

The effect of endurance training on changes in purine metabolism: a longitudinal study of competitive long-distance runners

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Abstract The purpose of the study was to characterize the changes in purine metabolism in long-distance runners in the main phases of their 1-year training cycle. Nine male athletes competing in distances 5 and 10 km at national/regional level, mean age 22.9 ± 0.6 years, practising sport for 8.6 ± 0.3 years, participated in the study. The changes in plasma concentrations of hypoxanthine (Hx), xanthine (X) and uric acid (UA) and the activity of the enzyme HGPRT in red blood cells haemolysate were followed in four characteristic points of the annual training cycle: preparatory phase (specific subphase), competition period, transition period and preparatory phase (intermediate subphase). Resting and postexercise plasma concentrations of X and, Hx and HGPRT activity changed significantly during 1-year training cycle. Significant changes in postexercise Hx values between training phases were found, from $9.3 \mu\text{mol l}^{-1}$ in competition period to $22.9 \mu\text{mol l}^{-1}$ in transition period (Friedmann's ANOVA, $P < 0.01$). Postexercise UA values ranged from 371 to $399 \mu\text{mol l}^{-1}$ and did not change significantly between training phases. An increase in resting (from 52.0 to $58.4 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$, $P < 0.05$) and postexercise (from 70.7 to $76.2 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$, not significant) HGPRT activity between the specific preparation and competition period was observed. In the transition period, Hx postexercise concentration

increased ($22.9 \mu\text{mol l}^{-1}$, $P < 0.01$) and HGPRT postexercise activity decreased ($58.8 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$, $P < 0.01$) significantly. The results indicate that the level of plasma Hx at rest and after standard exercise may be a useful tool for monitoring the adaptation of energetic processes in different training phases and support the overload/overtraining diagnosis.

Keywords Purine metabolism · Hypoxanthine · HGPRT · Endurance training · Runners

Introduction

Adenosine 5' triphosphate (ATP) is degraded during exercise. As a result of a reaction catalyzed by adenylate kinase, ATP and inosine monophosphate (IMP) are formed from adenosine 5' diphosphate (ADP). Depending on conditions and cell type, AMP deaminates to IMP (in skeletal muscle) or dephosphorylates to adenosine (in cardiac muscle). Inosine monophosphate, the concentration of which increases in muscle cells (Tullson et al. 1990), may undergo further transformations that result in formation of inosine.

Inosine is degraded by purine nucleoside phosphorylase (PNP) to hypoxanthine (Hx), which can efflux the muscle and be lost from the adenine nucleotide pool or be salvaged intramuscularly by enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT; Fig. 1). The conversion of Hx to xanthine (X) and further to uric acid (UA) is catalyzed by enzyme xanthine dehydrogenase (XDH) which may act also as xanthine oxidase (XOD) under hypoxic conditions, especially in ischaemia-related hypoxia (Jarasch et al. 1986; Parks and Granger 1986). Xanthine oxidase reaction is connected with the formation of free radicals (superoxide anions) (Sahlin et al. 1991; De Bono

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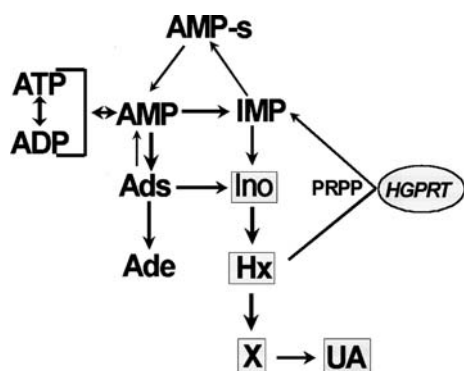


Fig. 1 Schematic view of the main metabolic pathways of adenine nucleotides

1994; Hellsten et al. 1997). Uric acid, a final compound of purine metabolism, is an antioxidant (Becker 1993) characterized by continuous slow increase of plasma concentration long after the termination of exercise. A schematic view of adenine nucleotides metabolic pathways is illustrated in Fig. 1.

In normal resting conditions, 95% of daily Hx turnover are converted to purine nucleotides via resynthesis and only 5% are excreted in urine (Murray 1971). Hypoxanthine is the only substance that may be re-converted and re-utilized in the nucleotide pool after being catalyzed by HGPRT. The activity of this enzyme plays the important role in maintaining ATP content effectively (purine synthesis de novo is relatively more energy consuming), and in the prevention of the formation of damaging free radicals in the subsequent degradation of Hx. In addition to muscle, HGPRT exists in the red blood cells and the brain (Rychlewski et al. 1994).

Hypoxanthine is considered to be an indicator of histotoxic hypoxia for a long time (Sutton et al. 1980; Banaszak and Rychlewski 1992). It is also regarded as a marker of adenine nucleotide degradation in muscle, a marker of energetic stress during exercise (Sahlin et al. 1999), an index of exercise intensity (Rychlewski et al. 1997), and it may be used in the classification of physical exercise (Bianchi et al. 1999).

