Hypoxanthine: A Universal Metabolic Indicator of Training Status in Competitive Sports

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ZIELINSKI, J. and K. KUSY. Hypoxanthine: a universal metabolic indicator of training status in competitive sports. Exerc. Sport Sci. Rev., Vol. 43, No. 4, pp. 214–221, 2015. Cardiorespiratory and biochemical indicators typically used by contemporary elite athletes seem to have limited applicability. According to some recent studies, purine metabolism better reflects exercise response and muscle adaptation in this group. We propose using purine derivatives, especially plasma hypoxanthine concentration, as indicators of training status in consecutive training phases in highly trained athletes.

Key Words: adenine nucleotide catabolism, hypoxanthine, 1-yr training cycle, metabolic indicator, HGPRT

INTRODUCTION

Some studies suggest that “classical” cardiorespiratory and metabolic indicators have limited applicability to training control in contemporary highly trained athletes. In elite long-distance runners, for example, aerobic capacity no longer appropriately pictures their athletic potential (14). Even if a high level of aerobic capacity is still a prerequisite for entry into elite, there is no clear relationship between maximum oxygen uptake and performance. In our previous articles (28–30,32), we reported that, in advanced athletes, maximal oxygen uptake remains unchanged during 1-yr or several years’ training cycles and does not permit discrimination between athletes of different sport levels and specializations. Also, no clear changes in blood lactate levels are observed between consecutive training phases in highly trained athletes, which is evidence of its questionable diagnostic value (29,30). Competitive endurance athletes show a relatively stable level of aerobic capacity across longer training cycles (29,30,32) and, consequently, their success is determined by anaerobic capacity (overtaking, accelerating, keeping high movement speed, or finishing fast) rather than aerobic potential. In speed-power disciplines, maximal aerobic capacity does not characterize the main desired training outcome, which is explosive performance (e.g., sprint run). Aerobic capacity is of some importance in the general preparation phase, but specific metabolic indicators would be more valuable for speed-power training adaptations.

The search for new metabolic indicators of training status based on anaerobic metabolism is justifiable. On the basis of our recent explorations (28–32), we propose using purine derivatives, especially plasma hypoxanthine (Hx) concentration and erythrocyte Hx-guanine phosphoribosyltransferase (HGPRT) activity, as indicators of training status in main training phases (general, specific, competition, and transition) in highly trained athletes. Our studies are probably the first attempts to encompass changes in purine metabolism in a whole 1-yr training cycle in competitive athletes. In this article, we briefly show the pathways of adenine nucleotide catabolism, then we present the results of our and other studies on exercise and training effects on plasma Hx and erythrocyte HGPRT levels, and, finally, we touch on practical perspectives.

ADENINE NUCLEOTIDE CATABOLISM

The main metabolic pathways for exercise-induced adenine nucleotide catabolism are shown in Figure 1. In a steady state, the rate of hydrolysis of adenosine-5’-triphosphate (ATP) equals the rate of its resynthesis. Short and prolonged exercises lead to increased muscle ATP catabolism (5,6,18,23,24). As a result, the rate of ATP degradation exceeds the maximal rate of its resynthesis, and inosine-5’-monophosphate (IMP) and ammonia accumulate in the skeletal muscle (9,16,27). IMP may be reaminated to adenosine-5’-monophosphate (AMP).
in the purine nucleotide cycle, restoring the adenine nucleotide pool, or be degraded (dephosphorylated) further to inosine in a reaction catalyzed by 5'-nucleotidase (4,9). However, the reamination of IMP to AMP occurs to a small extent during high-intensity exercise (11), and inosine is degraded further to Hx with the use of purine nucleotide phosphorylase (19). Both inosine and Hx efflux from the skeletal muscle into the blood and reduce the intramuscular adenine nucleotide pool. Hellsten et al. (4) demonstrated that the efflux of Hx and inosine from the muscle after high-intensity exercise until exhaustion depletes high-energy stores even by 9%. In the skeletal muscle, blood, and liver, Hx is degraded further to xanthine and uric acid in an irreversible reaction catalyzed by xanthine dehydrogenase (9,27). Inosine, Hx, and uric acid that accumulate in plasma can be removed via the kidneys and urine (3,21,23,26) or, in the case of uric acid, via gut (21).

