synthesis is considered to be responsible for the reduction in resting muscle ATP content. Assuming the elevation in purine excretion during recovery represents the loss of endogenous purines from the active muscle, the loss of purine metabolites in 24 hours recovery from an intense sprint bout represents around 2% of the resting ATP content in the untrained and trained state. This was estimated by calculating the extent of purine excretion relative to the active muscle. If skeletal muscle is 40% of body weight (76.1 kg) and the legs comprise 55% of the total muscle mass (Snyder et al. 1975), then the mean weight of the exercising muscle (both legs) is 3.85 kg d.m. (assuming 23% dry weight). The urinary purine loss is probably an underestimate, as uric acid can also cross into the gut (Sorensen and Levinson 1975). As well as reducing the production of purines in the muscle, the conservation of muscle purine nucleotides with sprint training could also be enhanced by an increased capacity of intramuscular purine recovery after sprint training. Evidence of an elevated activity of the purine salvage enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT) following sprint training (Hellsten-Westing et al. 1993b) supports this possibility and provides a mechanism to further reduce the loss of purines from the muscle following exercise in the trained state.

Interestingly, seven days of intense sprint training resulted in no differences in mean power, peak power or the fatigue index during a 30 sec maximal sprint cycle bout. These results are consistent with those reported after a similar training protocol (Hellsten-Westing et al. 1993a). The training protocol may limit any performance

improvement because there is inadequate recovery time between sessions to produce optimal training adaptation. In support of this, Parra et al. (2000) demonstrated that a training programme using the same training load but over a longer duration improved performance in a 30 s sprint test.

In conclusion, the present study demonstrated that sprint training reduced purine base accumulation in the muscle and plasma, and decreased urinary excretion of endogenous purines (inosine, Hx and uric acid) following maximal sprint exercise. An attenuated loss of muscle purine nucleotide degradation products after a sprint bout reduces the extent of replacement of the muscle nucleotide pool via the metabolically expensive purine *de novo* synthesis pathway.

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Acknowledgement

We thank M. Cooke and E. Rybalka for help with the tissue sample collection and A. Howarth, I. Fairweather and R. Stokes for excellent technical support. We also acknowledge P. Maddy at the Western General Hospital (Footscray, Australia) for assistance with some of the plasma and urinary biochemical analysis.

Table 1: Effect of seven days of sprint training on skeletal muscle metabolites at rest, after a 30s sprint and 10 min recovery.