During a moderate-intensity prolonged exercise, plasma Hx concentration increases but varies greatly between subjects and there are no significant changes after 5 min of recovery (Sahlin et al. 1999). We know from earlier research that Hx blood concentration increases after exercise, reaches maximum value after 10–20 min (Harkness et al. 1983; Hellsten-Westling et al. 1989; Sahlin et al. 1991) and is 2- to 4-fold higher than measured immediately after exercise. Rychlewski et al. (1997) found a significant correlation between an increased concentration of Hx in blood plasma and changes in the pH and base excess after physical exercise in canoeists and swimmers. Sjödin and

Hellsten-Westling (1990) proved that a critical point of Hx is observed at exercise intensity 107–115% of maximal oxygen uptake (VO_{2max}). After this threshold had been exceeded, a rapid increase in plasma Hx concentration was observed. Ketani et al. (1987) showed, that rapid increase in Hx concentration occurred at intensity below 90% of VO_{2max} . However, the discrepancy may result from different experimental protocols. These studies also show that the relationship between Hx and exercise intensity may be “shifted” to the right in competitive athletes like in the case of ventilation threshold (Sjödin et al. 1982).

Plasma Hx concentration increases also significantly (15- to 40-fold) after a maximal intensity intermittent sprint exercise repeated every 30 s, but the physiological responses are markedly influenced by sprint distance (Balsom et al. 1992). After 15-m sprints, the mean pre- and post-exercise Hx concentrations did not differ significantly, while postexercise values were significantly higher after 30- and 40-m sprints.

After 6 weeks of a high-intensity intermittent training, the level of Hx at rest and after exercise decreases considerably and HGPRT activity increases in habitually active males. It reflects muscle adaptation that leads to reduced loss of adenine nucleotides (Hellsten-Westling et al. 1993a, b). Lowered concentration of plasma purines after training allows athletes to exercise more intensively without the loss of purine nucleotide. The study on the effects of a 7-week field hockey-specific training on repeated-sprint ability, plasma Hx concentration and other blood parameters in 18 elite female field hockey players revealed a significant decrease in plasma Hx concentration (posttest minus rest values) (Spencer et al. 2004). The authors suggest that one adaptation of sport-specific repeated-sprint training may be to conserve the purine nucleotide pool. Moreover, the study by Stathis et al. (1994) demonstrates that inosine concentration, the precursor to Hx, is lower in the muscle following training as is the postexercise plasma Hx concentration after a 7-week sprint training. This provides evidence that Hx production and/or efflux from the muscle are reduced.

There are very few studies concerning the effect of endurance training on purine metabolism. Baldwin et al. (2000) compared endurance in trained and non-trained men who cycled for 60 min at a work rate of 70% VO_2 peak or at 95% of lactate threshold. There was a tendency for plasma Hx to be higher in non-trained subjects at 70% of VO_2 peak compared with trained subjects. However, plasma markers of metabolic stress were similar in both trained and non-trained group during exercise at 95% of lactate threshold.

Investigations that have been conducted so far relate mainly to single exercise or short programmes of sprint training that lasted for few weeks. The authors of the

present study are interested in the time course of purine changes during a longer training period. We presume that the analysis of changes in enzyme activity and plasma oxypurine concentration in the course of a real sport training may allow the use these biochemical parameters as an assessment of physical efficiency and metabolic response to sports training. Proportions of anaerobic exercise and thus possible increase of the metabolic stress on the adenine nucleotide pool in the muscle change considerably during subsequent training phases in endurance athletes. Thus, the aim of our study is to characterize the changes in purine metabolism in long distance runners in the main phases of their 1-year training cycle. We followed the changes in plasma concentrations of Hx, X and UA as well as the activity of the enzyme HGPRT in red blood cells haemolysate.

Methods

Subjects

Nine male long-distance Polish runners (specialized in distances: 5 and 10 km) competing at national and regional level, mean age 22.9 ± 0.6 (20–26) years, practising competitive sport for 8.6 ± 0.3 years, body mass 64.5 ± 14 kg, body height 177 ± 1.5 cm, maximal oxygen consumption (VO_{2max}) 67.3 ± 1.8 ml kg^{-1} min^{-1} , maximum heart rate 199 ± 1 beat min^{-1} , average levels of haemoglobin 15.2 ± 0.6 g dl^{-1} at rest and 15.5 ± 0.5 g dl^{-1} postexercise at the beginning of the study, participated in the research. No significant changes in body mass and height were observed during the study. Postexercise level of haemoglobin changed significantly between examinations (ANOVA = 8.1, $P < 0.05$) decreasing to a minimum value 14.6 ± 0.9 g dl^{-1} in the competition period. The aim of the research and testing methodology were explained to all subjects who gave their informed consent prior to their inclusion in the study. The project has been approved by Ethics Committee at the Karol Marcinkowski Medical University in Poznań and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Examinations

The study procedure was adapted to the annual plan of examined runners. The 1-year training cycle consisted of three main phases: preparatory, competitive and transition phase (Bompa 1999). Preparatory phase included three subphases. The aim of the general subphase was the development of general endurance on the basis of aerobic training means, improvement of running technique and progress in general fitness. In the intermediate subphase, athletes aimed

at the development of running endurance using aerobic–anaerobic training means. Specific subphase enabled athletes to develop specific endurance and specific physical fitness on the basis of interval and speed runs with predominance of anaerobic exercises. Competitive phase was characterized by decreasing training volume and increasing training intensity. Runners competed on their specialized and related distances, peaking their athletic shape. In the transition phase, training loads were small and the aim was physical and psychological regeneration and recovery from injuries. Athletes played team games, swam and did activity forms other than typical endurance running. Four consecutive examinations had been planned and performed in four characteristic points of the annual training cycle.