IMP is a purine nucleotide that cannot pass through the sarcolemma. Intramuscular stores of IMP can be resynthesized from Hx by salvage using muscle HGPRT (big dashed box) or by the energy-consuming de novo synthesis from purine bases (small dashed box). We studied the blood levels of purine bases that efflux the muscle (big gray box), especially plasma Hx, reflecting the loss of muscle adenine nucleotides during exercise. Erythrocyte HGPRT activity (small gray box) also is exercise and training dependent; however, mechanisms are not known yet. See the text for more explanation. ADP, adenosine-5'-diphosphate; AMP, adenosine-5'-monophosphate; AMP-s, AMP synthase; ATP, adenosine-5'-triphosphate; HGPRT, Hx-guanine phosphoribosyltransferase; Hx, hypoxanthine; IMP, inosine-5'-monophosphate; Ino, inosine; NH₃, ammonia; PNC, purine nucleotide cycle; PRPP, phosphoribosyl pyrophosphate; UA, uric acid; X, xanthine.

**EXERCISE AND TRAINING EFFECTS**

The Effect of Exercise on Plasma Hx Levels

Sutton et al. (26) were the first to examine the influence of exercise on purine metabolism in man. Rychlewski et al. (15) demonstrated that plasma Hx concentration depends on exercise intensity. Sahlin et al. (18) suggested that Hx may be an index of muscle adenine nucleotide degradation and a marker of exercise-induced energetic stress. Bianchi et al. (2) classified exercise types according to plasma Hx levels.

Resting plasma Hx concentrations range between 1 and 5 μmol·L⁻¹ (3,8,24,29). High-intensity or prolonged moderate-intensity exercise brings about 2- to 20-fold rise in plasma Hx levels depending on the exercise protocol and specific response of the individual (1,17–19). Exercise intensity and duration are the key parameters for purine nucleotide metabolism that determine postexercise plasma Hx concentration (7,17,19). Even prolonged moderate-intensity exercise results in a significant increase in plasma Hx concentration as early as 5 min after ceasing exercise (18). Highest plasma Hx levels are observed 10 to 20 min after exercise (3,17). After maximal sprint exercise, the concentration may rise 40-fold compared with resting conditions (1).

There is a critical point at the exercise intensity of 107% to 115% of maximum oxygen uptake at which a rapid increase in plasma Hx concentration occurs, interpreted as an expression of the predominance of ATP catabolism over ATP.
Effect of Short-term Training on Hx Changes

Some adaptations occur very rapidly with training. Muscle resting ATP levels already decreased within 1 wk (5,6) and 7 wk (22,24) of high-intensity training and influenced plasma Hx concentration after a sprint bout (23). High-intensity intermittent training increases muscle HGPRT activity and limits purine efflux from the skeletal muscle into the blood after intensive exercise. Consequently, resting and postexercise plasma Hx concentrations decrease (5,6). The recovery of muscle ATP (which degrades to Hx) after exercise may be relatively slow because of the rate-limiting de novo synthesis. In untrained individuals, muscle ATP is restored within 72 h of rest but in previously trained ones already within 3 h (6). It seems that the period immediately after exercise is critical for changes in plasma Hx that reaches its peak concentration about 20 min after a sprint bout and then returns to preexercise levels within 2 h (23). After sprint training, peak plasma Hx is lower and the recovery is faster than pretrained (23).

Available studies only analyzed purine metabolism in nonathletic individuals (5,6,23,24) but, most likely, strong training stimuli used by elite athletes are a greater metabolic challenge. Also, reduced training activity or rest is as important as exercise for scheduling training sessions in elite athletes. Thus, the exercise and recovery concentrations of Hx during heavy training and competition periods may serve as parameters to control the freshening up before a main event or “tapering.” Resting plasma Hx may be considered less valuable as a training tool because of its different fates: loss from the muscle (6,24) and subsequently excretion from the body via the urine as Hx or uric acid (23,25) combined with the increase in HGPRT activity (reflecting de novo synthesis) in the muscle (5), as presented in Figure 1. However, in the next section we report significant changes in resting plasma Hx in highly trained athletes during long-term training cycles.

