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Pathways of purine metabolism: effects of exercise and training in competitive athletes

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Abstract

Introduction. The main part of skeletal muscle adenosine-5'-triphosphate (ATP) is restored by inosine monophosphate (IMP) reamination in the purine nucleotide cycle. The intramuscular resources of IMP may be resynthesized via the quick and economical salvage pathway, in which muscle hypoxanthine (Hx) is reconverted to IMP by hypoxanthine-guanine phosphoribosyl transferase (HGPRT). IMP is subsequently reutilized in the adenine nucleotide (AdN) pool. Inosine and Hx, which flow out of the skeletal muscle, represent the loss of AdN precursors. In the latter case, full restoration of resting ATP levels depends on a slow and energy-consuming *de novo* pathway. Plasma Hx and erythrocyte HGPRT are indirect indicators of muscle metabolism, particularly of AdN degradation, that reflect exercise- and training-induced muscle energy status. Results. Our analyses of long-term training cycles in different sports show that plasma Hx concentration and erythrocyte HGPRT activity significantly change in consecutive training phases. Both high-intensity sprint training and endurance training incorporating high-intensity exercise lead to a decrease in plasma Hx levels and an increase in erythrocyte HGPRT activity. The lowest Hx concentration and the highest HGPRT activity are observed in the competition phase characterised by low-volume and high-intensity training loads. Training cessation in the transition phase brings about a reverse phenomenon: an increase in Hx levels and a decrease in HGPRT activity. Conclusions. Low plasma purine levels indicate that the administered training adapts the athletes to high-intensity exercise (more economical AdN use, limited purine efflux from muscle into the blood). Such an adaptation is of great importance for contemporary elite athletes. Purine metabolites are more sensitive markers of training status and better performance predictors than typical biochemical and physiological indicators (e.g. blood lactate and oxygen uptake) in highly-trained athletes of different specializations and ages. The use of Hx and HGPRT for monitoring and control of the training process is worthy of consideration.

KEYWORDS: adenine nucleotide catabolism, purine metabolism, hypoxanthine, one-year training cycle, muscle and metabolic indicator, HGPRT.

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What is already known on this topic?
Previous studies focused on changes in purine metabolism solely during short sprint training periods of 6-7 weeks. Such a training results in reduced loss of muscle adenine nucleotide pool, reduced resting and post-exercise Hx levels, increased muscle HGPRT activity and reduced efflux of purines from the muscle into the blood. These observations are evidence of metabolic adaptation to exercise demands, in particular, reduction in muscle adenine nucleotide loss.

Adenine nucleotide catabolism

The rates of degradation and synthesis of muscle adenosine-5'-triphosphate (ATP) are equal when energy balance is maintained. At rest, ATP constitutes

over 90% of the muscle adenine nucleotide (AdN) pool and is an important part of the process of energy transfer in cells. ATP catabolism intensifies regardless of exercise duration [19, 22, 47, 52, 53]. During metabolic stress, ATP molecule is dephosphorylated to adenosine 5'-diphosphate (ADP) and inorganic phosphorus (Pi), delivering energy for muscle contraction. This leads to an increase in ADP levels, intensification of the myokinase and adenosine 5'-monophosphate (AMP) deaminase reactions and, consequently, reduction in the AdN pool (∑ATP+ADP+AMP) [19, 53, 58]. In the myokinase reaction, one phosphate moiety is transported between two ADP molecules. As a result, one ATP and one AMP molecule is formed. AMP is then deaminated by AMP deaminase to inosine monophosphate (IMP) and ammonia (NH_3) that accumulate in the skeletal muscle [31, 45, 58]. An alternative pathway of AMP degradation is dephosphorylation to adenosine and ortophosphatase by 5'-nuceotidase (5'-NT) [39, 57]. However, this pathway is relatively weakly active in the skeletal muscle [39, 44, 48, 72].

IMP being accumulated in the muscle may be reaminated to AMP in the purine nucleotide cycle (PNC), restoring the AdN pool, or further be dephosphorylated to inosine (Ino) in a reaction catalysed by 5'-NT [16, 31]. However, the reamination is very limited during intense exercise [35] and Ino is mainly degraded to hypoxanthine (Hx) in a reaction mediated by purine nucleotide phosphorylase (PNP) [49].