First examination 15–16 of March, specific subphase of the preparatory phase, 147 ± 3.0 training sessions from the beginning of the 1-year training cycle, in total 213 h:56 min:15 s \pm 2 h:26 min:45 s net training time. Five main exercise zones, corresponding to estimates of the sources of energy for ATP resynthesis, were used within this total time in the following proportion: 73.0% in aerobic compensation zone; 20.6% in aerobic stimulation zone; 4.8% in combined aerobic–anaerobic zone; 0.9% in anaerobic lactacid zone and 0.7% in anaerobic non-lactacid zone.

Second examination 16–17 of June, competition period, 93 ± 1.6 training sessions from the first examination, in total 118 h:16 min:51 s \pm 3 h:25 min:48 s net training time. Subjects participated among others in the Polish Athletics Championships. Exercise zones proportion was 59.3:26.4:10.7:2.3:1.3%, respectively.

Third examination 9–10 of November, transition period, 135 ± 1.7 training sessions from the second examination, in total 188 h:01 min:59 s \pm 2 h:20 min:10 s net training time. Exercise zones proportion was 63.1:25.9:8.5:1.6:0.9%, respectively.

Fourth examination 2–3 of February, the next 1-year training cycle, the end of the general preparation (intermediate subphase) of the preparatory phase, 91 ± 1.2 training sessions from the third examination, in total 181 h:15 min:03 s \pm 4 h:29 min:41 s net training time. Exercise zones proportion was 77.6:18.8:2.2:0.7:0.7%, respectively.

Maximal oxygen uptake

All tests were conducted in the laboratory of the Department of Physiology of the University School of Physical Education in Poznań, in the morning, 2 h after consuming a light breakfast (bread and butter, water, without coffee or tea). Incremental running treadmill test (Woodway ES1, Waukesha, USA) was conducted. Athletes started at a

speed of 10 km h⁻¹. Subsequently, the travel speed of the moving strip was progressively increased by 2 km h⁻¹ every 3 min until volitional exhaustion. Respiratory parameters (VE, VO₂, VCO₂) were measured continuously by means of CPX-D computer system (Medical Graphics Corporation, St. Louis, USA). Heart rate was recorded every 5 s with a Polar Accurex Plus device (Polar Elektro, Finland).

Lactic acid

Capillary blood samples were obtained from the finger tip immediately before the exercise (at rest) and 3 min after exercise. Lactic acid (LA) concentrations were assayed enzymatically using a spectrophotometric Warburg method (Gutmann and Wahlenfeld 1974) that consists in measuring the increase in absorbance of NADH at wavelength 365 nm. A Marcel Media spectrofotometer (Poland) was used.

Hypoxanthine, xanthine, uric acid, HGPRT

Venous blood samples (5 ml) were taken from an antecubital vein at rest, immediately before the test exercise and 5 min after the exercise in order to obtain plasma and red blood cells. The plasma obtained was deproteinized with 1.2 mol l⁻¹ HClO₄ and centrifuged. An acid supernatant was neutralized with 1 mol l⁻¹ K₂CO₃ and centrifuged, and stored at -80°C before analysis. Blood cells were bathed three times in isotonic salt solution (0.9% NaCl), then haemolysed with a hypotonic solution of Tris buffer (hydroxymethylaminomethane), 10 mmol l⁻¹, pH 7.4. The prepared haemolysate was stored at -20°C before analysis for HGPRT activity.

Hypoxanthine, X and UA were assayed in a neutralized plasma extract by means of high-performance liquid chromatography (HPLC) according to the methodology of Wung and Howell (1980) modified by Banaszak (1999). Hewlett-Packard 1050 apparatus with UV detector (Ramsey, USA) was used. Separation was achieved by a Hypersil ODS 100 mm × 4.6 mm × 5 μm column and a Hypersil ODS 20 mm × 4 mm × 5 μm precolumn manufactured by Alltech (Deerfield, USA). The carrier phase was a buffer consisting of 1% methanol and 4% 100 mmol l⁻¹ KH₂PO₄ (potassium phosphate), pH 5.8. The flow rate was 1.0 ml min⁻¹. Substances were identified by comparing retention times with model UA, Hx, X and IMP compounds at known standard concentrations. In this system, UA, Hx and X were separated at retention times of about 2, 3 and 4 min, respectively. The measurement was done at wavelength 254 nm for Hx and X, and 280 nm for UA. The quantitative sample analysis was performed on the basis of a comparison of retention times and concentrations with standardized solutions.

HGPRT activity was measured in red blood cell according to the procedure described by Stolk et al. (1995) modified by Banaszak (1999). The incubation buffer consisted of 50 mmol l⁻¹ Tris pH 7.4, 10 mmol l⁻¹ PRPP, 7 mmol l⁻¹ NaF and 5 mmol l⁻¹ MgCl₂. The pre-incubation of the haemolyzate (100 μl) and the buffer lasted for 5 min in a 37°C water bath. The main 15-min incubation started after addition of 100 μl of 2.0 mmol l⁻¹ Hx solution into the pre-incubation mixture in order to initiate the enzymatic reaction. Immediately after Hx was added, 100 μl of incubation mixture was taken and added to 100 μl 1.2 mol l⁻¹ HClO₄ (perchloric acid). This sample served as “zero time” sample. The incubation was stopped after 15 min by renewed addition of 100 μl of mixture to 1.2 mol l⁻¹ HClO₄. Acid extracts were neutralized with 1 mol l⁻¹ K₂CO₃ and then used for HGPRT activity measurement by means of HPLC method. The buffer used as carrier phase consisted of 100 mmol l⁻¹ KH₂PO₄ with the addition of TBAS (tetrabutylammonium sulphate), final concentration 5 mmol l⁻¹ and pH 3.1. Separation was conducted at flow rate 1.2 ml min⁻¹. The mobile phase consisted of 15% of methanol and 50% of buffer pH = 3.1. An ODS Hypersil 150 mm × 4.6 mm × 5 μm column and an ODS Hypersil 20 mm × 4 mm × 5 μm precolumn manufactured by Alltech (Deerfield, USA) were used. The separation time was about 2.0 min for Hx and 5.0 min for IMP. The quantitative sample analysis was performed on the basis of a comparison of retention times with model Hx- and IMP-solutions at wavelength 254 nm. HGPRT activity is expressed as the amount of produced IMP (number of μmol IMP mg⁻¹ Hb min⁻¹) (Ralston et al. 1991). The blood concentration of Hb was measured by Cobas b121 apparatus (Roche, Mannheim, Germany).