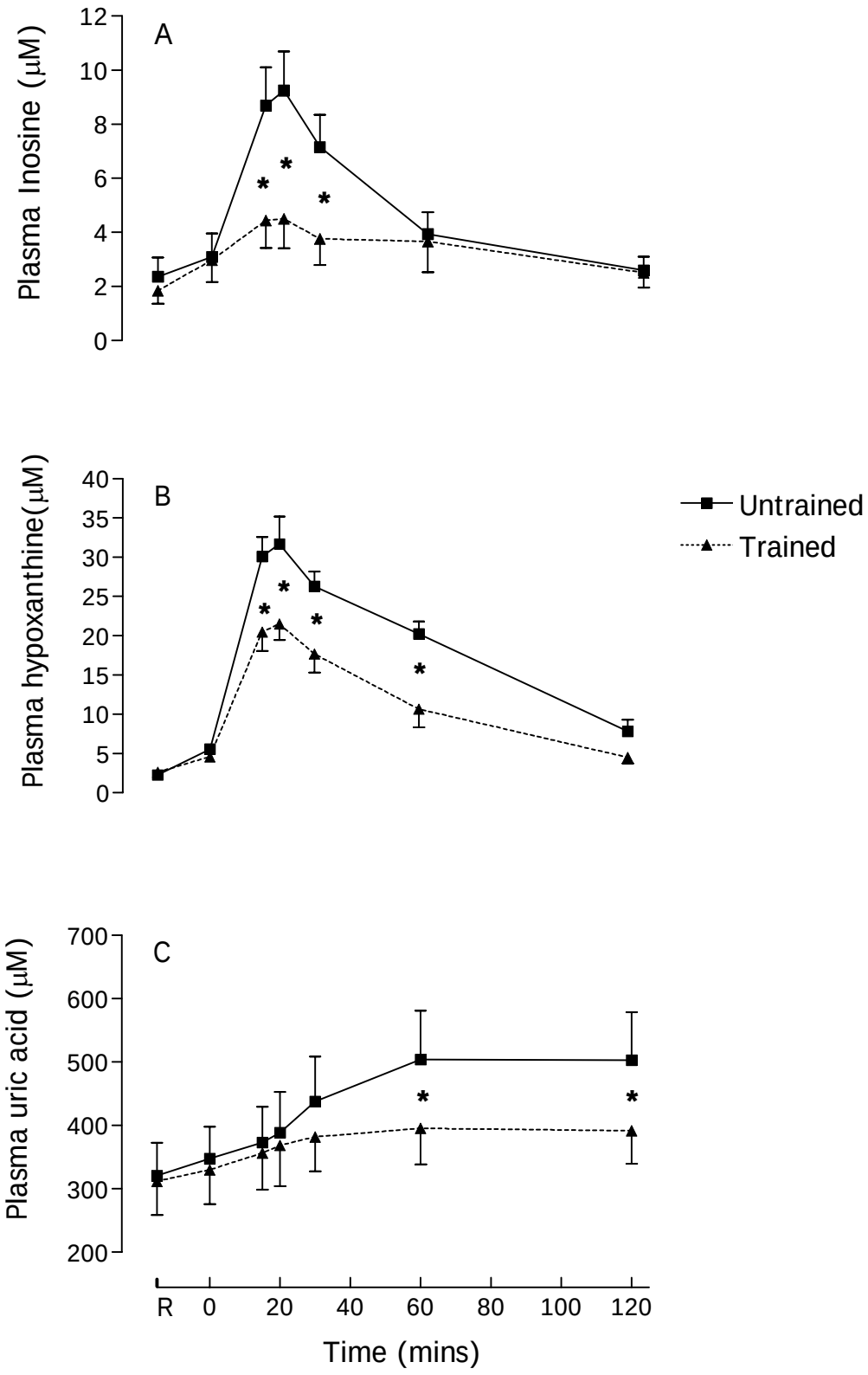
		Untrained			Trained	
	Rest	Exercised	10 min recovery	Rest	Exercise	10 min recovery
ATP	24.05±1.24	13.35±1.62 a	18.70±2.03 a,b	21.16±0.80	16.12±1.56* a	19.09±0.85 a,b
ATP-hplc	22.01±1.28	12.49±1.27 a	17.47±1.34	19.65±0.80	15.40±1.24* a	17.49±0.78 a,b
ADP	1.60±0.22	1.57±0.21	1.63±0.26	1.64±0.23	1.70±0.25	1.50±0.23
AMP	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.05±0.01
IMP	0.04±0.02	7.08±1.19 a	3.97±0.37 a,b	0.04±0.02	3.06±0.41* a	0.42±0.11 * a,b
Inosine	0.01±0.00	0.13±0.02	0.96±0.12 a,b	0.02±0.01	0.06±0.01*	0.42±0.07 *a,b
Hypoxanthine	<0.01	<0.01	0.04±0.00 a,b	<0.01	<0.01	0.03±0.01 a,b
PCr	82.0±3.7	27.3±4.4 a	71.9±3.8 a,b	87.4±3.7	31.5±4.1 a	83.8±4.3 b
Creatine	45.3±3.2	100.1±4.0 a	55.5±4.8 a,b	48.4±5.1	104.4±3.3 a	52.1±7.0 b
Lactate	5.51±0.63	91.90±7.51 a	49.37±6.46 a,b	5.53±0.99	83.94±5.32 a	39.24±2.53 a,b
AdN	23.67±1.34	14.13±1.35 a	19.16±1.56 a,b	21.36±0.96	17.17±1.41 * a	19.05±0.96 a,b
AdN + IMP + Inosine	23.72±1.34	21.33±0.61	24.10±1.40	21.41±0.97	20.28±1.21	19.89±0.87

Values are means ± SE; n=7; units are mmol.(kg dry weight)$^{-1}$ * different from untrained, $p<0.05$; a, different from Rest value $p<0.05$; b, different from Exercise value $p<0.05$.