Effect of Long-term Training on Hx Changes

Our research has focused on 1-yr training cycles in highly trained (national- and international-level) competitive athletes of different specializations. Young athletes were aged 19 to 29 yr (competitive experience 6–10 yr), and master runners were aged 39 to 51 yr (16 ± 5 yr). The athletes underwent an incremental exercise test until exhaustion on a treadmill (initial speed 10 km h⁻¹, increase by 2 km h⁻¹ every 3 min). The tests were performed after a minimum of 24 h of rest. Venous blood samples were taken at rest and 5 min after exercise. Hx and erythrocyte HGPRT activity were measured using high-performance liquid chromatography. Each subgroup (triathletes, distance runners, sprinters) consisted of 9 to 28 athletes depending on the study.

We have revealed (Figs. 2 and 3) significant changes in resting and postexercise plasma Hx concentration and erythrocyte HGPRT activity between main training phases (general, specific, competition, transition) in uniform groups of 9 young long-distance runners (31), 11 middle-distance runners (29), 10 sprinters, 10 triathletes (28), and 11 middle-aged runners (31). In all groups, the lowest postexercise increase in plasma Hx was observed in the competition phase. The increase was 3.1-fold, 3.4-fold, and 4.0-fold in young sprinters, middle-distance runners, and triathletes, respectively,
compared with resting values. In middle-aged runners, the increase was greater: 4.6-fold, 5.9-fold, and 7.0-fold in elite master, amateur, and recreational athletes, respectively (29–31). In contrast, the greatest differences between resting and post-exercise Hx concentration were found in the transition period (reduced training amount): 5.6-fold in sprinters, 5.8-fold in middle-distance runners, and 7.4-fold in triathletes (29,30).

These results indicate that the Hx response to a standard exercise as well as resting Hx changes with consecutive training phases. Thus, it can be considered a useful tool to monitor training metabolic adaptation and supports the diagnosis of physical overload and overtraining (29–32).

Effect of Long-term Training on Resting Hx

In Figure 2, we showed significant changes in resting Hx across a 1-yr training cycle in young sprinters, middle-distance runners, and triathletes (29,30,32). Training adaptation was manifested by the decrease in resting Hx levels (by ~10%) in the specific preparation and competition phases in sprinters and triathletes (29). An inverse phenomenon, that is, an increase in resting Hx, was characteristic of the transition period (up to 27% in middle-distance runners) (29,30,32). In line with our findings, Hellsten-Westing et al. (6) demonstrated an increase in maximal posttraining muscle HGPRT activity, supporting the reduced Hx efflux from the muscle into the blood.

A similar posttraining reduction in resting plasma Hx was demonstrated in elite female hockey players after a 7-wk training program (22). In contrast, in active untrained individuals, no significant change in resting plasma Hx was shown after a sprint training program of similar duration (24) or after a 1-wk program (23). The discrepancy likely is caused by differences regarding training load and workout schedule.

Effect of Long-term Training on Postexercise Hx

As shown in Figure 3, postexercise plasma Hx, measured 5 min after a standard maximal exercise test, significantly changed in a 1-yr training cycle in different groups: middle-distance runners, triathletes, and sprinters (29–32). We observed a considerable decrease in postexercise Hx concentration and a significant increase in erythrocyte HGPRT activity in the competition period (29–32). In this phase, sprinters had the lowest (8.1 μmol·L⁻¹) and triathletes the highest (14.1 μmol·L⁻¹) postexercise Hx concentration (28). In middle-aged runners, Hx levels were much lower in elite master athletes (14.6 μmol·L⁻¹) than in recreational participants (23.8 μmol·L⁻¹) (31). The reduction of training loads in the transition phase resulted in a reverse phenomenon: an increase in Hx levels and a decrease in erythrocyte HGPRT activity (29,30,32).

These results suggest that plasma Hx concentration and erythrocyte HGPRT activity may be used as training status indicators in consecutive training phases in athletes of different specializations and ages (28–32). A lower purge concentration in the competition period indicates that the administered training resulted in an adaptation to high-intensity exercise expressed as reduced purge efflux from the skeletal muscle. It represents a more economical energy distribution from ATP catabolism consisting of the posttraining decrease in resting muscle adenine nucleotide pool and exercise-induced ATP degradation (24).