Statistics

Values are presented as mean and standard error of the mean (±SEM). Friedmann's ANOVA was used to compare the longitudinal effect of training during the 1-year cycle. Wilcoxon test was used to assess the differences between related variables obtained from pre- and post-exercise measurements in the same training phase. All calculations were made in the Computation Center of our University School using STATISTICA v. 5.1 software.

Results

Physiological parameters

Mean values of VO_{2max} ranged from 65.0 ± 1.3 ml kg⁻¹ min⁻¹ in the transition period to 68.1 ± 2.0 ml kg⁻¹ min⁻¹ in the competitive phase. Changes in VO_{2max} between

examinations were statistically not significant. Nevertheless, they were crucial to training status (peaking athletic shape) and reflected normal desirable endurance fluctuations in the 1-year training cycle. Average distance covered until termination of the test exercise ranged from $2,942 \pm 48$ m in the transition phase to $3,067 \pm 99$ m in the competitive period and varied significantly between examinations (ANOVA = 8.9, $P < 0.05$).

Lactic acid

Mean resting values of LA in the 1-year training cycle ranged from 1.3 ± 0.1 mmol l⁻¹ in the competition phase to 1.7 ± 0.2 mmol l⁻¹ in the general preparation subphase (Fig. 2). Postexercise concentrations varied between 6.2 ± 0.4 mmol l⁻¹ in the transition phase and 7.0 ± 0.5 mmol l⁻¹ in the general preparation subphase. Both resting and postexercise values did not change during the 1-year cycle significantly. The rest–postexercise differences in LA concentrations ranged from 4.7 ± 4.0 mmol l⁻¹ in the transition phase to 5.5 mmol l⁻¹ during the competition period, but the 1-year variability of this parameter was not statistically significant. However, differences between resting and postexercise values were significant in each examined training phase.

Xanthine

The lowest average resting X concentration at 1.8 ± 0.1 $\mu\text{mol l}^{-1}$ was observed in the specific preparation phase and the highest concentration at 2.8 ± 0.1 $\mu\text{mol l}^{-1}$ was measured in the competition period (Fig. 3). Resting mean values decreased along with consecutive training phases and the differences turned out to be statistically significant (ANOVA = 14.1, $P < 0.01$). Postexercise X concentrations

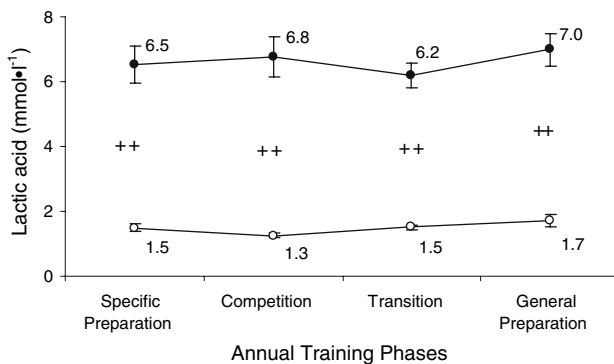


Fig. 2 Changes in lactic acid blood concentrations (mean and SEM, $n = 9$) during the annual training cycle in competitive long-distance runners for pre (open circle) and post (closed circle) exercise. Significant differences between successive training phases are denoted with single asterisk ($P < 0.05$) or double asterisks ($P < 0.01$) and between pre- and post-exercise values of the same phase with + ($P < 0.05$) or ++ ($P < 0.01$)

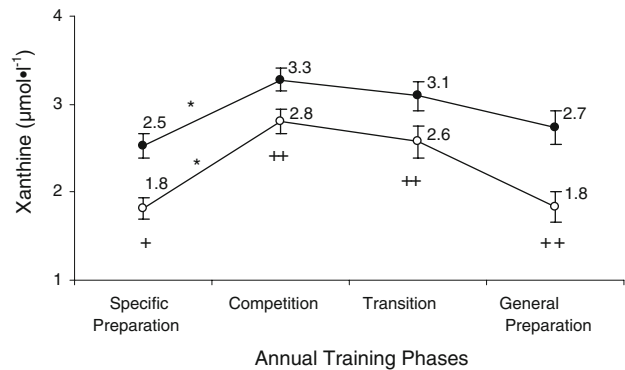


Fig. 3 Changes in xanthine blood concentrations (mean and SEM, $n = 9$) during the annual training cycle in competitive long-distance runners for pre (open circle) and post (closed circle) exercise. Significant differences between successive training phases are denoted with single asterisk ($P < 0.05$) or double asterisks ($P < 0.01$) and between pre- and post-exercise values of the same phase with + ($P < 0.05$) or ++ ($P < 0.01$)

were also lowest in the specific preparation phase and highest in the competition period averaging 2.5 ± 0.1 $\mu\text{mol l}^{-1}$ and 3.3 ± 0.1 $\mu\text{mol l}^{-1}$, respectively. A permanent decline of mean value was noted from the competition period to the general preparation phase. The changes in postexercise X concentration during 1-year cycle were statistically significant (ANOVA = 11.0, $P < 0.05$). The variability of the difference between resting and postexercise X values in 1-year cycle did not reach statistical significance. This parameter ranged from 0.47 ± 0.1 $\mu\text{mol l}^{-1}$ in the competition period to 0.9 ± 0.1 $\mu\text{mol l}^{-1}$ in the general preparation phase and was relatively constant. Nevertheless, resting–postexercise differences were statistically significant in each separate training phase, as demonstrated by means of Wilcoxon test in each training phase (Fig. 3).