Figure Legends

Fig. 1. Forearm venous plasma inosine (A), hypoxanthine (B), and uric acid (C) concentrations at rest (R) and during recovery from a 30s sprint bout before and after one week of sprint training. Values are means ± SE, n=7, * different from untrained; $P<0.05$. (note difference in scale).

Fig. 2. Exercise endogenous urinary inosine, hypoxanthine, uric acid and total purine excretion after (A) 2 hours, and (B) 24 hours, of recovery from a 30s sprint bout before and after one week of sprint training. Values are means ± SE, n=7. * different from untrained, $P<0.05$.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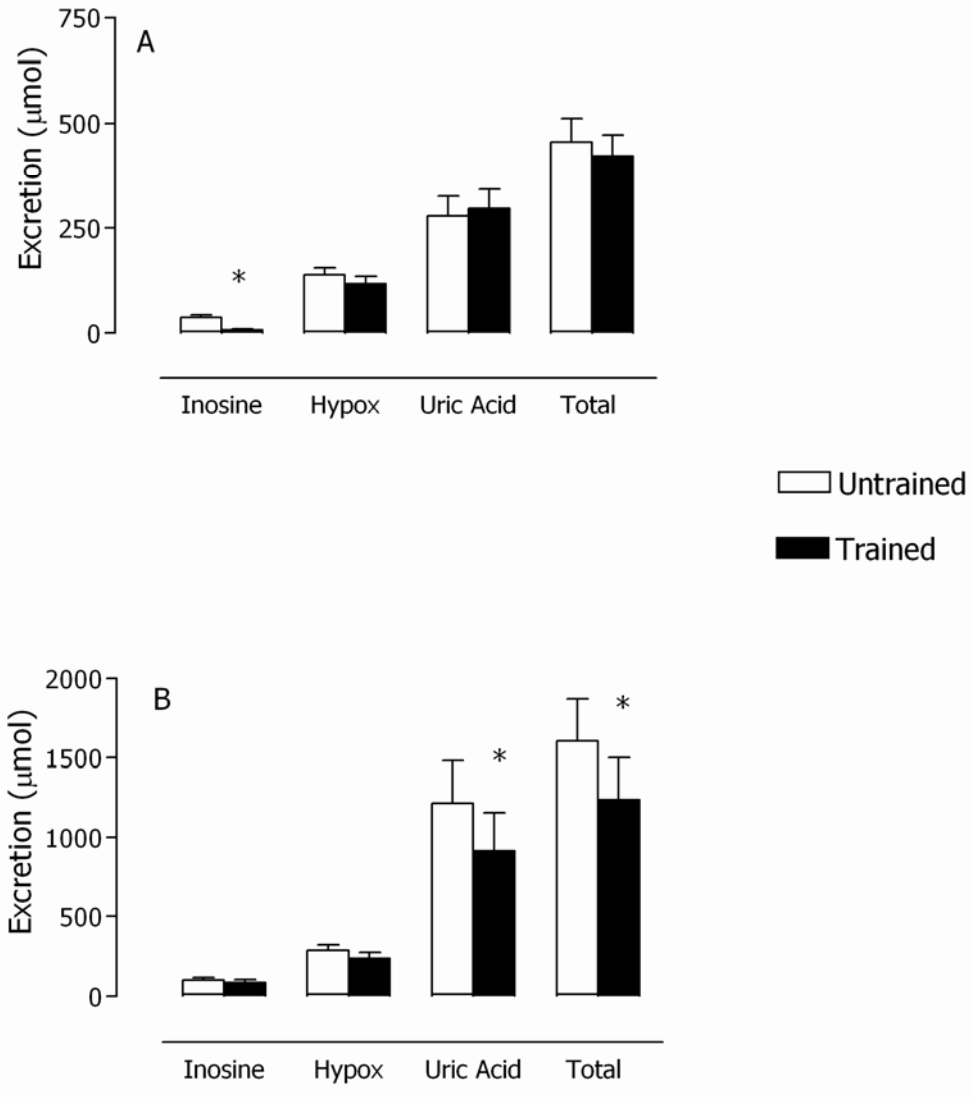
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Total
Untrained
Trained
B
2000
1500
1000
500
0
Excretion (µmol)
*
*
Inosine
Hypox
Uric Acid
Total