Effect of Specific Training Loads on Hx Changes in a 1-Yr Cycle

We were the first to study the effect of training loads on purine metabolism in 1-yr training cycles in competitive athletes. We also analyzed the contribution of specific training loads in consecutive training phases. In the study on middle-distance runners (30), we divided the training loads into five zones: zone 1, low intensity, heart rate 130 to 150 beats min⁻¹; zone 2, moderate intensity, heart rate 150 to 160 beats min⁻¹; zone 3, high intensity, heart rate 160 to 170 beats min⁻¹; zone 4, very high intensity, heart rate 170 to 180 beats min⁻¹; zone 5, maximal intensity, heart rate more than 80 beats min⁻¹. The net exercise time spent in each zone was recorded and accumulated for each training phase to obtain the structure of training loads and its changes.

We revealed that the indices of purine metabolism changed in line with training load modifications. In the competition period, the load in zone 1 decreased by 65% and in zones 2 and 3 by 21%, whereas the load in high-intensity zones 4 and 5 increased by 133% and 75%, respectively. This coincided with a significant decrease in postexercise Hx concentration by 39% (Fig. 3, solid line) and an increase in postexercise erythrocyte HGPRT activity by 7.5%. In the transition phase, the proportion of training time in low-intensity zones 1 and 2 increased by 114% and 65%, respectively, whereas the contribution of high-intensity zones 4 and 5 decreased by 39% and 41%, respectively, compared with the competition period. Again, plasma Hx and erythrocyte HGPRT reflected the new levels of training loads: postexercise plasma Hx concentration increased by 176% and erythrocyte HGPRT activity decreased by 17%. No significant changes were observed in healthy untrained controls.

We have confirmed in other studies (29,32) that the increase in high-intensity exercise in the competition phase results in a significant decrease in plasma Hx levels (Figs. 2 and 3) and an increase in erythrocyte HGPRT activity. The training reduction entails a reverse response, that is, a significant increase in plasma Hx levels and a decline in erythrocyte HGPRT activity. The effect of high-intensity training loads is significant, although the total net training time they take is very short (~8% of the net time). The raised erythrocyte HGPRT activity in the competition period shows an adaptation that may be characterized as a “permanent readiness” to purine salvage. Purine markers can be, thus, regarded as useful metabolic indicators, sensitive to anaerobic high-intensity training loads.

In our other study, we showed that the relation between training load and purine metabolism is true not only for young but also for middle-aged (39–51 yr old, N = 11) elite athletes (31). In the latter group, the contribution of aerobic, mixed, and anaerobic high-intensity exercise was visibly altered, depending on the period of their 1-yr training cycle. Amateur runners (N = 9) used aerobic and mixed but not anaerobic loads. Recreational runners (N = 10) only practiced low-intensity aerobic training forms. Plasma Hx concentration and erythrocyte HGPRT activity were measured across the main training periods (general, specific, competition). In
elite runners, significant changes in purines were observed. The lowest level of plasma Hx and the highest erythrocyte HGPRT activity were present in the competition phase when the contribution of high-intensity loads was maximal. A similar significant change also was observed in amateur runners between general and specific preparation but not in the competition period because the load intensification was not continued. In recreational runners, plasma Hx levels and erythrocyte HGPRT activity remained unchanged during the whole annual cycle with invariable training. In general, the lowest plasma Hx concentration and the highest erythrocyte HGPRT activity were shown in elite master runners comparing with less advanced groups.

In athletes specializing in longer distances, the contribution of high-intensity loads and posttest elevations of plasma Hx are lower. Athletes specialized in shorter distances who use high-intensity exercise more often reduce the efflux of Hx into the blood more efficiently (29–32) (Figs. 2 and 3: competition period). In contrast, in recreationally active subjects who use anaerobic high-intensity exercise to a much lesser extent or not at all, the release of Hx into the blood is considerably higher than that in advanced athletes (30,31). Based on these results, one can assume that long-term training, in which new high-intensity loads are incorporated, brings about proportional changes in purine metabolism, whereas low-intensity training does not.