Hypoxanthine

Mean resting values of Hx concentrations ranged from 3.0 ± 0.2 $\mu\text{mol l}^{-1}$ in the specific preparation phase to 4.4 ± 0.4 $\mu\text{mol l}^{-1}$ in the transition phase (Fig. 4). The most visible change occurred between these two phases. The variability of resting Hx values along with training phases was statistically significant (ANOVA = 10.0, $P < 0.05$). Average postexercise values changed during the 1-year cycle within a very wide range from, 9.3 ± 0.6 $\mu\text{mol l}^{-1}$ in the competition period to 22.9 ± 2.1 $\mu\text{mol l}^{-1}$ in the transition phase. The analysis of variance revealed statistical significance of these changes (ANOVA = 20.5, $P < 0.01$). Average resting–postexercise differences changed also considerably during the annual training cycle (ANOVA = 19.8, $P < 0.01$). Highest difference was noted in the transition phase (18.5 ± 1.9 $\mu\text{mol l}^{-1}$, Wilcoxon test = 2.7, $P < 0.01$) and the lowest,

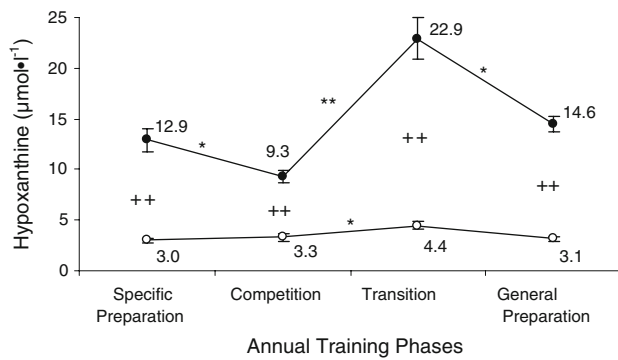


Fig. 4 Changes in hypoxanthine blood concentrations (mean and SEM, $n = 9$) during the annual training cycle in competitive long-distance runners for pre (*open circle*) and post (*closed circle*) exercise. Significant differences between successive training phases are denoted with *single asterisk* ($P < 0.05$) or *double asterisks* ($P < 0.01$) and between pre- and post-exercise values of the same phase with + ($P < 0.05$) or ++ ($P < 0.01$)

but still significant difference, in the competition period ($6.1 \pm 0.7 \mu\text{mol l}^{-1}$, Wilcoxon test = 2.7, $P < 0.01$).

Uric acid

Resting concentration of UA averaged from the lowest level in the general preparation period ($264 \pm 17 \mu\text{mol l}^{-1}$) to the highest value in the specific preparation period ($331 \pm 13 \mu\text{mol l}^{-1}$). Postexercise values ranged from $371 \pm 17 \mu\text{mol l}^{-1}$ in the general preparation phase to $399 \pm 14 \mu\text{mol l}^{-1}$ in the specific preparation phase (Fig. 5). The changes between training phases were not significant (at rest: ANOVA = 3.5, not significant; postexercise: ANOVA = 2.2, not significant). However, a trend towards lower UA concentrations during the yearly training cycle and a single significant difference between resting values in the specific preparation and the transition period are

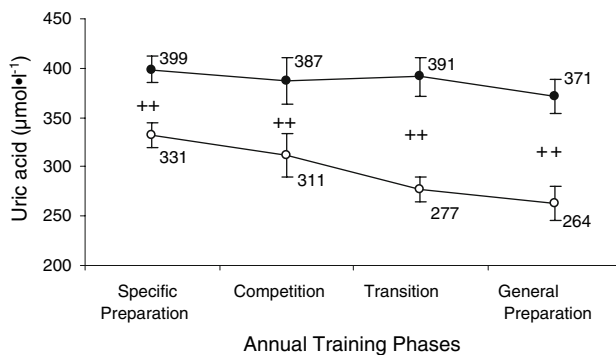


Fig. 5 Changes in uric acid blood concentrations (mean and SEM, $n = 9$) during the annual training cycle in competitive long-distance runners for pre (*open circle*) and post (*closed circle*) exercise. Significant differences between successive training phases are denoted with *single asterisk* ($P < 0.05$) or *double asterisks* ($P < 0.01$) and between pre- and post-exercise values of the same phase with + ($P < 0.05$) or ++ ($P < 0.01$)

to be noticed (331 vs. $277 \mu\text{mol l}^{-1}$, Wilcoxon test = 2.1, $P < 0.05$). The resting–postexercise difference changed significantly during the whole training cycle (ANOVA = 8.6, $P < 0.05$). Similarly, resting–postexercise differences in each separate examination were statistically significant and they were decreasing to a minimum in the specific preparation phase ($67 \pm 22 \mu\text{mol l}^{-1}$, Wilcoxon test = 2.7, $P < 0.01$) and reaching maximum value in the transition phase ($114 \pm 21 \mu\text{mol l}^{-1}$, Wilcoxon test = 2.7, $P < 0.01$).