In the skeletal muscle, Hx is degraded to xanthine (X) and uric acid (UA) in an irreversible reaction catalysed by xanthine dehydrogenase (XDH) [31, 58]. The efflux of Ino [14] and Hx [2, 14, 16] from skeletal muscle into the blood after a high-intensity exercise until exhaustion may even deplete high-energy resources by 9% [16]. In resting conditions, 80-90% of this enzyme occur as dehydrogenase [4, 40] but under metabolic stress the dehydrogenase form is transformed into oxidase form [8]. Regardless of the form, XDH catalyses the degradation of Hx to X and then to UA. Moreover, both forms play an important role in the formation of free radicals that can cause tissue damage by triggering specific chemical reactions. The transformation of dehydrogenase into oxidase is mediated by nicotinamide adenine dinucleotide (NAD), an electron acceptor, resulting in the formation of superoxide radicals [30]. XDH is present in the endothelium of most human tissues including liver, intestine, heart, lungs and skeletal muscles [19, 25] but its activity differs between tissues [21]. The highest activity was shown in endothelial liver cells, which suggests that liver substantially contributes to Hx degradation to UA [21]. Plasma purine metabolites (Ino, Hx and UA) are excreted via kidneys [14, 50, 52, 55] or, in the case of UA, via gut [50].

Pathways of adenine nucleotide salvage

The main part of skeletal muscle ATP is restored by IMP reamination in the PNC cycle. The intramuscular resources of IMP may be, in turn, resynthesized via the salvage pathway [37]. Hx is the only compound that can be reconverted by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and reutilized in the AdN pool [33, 37]. Ino and Hx, which are moved out of the skeletal muscle, represent the loss of AdN precursors. In the latter case, the full restoration of resting ATP levels depends on a relatively slow and energy-consuming *de novo* pathway [33, 58] that needs six high-energy phosphate bonds [38]. IMP is the final product of both salvage and *de novo* pathways.

IMP reamination

The restoration of the AdN pool using IMP is a two-stage reamination during the PNC. The reactions are catalyzed by adenylosuccinate synthetase (AdSS) and adenylosuccinate lyase (AdSL). AdSS catalyses the transformation of IMP and aspartate to adenylosuccinate. The availability of specific substrates, i.e. IMP, guanosine 5'-triphosphate (GTP) and aspartate, regulates the AdSS activity [54]. However, only IMP significantly affects AdSS during muscle activity [54]. Resting muscle IMP concentration is about 0.07 mmol. Thus, even a small increase in muscle IMP levels elevates AdSS activity [10]. In spite of this fact, increased IMP concentration inhibits the rate of the reaction *in vitro* [10, 54]. The regulation of AdSS activity is a complex process, and its activation seems to depend on the balance of activators and inhibitors. Meyer and Terjung [35] demonstrated in rats that during high-intensity exercise AdSS is inhibited and PNC is not effective. As a result, reamination occurs only during recovery, as was also confirmed in humans [16, 64]. AdSL, in turn, catalyses two reactions leading to the formation of purine nucleotides. The first reaction transforms succinylaminoimidazolecarboxamide ribotide (SAICAR) to aminoimidazolecarboxamide ribotide (AICAR) and fumarate. The second reaction the AdSL regulates is transformation adenylosuccinate to AMP and fumarate.

Purine nucleotide salvage

The salvage pathway, involving purine bases, allows the avoidance of the energy-consuming *de novo* synthesis of nucleotides [33] and limits purine loss from skeletal muscle by IMP resynthesis from Hx. The latter reaction combines Hx with 5-phosphoribosyl-1-pyrophosphate (PRPP) to form IMP and is catalysed by HGPRT [37, 60]. In resting conditions, HGPRT is responsible for restoration of about 75% of intramuscular Hx [9], but its role in post-exercise purine recovery is not clear. Kim et al. [28] showed that PRPP availability limits the rate of IMP formation, and that ribose-5-phosphate provision regulates PRPP availability. This was supported by Harmsen et al. [15], who elicited an intensified synthesis of purine nucleotides from Hx when ribose was added to the fluid perfusing the myocardium. Ribose supply to rat hindlimb preparations brought about a 3- to 8-fold increase in the rate of Hx salvage [6]. Factors affecting muscle HGPRT activity have not been identified until now, however, ribose supplementation increases the rate of muscle ATP recovery in the 72 hours after a 1-week high-intensity sprint training [18].