**Differences between Sprint and Endurance Athletes**

In principle, sprint and endurance training significantly affects purine metabolism in highly trained athletes provided that anaerobic exercise is incorporated (29). We observed a typical decrease in Hx concentration in the competition period and an increase in the transition period in both sprinters and triathletes. However, across the whole 1-yr training cycle, postexercise plasma Hx levels were lower in sprinters (8.1–18.0 μmol·L⁻¹) than in triathletes (14.1–24.9 μmol·L⁻¹) (Fig. 3). Resting Hx concentration was lowest in sprinters and highest in triathletes in both the competition (2.6 vs 3.5 μmol·L⁻¹, respectively) and transition (3.3 vs 4.4 μmol·L⁻¹, respectively) training phases (Fig. 2). Erythrocyte HGPRT activity increased in the competition period and decreased in the transition period, but sprinters were characterized by a significantly higher erythrocyte HGPRT activity in all training phases in comparison with triathletes, with the largest relative difference of 8.4% (P = 0.000) in the specific preparation and the smallest difference in the transition period (4.6%, P = 0.007). The differences between triathletes and sprinters resulted from differences in the use of high-intensity exercise rather than from differences in the whole training volume.

The results obtained in sprinters and triathletes confirm that plasma Hx and erythrocyte HGPRT may be regarded as sensitive metabolic indicators of training status in quite different sports. Lower plasma Hx levels and higher erythrocyte HGPRT activity in the competition period reflect the metabolic readiness to purine salvage and, consequently, to a more efficient ATP resynthesis. Plasma Hx and erythrocyte HGPRT seem to be adequate indicators that indirectly provide information about the energetic status of the muscle. This is particularly important in speed-power or sprint disciplines, in which no adequate metabolic indicators have been developed so far that would allow monitoring exercise responses and adaptation changes resulting from intense anaerobic stimuli. The use of commonly recognized biochemical and physiological indicators (lactate, maximal oxygen uptake, “anaerobic threshold” measures) is not effective in sprint-trained and speed-power athletes.

**Sport Performance Prediction**

We assessed the usefulness of plasma Hx concentration in predicting the actual sport performance in highly trained athletes (Fig. 4) (28). Resting and postexercise (incremental test until exhaustion) concentrations of plasma Hx, xanthine, uric acid, and lactate were assayed as well as resting erythrocyte HGPRT activity. Season-best race times were analyzed, achieved in actual athletic competition by 28 triathletes, 12 long-distance runners, 13 middle-distance runners, and 18 sprinters over standard triathlon, 5000 m, 1500 m, and 100 m, respectively, within 1 month after our examination.

In Figure 4, we present different prediction models (multiple regression), combining “classical” variables and purine metabolites, to obtain as high coefficient of determination ($r^2$) as possible. It was revealed that Hx alone explained a much greater proportion of variance in sport performance in all athletic groups ($r^2 = 0.81$ in triathletes, 0.81 in long- and middle-distance runners, and 0.78 in sprinters). In other words, the best performed athletes had the lowest postexercise Hx. The models based on respiratory variables (maximum and threshold oxygen uptake) and lactate yielded a relatively weak effect ($r^2 = 0.51, 0.37, 0.59,$ and 0.31, respectively). The models combining respiratory measures (maximum and threshold oxygen uptake) with purine derivatives (xanthine, Hx, HGPRT) yielded the strongest performance prediction in all groups ($r^2 = 0.86, 0.93,$ and 0.93, respectively). Notably, in sprinters, only the variables related to purine metabolism (postexercise Hx and xanthine and resting erythrocyte HGPRT) entered the best prediction model ($r^2 = 0.91$), and none of the cardiorespiratory variables remained significant.

We can conclude that (i) Hx is a strong predictor of sport performance in highly trained athletes regardless of discipline profile, (ii) combining purine and cardiorespiratory variables may strengthen the prediction, and (iii) purine metabolites alone are effective biochemical performance predictors in sprint disciplines.

**PRACTICAL APPLICATION**

Our research suggests that purine derivatives can be used as training status indicators in different training phases of a 1-yr training cycle in highly trained athletes of different specializations and ages. Hx may be regarded as a marker of anaerobic metabolism. It seems that the commonly used “anaerobic threshold” conception and derived training recommendations should be revised in the context of the new knowledge about training-related changes in purine metabolism. There is a possibility to obtain diagnostic data similar to those obtained from muscle biopsy (the use of which is limited because of invasive methods and institutional ethical constraints).