HGPRT

Resting HGPRT activity averaged from $50.6 \pm 1.1 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$ in the transition phase to $61.0 \pm 3.0 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$ in the general preparation period (last examination, Fig. 6). The changes were significant (ANOVA = 13.4, $P < 0.01$). Postexercise HGPRT activity ranged from $58.8 \pm 1.7 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$ in the transition phase to $76.2 \pm 1.7 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$ in the competition period. The values varied also significantly with successive training phases (ANOVA = 14.5, $P < 0.01$). Resting–postexercise differences did not undergo considerable changes during 1-year training cycle but still remained significant in separate examinations. The largest difference between resting and postexercise values was observed in the specific preparation phase ($18.7 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$, Wilcoxon test = 2.7, $P < 0.01$) and the smallest in the transition phase ($8.22 \text{ IMP mg}^{-1} \text{ Hb min}^{-1}$, Wilcoxon test 2.7, $P < 0.01$).

Discussion

The research that has been conducted so far has focused on the effect of single exercise sessions or short training

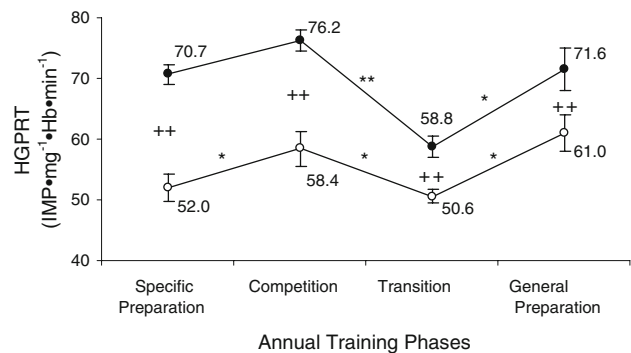


Fig. 6 Changes in HGPRT blood concentrations (mean and SEM, $n = 9$) during the annual training cycle in competitive long-distance runners for pre (*open circle*) and post (*closed circle*) exercise. Significant differences between successive training phases are denoted with *single asterisk* ($P < 0.05$) or *double asterisks* ($P < 0.01$) and between pre- and post-exercise values of the same phase with + ($P < 0.05$) or ++ ($P < 0.01$)

programmes in subjects practising different types of sport and in the non-trained. In our study, we analysed the effect of an 1-year training cycle on the purine metabolism in individuals with a high level of physical efficiency (competitive long-distance runners). We believe that our results demonstrate that changes in purines could be an assessment of the metabolic response to sports training.

According to Green et al. (1995), endurance training reduces the increase of muscle IMP, probably due to the increase of aerobic potential of muscles. Baldwin et al. (2000) described the effect of endurance training on Hx level, showing the difference in Hx concentration between trained and non-trained subjects at 95% of VO_{2max} . Due to the lack of studies on endurance-trained subjects, particularly on competitive endurance athletes during a longer training period, only comparisons with sprint training are possible. Repeated high-intensity training lasting for 6 weeks reduces considerably the resting and postexercise Hx concentration and increases HGPRT activity in the human muscle (Hellsten-Westing et al. 1993a). This reflects the muscle adaptation which reduces in turn adenine nucleotides loss (Hellsten-Westing et al. 1993a, b). Similar results were obtained by Stathis et al. (1994) in active but nonspecifically trained subjects (men and women) and Spencer et al. (2004) in elite female field hockey players. Stathis et al. (1994) demonstrated significant differences in muscle and plasma Hx concentration between the trained and the non-trained group as well as between the pre- and post-training values after a 7-week sprint training. At the same time, resting muscle ATP and total adenine nucleotide content, postexercise ATP depletion as well as accumulation of muscle IMP and inosine, precursors to Hx production, were reduced after training. Moreover, an increase in performance of the 30-s sprint bout following training was noted. On the basis of these studies, one may suggest that a lower plasma purine concentration after a training period allows athletes to exercise at a higher intensity without the loss of purine nucleotide.

The trends in plasma Hx concentration in our endurance 1-year training study are similar to those observed in short-duration sprint training (Hellsten-Westing et al. 1993a, b; Stathis et al. 1994; Spencer et al. 2004). In our study, the lowest postexercise concentration of Hx was observed during the competition phase when the proportion of anaerobic training loads was highest, that is the training was characterized to a larger extent by high-intensity exercises. The postexercise Hx concentration was markedly higher in training phases with less stressed anaerobic content.

Significant changes in average X concentration were observed at rest ($P < 0.01$). Plasma X concentration remained within the range of standard values obtained by Boulieu et al. (1983) with HPLC method. Bianchi et al. (1999) observed similar resting X concentrations in

marathon runners and Sahlin et al. (1999) in men practicing different leisure sports. The changes of the resting–postexercise value were not significant in our study.