***De novo* synthesis of adenine nucleotides**

The formation of one IMP molecule in *de novo* pathway is energy-consuming and demands the use of six high-energy phosphate bonds [38]. The initial substrate for *de novo* synthesis is PRPP and then, in a series of complex reactions, carbon and nitrogen atoms – delivered by glutamine, aspartate, glycine, formate and bicarbonate ions – are added to PRPP to produce IMP. The latter is incorporated into the AdN pool via the PNC [38]. The availability of PRPP (and other substrates) seems to be the factor limiting the rate of *de novo* synthesis *in vivo* [15, 58, 71]. PRPP synthesis is catalysed by PRPP synthetase, an enzyme inhibited by various nucleotides, particularly by AMP, ADP and guanosine 5'-diphosphate (GDP) [34]. The rate of *de novo* synthesis may be limited by the flow through the hexose monophosphate (HMP) shunt, which produces ribose necessary for PRPP formation [70]. Tullson and Terjung [58] proposed that *de novo* synthesis of AdN occurs under favourable metabolic conditions, especially at a high ATP/ADP ratio.

Muscle purine efflux into the blood

After exercise, the accumulated muscle IMP is dephosphorylated in an irreversible reaction catalysed by 5'-NT. As a result, muscle inosine content increases after 3-10 min recovery [16, 53, 64]. Ino, in turn, may degrade to Hx in the reaction catalysed by PNP. The concentration of muscle Hx and Ino at rest is small, approximately 0.01 $mmol \cdot kg^{-1}$ w.w. (wet weight), and rises following intense exercise to peak at 8 and 35 $\mu mol \cdot min^{-1}$, respectively [16]. After a short exercise until exhaustion, muscle Hx and Ino concentrations elevate 2- and 4-fold, respectively. In contrast, muscle IMP post-exercise concentration increases 135-fold compared to resting conditions (about 0.015 $mmol \cdot kg^{-1}$ w.w.). The release of Ino and Hx from the muscle at rest is small and increases after intense exercise [16]. Hx accumulates in the muscle not only after high-intensity exercise but also during long-lasting endurance exercise until exhaustion. Intramuscular Hx accumulation is not observed after intense short-time exercise [13, 16, 53, 64].

Purine bases flow out of the muscle across biological membranes into the blood. Two main types of nucleotide transporters (NTs) are probably involved. These are equilibrative nucleoside transporters (ENTs) of the SLC29 family and concentrative nucleoside transporters (CNTs) of the SLC28 family [7, 62]. ENTs are facilitated transport carrier proteins that translocate nucleosides and nucleobases down their concentration gradients [24, 61]. CNTs are secondary active transporters that co-transport nucleosides and sodium unidirectionally against the nucleoside concentration gradients [7]. In humans, three ENTs are reported: hENT1 [11], hENT2 [61] and hENT3 [24], of which hENT2 have been found in skeletal muscle [42, 61, 62, 63]. The understanding of their function is, as yet, limited in spite of the fact that their expression level in muscle tissue is high [32, 41] and they actively support metabolism, as evidenced by high PrN turnover and increased vasodilation in response to exercise demands [21]. Adenosine (and probably Ino) may play an important role in the regulation of blood glucose transport to the muscle [12]. It is believed that adenosine formation occurs extracellular by ecto-5'-nucleotidase activity [5]. This suggests that the main role of NTs in the muscle is the salvage of purine nucleosides and their metabolites to be further transformed. As in other tissues, extracellular muscle adenosine seems to act through A1 receptors which signalize the increase of the insulin-stimulated glucose extraction [56]. The role of NTs as modulators of these processes is not

known. The studies of Hellesten et al. [16, 17] revealed a unidirectional Hx flow from the muscle into the blood, which limits the possibility to transport purine bases across cell membranes.