The changes in Hx reflect IMP degradation and, thus, purine resynthesis after exercise. It seems that purine metabolites...
are sensitive indicators of training status in advanced athletes and provide information about adaptations determining a high level of performance. Measurements done under real training conditions could provide coaches with almost instant information. At present, the use of biochemical analyses presented in our studies is not possible under field conditions. However, possible development of specific technologies could result in the invention of and manufacturing of portable measuring devices that would enable an easy measure and feedback. In such a case, exercise modification could be implemented just during training sessions based on metabolic response.

**LIMITATIONS AND STRENGTHS**

In our studies, we drew only single blood samples 5 min after a maximal exercise test. It is known that Hx concentration reaches maximum 10 to 20 min after intensive exercise (3,17). Thus, prolonged sampling would give a more detailed picture of postexercise and training-related changes (23,24). On the other hand, Hx levels rise significantly immediately after exercise as studies conducted so far revealed (15,18,28), which seems to be sufficient for practical purposes.

We took blood samples, not direct muscle samples (biopsy). There are only few studies that used muscle biopsy to determine the changes in Hx after training (5,23,24). Such invasive procedures are not possible in competitive elite athletes. In many countries, muscle biopsy is forbidden or may be only administered for strictly medical purposes. Therefore, some questions regarding muscle status and mechanisms of purine metabolism are difficult to study in competitive athletes. It is not clear whether the intensified conversion of Hx to IMP or the lower Hx production in the muscle is behind the reduced Hx efflux from the muscle after sprint training (5). The first possibility, reduction of purine loss via salvage, is an advantageous adaptation of the muscle subjected to metabolic stress. The second scenario consists of the reduced degradation of adenine nucleotides because of the increased ability to ATP resynthesis in the muscle during anaerobic glycolysis. It is possible that both these phenomena occur simultaneously. In addition, the role of erythrocyte HGPRT in the exercise- and training-induced adaptation is not evident, for example, erythrocytes do not have de novo capacity (5) and ATP cannot be resynthesized from Hx because a mature erythrocyte does not have enzymes transforming IMP into AMP (20). On the other hand, training induces erythrocyte HGPRT activity in line with muscle HGPRT activity, which indicates that a (still unknown) link exists between erythrocyte and muscle purine metabolism.

Our research mainly applies to individual sports and measurable athletic performance. However, team sports with a greater contribution of tactical skills or disciplines scored by judges also can take advantage of our results.

Regardless of ambiguities regarding metabolic pathways and technical aspects of sampling, the changes in Hx and other purine metabolites actually reflect training adaptation and predict specific sport performance (28). Importantly, long training cycles were studied in highly trained athletes and the changes in training loads were analyzed. Moreover, the results of our studies are comparable because they were obtained in the same laboratory using the same exercise and sampling protocols.

**SUMMARY**

In the series of our studies, training loads that changed in consecutive phases of 1-yr training cycles were the leading
stimuli that affected purine metabolism. We demonstrated that planned long-term training in competitive athletes, especially training incorporating high-intensity exercise, brought about the decline in plasma Hx concentration and the increase in erythrocyte HGPRT activity in the competition period. The extent of the changes was determined by the amount of anaerobic exercise. The reduction in high-intensity training produced reverse changes: a plasma Hx increase and a decrease in erythrocyte HGPRT activity.

In Figure 5, we summarize the areas of potential use of plasma Hx in competitive sports. Hx levels mirror the metabolism of the exercising muscle under anaerobic conditions. Training status and training adaptation in consecutive phases of long training cycles may be evaluated based on Hx concentration. Purine metabolism visibly responds to anaerobic high-intensity exercise and may be used as a tool for training control. Furthermore, the level of blood Hx is a reliable biochemical predictor of sport performance. Importantly, the diagnostic value of Hx extends to quite different sport types (sprint and endurance) and both young and older athletes.

Changes in plasma Hx levels and other purine derivatives across a long-term training cycle reflect adaptation to high-intensity exercise. This is a key adaptation for contemporary elite athletes that enables a more economical distribution of energy sources for ATP resynthesis during and after exercise. Plasma Hx and other purine metabolites, reflecting the exercise-induced adenine nucleotide degradation and resynthesis in the muscle, may be regarded as adequate metabolic indicators of training status in highly trained athletes.

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References