In the present study, changes in resting concentration of Hx ($P < 0.05$) and in resting–postexercise values ($P < 0.01$) during 1-year training cycle have been shown. Similar resting values were observed in long- and middle-distance runners in the study of Bhattacharya et al. (1983). In long-distance runners, we found the highest Hx concentration in the transition period, after the end of a long competition period and of the whole 1-year training cycle, both at rest ($4.43 \mu\text{mol l}^{-1}$), and after exercise ($22.9 \mu\text{mol l}^{-1}$). The lowest concentration of Hx was noted in the competition period when runners were peaking their athletic shape. It is important that the changes in Hx concentration were inversely proportional to the distance covered in the maximum oxygen consumption test. The best average performance was noted in the competition period ($3,067.0 \pm 350.5$ m) and the shortest distance in the transition phase ($2,641.8 \pm 97.9$ m). The higher anaerobic content during training of the competition phase could potentially increase purine loss during the training challenge on the muscle. Stathis et al. (1994) demonstrated both a reduced ATP content at rest in the 7-week sprint trained state and a substantially reduced degradation of ATP to IMP during 30 s of maximal sprint exercise, with increased performance measures. Thus, as with sprint training, the adenine nucleotide content in our subjects may be lower at rest, following training in the competitive phase, and not degrade to the same extent following exercise, even with an increased performance measure in distance covered. This could explain the reduced plasma Hx concentration in recovery.

These data suggest that Hx plasma concentration may be a measure of training status, overload or overtraining. This is supported by the lack of significant changes in LA concentrations in consecutive examinations, neither for resting nor for postexercise values. Thus, plasma Hx concentration may be the source of an additional specific and important information on training status, better than classic measures like VO_{2max} and LA concentration.

The HGPRT activity in red blood cell at rest varied greatly during the investigated training cycle ($P < 0.01$). The lowest activity was found in the transition phase of the training cycle and the highest activity in the competition period. In support of our results, Banaszak and Rychlewski (1989) found a positive correlation between HGPRT activity and VO_{2max} and a negative correlation between HGPRT activity and Hx concentration. Similar nature of changes was revealed in our study. Along with increase of HGPRT activity, VO_{2max} increased and Hx concentrations decreased.

Using metabolites of purine degradation in the plasma at rest has limitations. Their concentrations are in a constant state of flux as purines are produced, entering the blood

from the muscle and being excreted. The level and the rate of excretion of the different purines can change following exercise and training (Stathis et al. 1999, 2006). However, in the research of Stathis et al. 2006 pre- and post-training LA and Hx concentrations did not differ significantly. Probably due to a very short-term training cycle (1 week), the adaptation processes could not develop. There was no performance change after training (no changes in power and fatigue index were observed). In contrast to this, the research of Spencer et al. 2004 on female national field hockey players revealed a considerable decrease of resting (as well as postexercise) Hx concentration after training lasting for 7 weeks. This suggests that changes in resting concentrations occur only after a longer period of training. In our study, we consider a long-term training cycle in competitive athletes with examinations made at intervals of 3–5 months. The resting (and postexercise) values were determined by a regular, almost daily training in a long period. In competitive athletes, the dominant factor influencing metabolic changes seems to be specifically planned and structured training load (intensity, volume, frequency), according to the aim of each training phase. This could be the reason why resting Hx and X concentrations and HGPRT activity changed significantly. With standard exercise protocol, it is a good basis for monitoring training status at chosen points of training cycle in individual athletes (in competitive sport we follow the individual parameters rather than average “team” values). Unexpected changes of concentrations at rest could be evidence for unfavourable metabolic changes, e.g. overload/overtraining. It is particularly important in the light of the fact that LA concentrations do not change along with training phases in our study, i.e. LA is of limited diagnostic value in competitive athletes. So far, other studies on the long-term (1-year, many years’) changes in purine metabolism have not appeared and the problem needs further investigation.

Hellsten-Westling et al. (1993a) hypothesize that the decreased Hx release from muscle may be explained by intensified conversion of Hx to IMP or by decrease in Hx formation. The first possibility, reducing nucleotide loss, could be considered as an advantageous adaptation of muscles being subject to metabolic stress. The reduction of Hx release may be also explained by the decrease in adenine nucleotide degradation. Such a reduction could be achieved by the increase of muscle ability to ATP resynthesis in the process of anaerobic glycolysis and, in this way, by the redress of the balance of ATP consumption and resynthesis at high exercise intensity. Considerable decrease in the activity of myoadenylate (AMP) deaminase could also slow down the rate of AMP deamination and the rate of IMP degradation to purine at the same time.

In our study, the higher erythrocyte HGPRT activity and the decreased Hx blood concentration after a specific

endurance training could suggest that the rephosphorylation of intracellular Hx to IMP increased. However, muscle HGPRT was not measured and the main limitation of this kind of speculation is the lack of evidence that muscle and erythrocyte HGPRT activity are directly related. It seems that the HGPRT plays different role in the erythrocytes (they do not have de novo capacity) compared with muscle.

Significant differences between resting and postexercise concentrations of UA in each training phase were observed in our study ($P < 0.05$). Moreover, the resting–postexercise difference changed significantly during the whole training cycle (ANOVA $P < 0.05$). The concentrations remained within the normal (medical) reference range at rest as well as postexercise. No significant 1-year changes of UA were found in resting and postexercise values considered separately. However, a visible but non-significant trend towards lower UA concentrations during the yearly training cycle was noticed. Earlier studies on animals revealed that the liver has a substantial potential for Hx uptake and UA production (Moyer and Henderson 1983). Purines (to a large extent as hypoxanthine) are released from exercising muscle into blood. The increase in plasma urate stems largely from oxidation of Hx in the liver, whereas the formation of UA in skeletal muscle seems to be limited (Hellsten-Westling et al. 1994; Sahlin et al. 1991; Bangsbo et al. 1992). After release from muscle, the liver extracts Hx from the blood, oxidizes it to urate and releases UA into the blood. Uric acid in blood may be then extracted by exercised muscle to replenish the muscle urate stores, may be utilized as a free radical scavenger and oxidized to allantoin during exercise (Hellsten et al. 1997; Hellsten et al. 2001). Hypoxanthine does not accumulate in plasma to an appreciable degree because it is continuously degraded to UA. This may explain resting–postexercise differences in UA concentrations being 3–10 times higher than combined X and Hx magnitudes. In support of our results, Bianchi et al. (1999) showed the increase of UA postexercise concentration by $33 \mu\text{mol l}^{-1}$ in soccer players and by $73 \mu\text{mol l}^{-1}$ in marathon runners (3–6 times higher than at rest) at 5 min of recovery.