Plasma purines

As described above, purines (Hx, X, UA) flow out of the muscle into the blood across cell membranes. However, plasma purines have various sources and the doubt arises if the exercising muscle is their main source. To decide this question, arterial and venous blood samples were simultaneously drawn from vessels supplying an active muscle. It was found that the active muscle is the main source of plasma Hx, but UA and Ino are only released from working muscle to a small extent. Similar measurements were made in liver circulation, which showed that liver extracts Hx from plasma after exercise when arterial Hx concentration is increased. Liver releases a substantial amount of UA post exercise and significantly contributes to the increase in plasma UA concentration, even if other important sources of UA exist [21]. The elevated production of UA in liver compared to that of muscle production may be explained by the differences in XDH activity. Human muscle is characterized by a much higher level of this enzyme than liver [19, 25]. Separate sources of Hx and UA explain the considerable time shift in peak plasma concentrations of these purines. Maximum plasma Hx concentration is observed 10-20 min and UA 45-60 min after exercise. The delay in plasma UA increase is due to the time the liver needs to extract Hx, produce UA and release it into the blood [21].

Accumulation of purine metabolites in plasma

Inosine

Resting plasma Ino concentration is approximately 1 mmol and increases to 4-6 μmol during maximal exercise [16]. Although during intense exercise the increase in muscle Ino surpasses that of Hx, plasma Ino accumulation is small. It may be due to the high activity of PNP both in muscle and endothelial cells. Plasma Ino accumulation is intensity-dependent. Kono et al. [29] showed that cycling at low intensity did not increase plasma Ino concentration in healthy subjects, but a 2-minute intense cycling did [14]. Also, specific local exercise using extremities brings about significant plasma Ino elevation, e.g. 2-minute ischemic forearm exercise [36] and leg extension to fatigue [16].

Hypoxanthine

Resting plasma Hx concentration is about 2-5 $\mu mol \cdot l^{-1}$ [14, 27, 36, 46, 65, 66, 67, 69]. During maximal exercise, plasma Hx concentration elevates, peaking even to 80 $\mu mol \cdot l^{-1}$, thus exercise intensity is the main determinant of plasma Hx levels [14, 23, 27, 46, 49, 52, 53]. Hx is an important precursor of nucleotides for many cells, such as erythrocytes and central nervous system cells. Therefore, the extraction of Hx from plasma by various tissues probably intensifies with exercise intensity, as indicated by elevated erythrocyte Hx levels after intense exercise.

During repeated sprint exercise, the rate of plasma purine accumulation is determined by the recovery duration between consecutive bouts. The accumulation increases with shortening recovery time. One explanation is that AMP deamination is related to muscle PCr content: fast rate of AMP deamination is accompanied by increased AMP turnover and low PCr levels [19, 52, 53]. Sjödin and Hellsten-Westing [49] demonstrated that during incremental exercise a critical point exists, at which a rapid increase in plasma Hx concentration occurs. This point is reached at the intensity of about 110% of maximum oxygen uptake (VO_2max). Ketai et al. [27] revealed that plasma Hx elevates at intensities above ventilatory threshold (VT). Maximal-intensity sprint bouts do not induce the increase in plasma Hx concentration immediately after exercise. The peak increase is noticeable after 10-20 min of recovery [20]. Elevated plasma Hx concentration maintains at least 30 min after a 30-second maximal cycling [53] and 120 min after a 2-minute maximal-intensity run [20].

Plasma Hx concentration during and after exercise is determined by several main factors: (1) production in the muscle, proportional to exercise intensity and type; (2) reconversion to IMP by muscle HGPRT activity; (3) reconversion to IMP by erythrocyte HGPRT activity; (4) extraction from blood by liver and; (5) removal with urine [21, 26, 37].

Xanthine

Xanthine is a link between two reactions catalysed by xanthine oxidase: degradation of Hx to X and then X to UA [21]. Resting and post-exercise plasma X concentrations are 1.4-2.8 $\mu mol \cdot l^{-1}$ and 2.3-3.3 $\mu mol \cdot l^{-1}$, respectively. Our several studies revealed a significant increase in plasma X concentration after exercise until exhaustion [65, 66, 67, 68, 69]. Also, both resting and

post-exercise plasma X levels changed considerably in a 1-year training cycle in long-distance runners [69].