Smallest differences between rest and postexercise UA concentrations were revealed in the specific preparation and competition phase (first and second examination) when the contribution of anaerobic training loads was largest. Uric acid is the end point of purine metabolism and can accumulate in the plasma. Its blood concentration may be considered as a metabolic indicator of the changes which occurred in the preceding exercise. A rise in the plasma UA from rest to postexercise level is also likely to indirectly represent adenine nucleotide degradation during exercise. Less plasma UA accumulation postexercise (smaller difference between rest and postexercise concentration) appears to be

caused by less Hx efflux from the muscle (less production or better salvage).

Lower postexercise purine concentration in competition period indicates that the training allowed the athletes to exercise at higher intensity but, at the same time, purine turnover in skeletal muscle was reduced. Such an adaptation is crucial from the practical point of view, especially with reference to competitive athletes often performing high-intensity exercise. Decrease in adenine nucleotides loss means less cell damage and better sport performance. This was confirmed by Hellsten-Westing et al. (1993b) who revealed that plasma purine bases concentration decreases along with the increase of physical efficiency (endurance level). Stathis et al. (1994) reported lower IMP and inosine concentration in the muscle after training and provided direct evidence of reduced purine base accumulation in, and loss from the muscle in the light of improved performance. Furthermore, Sahlin et al. (1999) proved that plasma Hx is positively correlated with plasma ammonia (NH₃) and LA concentration, and negatively correlated with endurance level.

Our study revealed that, in long-distance runners, endurance training leads to an increase in erythrocyte HGPRT activity and to a decrease in Hx formation in the competition period. The process of re-utilization of Hx and its conversion to IMP may be preferred with increase in endurance level (training status). Thus, a more “economical” metabolism is favoured that aims to salvage the purine ring, biosynthesis of which is very energy consuming. This may bring about a lower level of oxypurine in the blood of trained than in the blood of non-trained individuals after the same exercise. Oxypurine concentration may decrease as a result of the reaction of Hx with phosphoribosylpyrophosphate (PRPP) catalyzed by HGPRT, leading to IMP formation. Consequently, the level of IMP, being a link in the purine nucleotide cycle, increases.

The potential limitation of our study is a single blood sampling point at 5 min of recovery. It is known that Hx concentration increases after exercise and reaches maximum value after 10–20 min (Harkness et al. 1983; Hellsten-Westing et al. 1989; Sahlin et al. 1991) and UA concentration even later (Stathis et al. 1994, 2006). Moreover, no significant changes after 5 min of recovery were found in plasma Hx concentration during a moderate-intensity exercise (Sahlin et al. 1999). From this point of view, further postexercise samples during 15–20 min would have been optimal. However, at the same time, other studies reveal significant differences in metabolic response to exercise between groups of subjects, which is of great importance in the context of sports training. Bianchi et al. (1999) showed that postexercise plasma oxypurines (at 5 min of recovery) were more precise predictors of muscle energy exhaustion than strain intensity or duration in athletes specialized in different sports. Bhattacharya et al. (1983)

revealed a differentiation of pyruvate exercise response in short-, middle- and long-distance runners (2.5–3 min after exercise). Rychlewski et al. (1997) used plasma Hx (5 min after exercise) as an indicator of exercise intensity in athletes. When the practical application of measured parameters in individual athletes is considered, information must be provided almost instantly to an athlete/coach. Short time between exercise end point and blood sampling might enable current (during the training session) modification of the training load depending on metabolic response. Thus, such a laboratory procedure seems to be justifiable and useful.

In summary, the results of the present study demonstrated that resting and postexercise plasma concentrations of X and, Hx and erythrocyte HGPRT activity change significantly in competitive long-distance runners during 1-year training cycle. Significant changes in resting–postexercise values between training phases were found for Hx and UA. Endurance training in competitive long-distance runners was the cause of a significant decrease in postexercise plasma X concentration and resulted in an increase in resting and postexercise HGPRT activity in the competition period. During the transition period, Hx concentration increased and HGPRT activity decreased significantly. The effect of our study may be that the level of plasma Hx at rest as well as after standard exercise may be a useful tool for monitoring the adaptation of energetic processes in different training phases. The analysis of diverse reactions of plasma Hx could also support the overload and overtraining diagnosis. Moreover, collecting blood samples within 5 min after exercise seems to be more suitable to sport practice than after a longer time. A quick measurement and the possibility of analysis would enable both coach and athlete to control the effects of training loads immediately after the exercise and to modify it possibly in the course of a training session or a training period. In the future, one could analyse the effects of longer training cycles in athletes practising other competitive sports to measure the changes in purine metabolism and its application to training in a more detailed way. It would be interesting to investigate the different types of training and loads which would cause the most advantageous changes in post-training purine concentrations in competitive athletes.

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