Uric acid

The resting concentration of UA in blood is approximately 250-350 μM in healthy humans [20, 49, 67]. At rest, two-thirds of circulating UA is removed via kidneys and 1/3 via gut [50]. The increase in plasma UA levels is observed 20-30 min after intense [23, 49, 53] or maximal exercise [65, 66, 67, 68, 69]. Exercise intensity [49] and duration [23] increase blood UA concentration. However, Hx and UA levels do not change significantly if the intensity is below 115% of VO_2max [49]. Moreover, peak UA concentrations are only observed 45 min after exercise termination [20]. The slow accumulation of UA is probably due to xanthine oxidase that is responsible for UA production in endothelial cells of blood vessels in the human muscle [25] and other tissues [59]. The xanthine oxidase activity is lower in skeletal muscle compared to other tissues and organs, particularly liver [59], which is the main source of UA production [21].

Purine metabolites in sport

Purines only accumulate in muscle in small amounts, as explicated above, because they flow out into the blood across cell membrane. There is no evidence that the permeability of cell membrane is increased. Therefore, the exercise-induced production and release of purines (especially Hx) into the plasma is expected to be the main reason for the decrease in muscle nucleotides levels.

Main determinants of plasma Hx concentrations are exercise intensity and duration [23, 46, 49]. As early as in 1980, Sutton et al. [55] proposed that Hx could be used as an indicator of ischemic tissue hypoxia. Two years later Balsom et al. [1] demonstrated that plasma Hx concentration substantially (15-40-fold) increased after intense sprint exercise. Then, Hx was proposed as an exercise intensity indicator [43] and a criterion for exercise classification [3]. Hx was also identified as a marker of metabolic stress during exercise and a marker of muscle AdN degradation [47].

Effects of short-term training on purine metabolites

Earlier studies focused on changes in purine metabolism during short sprint training periods (6-7 weeks). It was revealed that such a training resulted in reduced loss of muscle AdN pool [52, 53], reduced Hx levels both at rest and after exercise [22, 51, 52, 53], increased muscle HGPRT activity [19] and reduced efflux of purines from the muscle into the blood, limiting the loss of muscle purine nucleotides [19]. Lower resting concentration of Ino, precursor of muscle Hx, was related to lower post-exercise plasma Hx concentration. This has been supported in studies examining female hockey players [51] and male sprinters [53]. All the changes are evidence of metabolic adaptation to high-intensity exercise demands, particularly reduction in muscle AdN loss.

Effects of long-term training on purine metabolites

Zieliński et al. [69] revealed that resting as well as post-exercise Hx and X plasma concentrations and erythrocyte HGPRT activity significantly changed during a 1-year training cycle in competitive long-distance runners specializing in races 5 and 10 km. Post-exercise Hx levels changed from 9.3 $\mu mol \cdot l^{-1}$ in the competition phase to 22.9 $\mu mol \cdot l^{-1}$ in the transition period (Friedman's ANOVA, $p < 0.05$). In the transition phase, the post-exercise HGPRT activity was reduced (58.8 $nmolIMP \cdot mgHb^{-1} \cdot h^{-1}$) compared to the competition phase (76.2 $nmolIMP \cdot mgHb^{-1} \cdot h^{-1}$, $p < 0.01$). Post-exercise UA levels did not significantly change across the annual cycle and ranged between 371 and 399 $\mu mol \cdot l^{-1}$. This study suggests that plasma Hx concentration at rest and after standard exercise may be a useful tool for monitoring metabolic adaptation in different training phases as well as may support overtraining diagnosis.

Changes in training loads and purine metabolism

We studied the effects of training loads on purine metabolism in competitive middle-distance runners and healthy recreational runners [66]. Apart from Hx, X, UA and HGPRT levels, training loads were analyzed in four consecutive phases of a 1-year training cycle. In competitive runners, the competition phase was characterized by a significant decrease in the amount of low- and moderate-intensity aerobic exercise (by 65.4% and 20.5%, respectively) and a considerable increase in high- and maximum-intensity exercise (by 132.5% and 74.6%, respectively). In this phase, a significant decrease in post-exercise plasma Hx was found (by 6.2 $\mu mol \cdot l^{-1}$), accompanied by an increase in HGPRT activity (by 4.9 $nmolIMP \cdot mgHb^{-1} \cdot h^{-1}$). The effect of high-intensity training loads was critical, although their contribution to the total load (8.2% of

net exercise time) was relatively small. In the transition phase, reverse changes were observed, i.e. increases in Hx levels and a reduction in HGPRT activity. No significant changes were revealed in the recreational group, in which training loads were relatively constant across the whole year.

The increased HGPRT activity in the competition phase suggests adaptation changes that are connected with permanent systemic readiness to purine restoration. Training cessation in the transition phase brings about reverse changes. Thus, plasma Hx concentration and erythrocyte HGPRT activity may be considered useful indicators sensitive to metabolic changes induced by high-intensity anaerobic exercise in competitive athletes.

Training modality and purine metabolism

We also analysed the effects of training loads on purine metabolism in highly-trained sprinters and triathletes in a 1-year cycle [65]. Plasma Hx and resting erythrocyte HGPRT activity were assayed in four training phases: general (increase in volume and intensity of the training load), specific (decrease in volume, increase in intensity), competition (low volume, high intensity) and transition (reduction in volume and intensity, training cessation). In all the phases, Hx concentration was lower in sprinters (8.1-18.0 $\mu mol \cdot l^{-1}$) than in triathletes (14.1-24.9 $\mu mol \cdot l^{-1}$), even though the pattern of Hx changes was the same, i.e. a significant decrease occurred in the competition phase and an increase in the transition phase. In sprinters, HGPRT activity was higher than in triathletes across the whole cycle (e.g. 71.8 vs 66.6 $nmolIMP \cdot mgHb^{-1} \cdot h^{-1}$ in the competition phase, respectively). In both groups, HGPRT activity increased in the competition phase and decreased in the transition phase.

The results of the above study suggest that the long-term sprint training leads to a more pronounced decrease in resting and post-exercise Hx levels and a higher erythrocyte HGPRT activity than endurance training. It could be due to a more efficient use of anaerobic energy sources in response to sprint training, based on high-intensity exercise.

The changes in plasma Hx and erythrocyte HGPRT activity may be used as a sensitive indicator of training status. They reflect adaptation changes consisting in the readiness to purine salvage, and provide indirect information about the metabolic status of muscles in highly-trained athletes, in which adaptation changes are hardly detectable using popular acknowledged biochemical and physiological measures (lactate, maximal and threshold oxygen uptake).

Effects of performance level and age on purine metabolism

In another study, we also showed the effect of a 1-year training cycle on changes in plasma Hx and erythrocyte HGPRT in middle-aged master runners of different performance levels: highly-trained competitive athletes (46.0 ± 3.8 years old), amateur (45.1 ± 4.7 years) and recreational runners (45.9 ± 6.1 years) [67]. The athletes also differed in training loads. The highly-trained runners used aerobic, mixed and anaerobic exercise in their training routine. Amateur runners used aerobic and mixed (but not anaerobic) loads, and recreational runners only aerobic training loads. Hx concentration and HGPRT activity were measured in three consecutive training phases, in which significant changes and between-group differences were shown.

In the highly-trained group, the lowest Hx concentration and the highest HGPRT activity were revealed across the whole training cycle, compared to both less trained groups. Analogous differences were shown between the amateur and recreational groups, but solely in the specific preparation phase. In competitive runners, the changes were visible between all training phases with the lowest Hx concentration and the highest HGPRT activity in the competition phase. In the amateur group, the change was only significant between general and specific preparation without any further change in the competition phase. In recreational runners, who did not use anaerobic exercise, no significant changes were revealed during the whole cycle.

In competitive athletes, who use anaerobic exercise to a greater extent, muscle Hx production and release into the blood are attenuated. Therefore, Hx may be reconverted to IMP (increased HGPRT activity) both in muscle and plasma. Probably, less Hx is also excreted with urine. The post-training decrease in Hx concentration is indirect evidence that anaerobic exercise reduces resting muscle Hx production and efflux, which was previously demonstrated by Stathis et al. [52, 53]. Our research demonstrates that incorporation of high-intensity (anaerobic) exercise in long-term endurance training causes significant changes in purine metabolism, whereas a low-intensity (aerobic) training does not. Therefore, plasma Hx

concentration and erythrocyte HGPRT activity may be considered sensitive markers of training adaptation and training status as well as a criterion for training control.

Purine metabolites as predictors of sport performance

We assessed the usefulness of plasma Hx levels for the prediction of sport performance [68]. Season-best actual race times were considered in triathletes (standard distance), long-distance runners (5000 m), middle-distance runners (1500 m) and sprinters (100 m). Resting and post-exercise (treadmill test until exhaustion) plasma concentrations of Hx, X, UA and lactate were measured as well as resting erythrocyte HGPRT activity. Statistical models (multiple regression) were built and evaluated. It was revealed that Hx as an independent predictor better predicted performance in the four above groups (coefficient of determination: r^2 = 0.81, 0.81, 0.88 and 0.78, respectively) than in models based on aerobic capacity and lactate (r^2 = 0.51, 0.37, 0.59 and 0.31, respectively). Models that combined purine metabolites and cardio-respiratory variables were most effective in performance prediction (r^2 = 0.86, 0.93, 0.93 and 0.91, respectively). Moreover, in sprinters only purine metabolites were able to accurately predict performance. The results indicate that Hx may be used as a strong predictor of sport performance in highly-trained athletes regardless of training and competition specificity: from speed-power to endurance disciplines. However, at present, this conclusion only applies to measurable performance (time, distance etc.) in individual sports. Further research is needed to evaluate the prediction strength of purines in team and other sports where a more complex set of factors (e.g. team tactics and co-operation) determines the performance level, and where competition results are expressed as scores, relative measures (ranking) or quasi-subjective assessment (panel of judges).

Summary

The analysis of long-term training cycles showed that indicators of purine metabolism, i.e. plasma Hx concentration and erythrocyte HGPRT activity, significantly change across consecutive training phases in athletes of different specializations, performance levels and ages. The changes result from intentional modifications of training loads during longer training cycles. Long-term high-intensity sprint training, but also endurance training incorporating high-intensity exercise, both lead to a decrease in plasma Hx levels and increase in erythrocyte HGPRT activity. The extent of these changes is determined by the amount of intense anaerobic exercise used by athletes. The lowest Hx concentration and the highest HGPRT activity are observed in the competition phase characterised by low-volume and high-intensity training loads. Training cessation in the transition phase brings about a reverse phenomenon: increase in Hx levels and decrease in HGPRT activity.

Plasma Hx is an indirect indicator of muscle metabolism, particularly of AdN degradation, reflecting exercise- and training-induced muscle energy status. Lower plasma purine concentration in the competition phase indicates that the administered training adapts the athletes for high-intensity exercise (more economical AdN use expressed as attenuated purine efflux from muscle into the blood). Such an adaptation is of great importance for contemporary elite athletes using high-intensity exercise more and more often in their training and competition.

Practical aspects

Purine metabolites, especially Hx and HGPRT, could be used as indicators of the training status change in different training phases, regardless of sport discipline. Hx and HGPRT are sensitive markers of training status in highly-trained athletes. They enable assessing training effectiveness and status in both young and older athletes. It is particularly important in view of the fact that "classic" measures such as blood lactate or VO_2max are less sensitive to training load modifications and do not change significantly across consecutive training phases in highly-trained athletes. Moreover, typical biochemical and physiological measures of aerobic capacity are unable to describe and evaluate the outcomes of the sprint training aimed at reaching a high explosive capacity (maximum intensity, extremely short time). It seems that the lactate or ventilatory threshold conception, currently "in force", and derived training recommendations should be revised in the context of purine metabolism. Although the procedures outlined in this article are not yet possible during training sessions outside laboratory, the future technological development may bring field methods based on portable devices. In such a case, exercise modification could be implemented on the spot, after obtaining information about metabolic reaction. Using purine metabolites, particularly Hx,

for monitoring and control of training status in highly-trained athletes is worthy of consideration.

What this study adds?

We are the first to show the effects of long-term (one-year) training cycles on purine metabolism in highly-trained athletes of different specializations and ages. We demonstrate that plasma purines may be a useful tool for monitoring metabolic adaptation because their levels significantly change across consecutive training phases depending on modifications in exercise volume and intensity. Long-term sprint training leads to more favourable changes in purine metabolism than endurance training but the annual pattern of variations is the same for both modalities. Purine metabolites are, thus, sensitive markers of training adaptation and status. Moreover, they are strong predictors of sport performance in highly-trained athletes practising individual sports, regardless of specialization: from speed-power to endurance disciplines.

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