Training-induced adaptation in purine metabolism in high-level sprinters vs. triathletes

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Spritz training consists in repeating short intensive intermittent exercise. The exercise of this kind leads to a higher rate of ATP hydrolysis than its resynthesis. The catabolism of ATP causes the increase in free adenosine diposphate (AMP) which stimulates the myokinase reaction and the adenosine monophosphate deaminase (AMPD) reaction. Consequently, the adenine nucleotide pool (AdN = ATP + ADP + AMP) decreases (37). After the phosphat moiety is moved from one ADP molecule to another, one ATP molecule and one AMP molecule are formed (myokinase reaction) and the AMP is subsequently deaminated to IMP via AMP deaminase in skeletal muscle (41).

The IMP accumulated in skeletal muscle may be reaminated to AMP in the purine nucleotide cycle, restoring the AdN pool, or may be dephosphorylated to inosine by 5’-nucleotidase (26).

However, the rate of reamination is slow during intense exercise (28) and hence inosine degrades to hypoxanthine (Hx) with the participation of purine nucleotide phosphorylase (33). Then, both inosine and Hx may flow out of the muscle, decreasing the AdN pool, which has to be restored after exercise. Hellsten at al. (15) demonstrated that inosine and Hx efflux from human skeletal muscle after intensive exercise (until exhaustion) might reduce the ATP pool by as much as 9%.

The intramuscular IMP resynthesis from Hx, being the pathway of purine salvage, is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) that binds Hx with phosphoribosylpyrophosphate (PRPP) and transforms it into IMP (29). In this way, the energetic cost of ATP restorations is lower due to the use of purine bases for nucleotide synthesis (27) and the expensive de novo metabolic pathway can be avoided. Hx may be then converted to xanthine (X) and uric acid (UA) by X dehydrogenase in an irreversible reaction.

The purine metabolites that accumulate in the plasma, i.e., inosine, Hx, and UA, may be removed via kidney (13, 34, 36, 39). Additionally, UA is excreted via gut (34). Thus the net efflux of purine bases represents the loss of molecules being AdN precursors in the muscle. The complete restoration of the resting ATP level relies on a relatively slow and costly de novo synthesis (40).

Hx is the only compound that may be reconverted and reutilized in the nucleotide pool after being catalyzed by HGPRT. Plasma Hx concentration increases significantly (15- to 40-fold) after a maximal intensity intermittent sprint exercise (2). For that reason, it is regarded as a marker of muscle AdN degradation, a marker of energetic stress during exercise (32), and an index of exercise intensity (30). It may be used in the classification of physical exercise (5). Hx is also considered to be an indicator of chronic hypoxia (39).

The research on the effect of high intensity training on purine metabolism was initiated by Hellsten-Westling et. al. (18) who showed that repeated sprint training limited the efflux of purines to the plasma after an intensive exercise and thereby reduced the muscle nucleotide loss in habitually active men. It was found that after a 6-wk high-intensity intermittent training, the level of Hx both at rest and after exercise decreased considerably (and HGPRT activity increased). This reflects the muscle adaptation that leads to reduced loss of adenine nucleotides (18, 21). Subsequent studies concentrated on competitive athletes. Spencer at al. (35) demonstrated the effects of a 7-wk field-hockey-specific training on repeated sprint ability and plasma Hx concentration in 18 elite female field-hockey players. A significant decrease in plasma Hx concentration was revealed. The authors suggested that one adaptation of sport-specific repeated-sprint training may be to conserve the purine nucleotide pool. The study by Stathis et al. (37) provided...
evidence that inosine concentration, the precursor to Hx, is lower in the muscle following training as is the postexercise plasma Hx concentration after a 7-wk sprint training. This, in turn, suggest that Hx production and/or efflux from the muscle are reduced. Indeed, it was then demonstrated that sprint training reduces the estimated net muscle purine loss measured as purine increase in urine and plasma after an intensive sprint exercise (36).

The studies mentioned above encompassed only a small part of a training cycle. In reality, competitive professional athletes use much longer training periods divided in subphases aimed at specific goals. So far, only our recent studies (46, 47) have presented the changes in purine metabolism in a 1-yr training cycle in middle- and long-distance runners, taking into consideration the quantity and quality of training loads. We revealed that resting and postexercise plasma concentrations of X, Hx, and erythrocyte HGPRT activity change significantly in competitive runners in consecutive training phases (general, specific, competition, and transition). The increase in anaerobic training load during the specific preparation phase results in a significant decrease in postexercise plasma Hx concentration and in a significant increase in both pre- and postexercise erythrocyte HGPRT activity. The effect is noticeable despite a very short total duration of anaerobic training loads. Elevated preexercise erythrocyte HGPRT activity in the competition phase suggests adaptation occurring that supports increased purine salvage. The detraining reverses the adaptation changes in the transition phase. Obviously, HGPRT plays a different role in the erythrocytes (they do not have de novo capacity) compared with skeletal muscle. Admittedly, it is not clear how erythrocyte purine salvage affects or contributes to muscle metabolism but it seems that the analysis of HGPRT activity may be useful because the changes in erythrocyte and muscle are parallel and unidirectional (18, 47).

Until now, our research concentrated on typical endurance-trained athletes. The results mentioned above suggest, however, that in sprint-trained athletes even a greater variation in Hx and HGPRT should occur along consecutive training phases. The lower plasma purine concentration and higher erythrocyte HGPRT activity in the competition period indicates that anaerobic loads evoke a metabolic adaptation that allows exercising at high intensity without an excessive purine efflux from muscle. In speed-power athletes, anaerobic exercise is used in a much larger proportion than in endurance-trained individuals; therefore, this adaptation is of greater importance in sprint training because it directly affects the performance through reduced AdN loss and diminishes impairment of muscle cell to damage. As a marker of energetic stress, Hx may be a more sensitive physiological indicator of training status in sprint-trained athletes, especially in elite competitors, in which commonly recognized diagnostic parameters (e.g., maximal oxygen uptake or anaerobic threshold measures) do not change significantly along with specific exercise loads in different training periods or are of little importance. Thus the aim of this study is to assess the effect of training loads on plasma purine concentration and erythrocyte HGPRT activity in highly trained sprinters in a 1-yr cycle compared with the endurance-training mode in triathletes.

METHODS

Subjects. Ten male sprinters (specialized in distances: 100 m, 200 m, and 4 × 100 m relay) competing at national and Olympic level, aged 20–29 yr, practicing competitive sport for 9.4 ± 3.3 yr, weight of 77.9 ± 7.9 kg, height of 182.8 ± 8.2 cm, maximal oxygen consumption (\(\dot{V}_\text{O}_{2}\max\)) of 49.3 ± 3.8 ml·kg\(^{-1}\)·min\(^{-1}\), maximum heart rate (HR\(_{\text{max}}\)) of 184.2 ± 5.1 beat·min\(^{-1}\), and average levels of Hb of 14.8 ± 0.9 g/dl at rest and of 15.1 ± 1.0 g/dl postexercise at the beginning of the study, participated in the research. A comparative group consisted of 10 triathletes at regional level, aged 21–28 yr, practicing competitive sport for 9.6 ± 2.1 yr, weight of 77.7 ± 3.2 kg, height of 181.4 ± 3.2 cm, \(\dot{V}_\text{O}_{2}\max\) of 62.1 ± 5.7 ml·kg\(^{-1}\)·min\(^{-1}\), HR\(_{\text{max}}\) of 193.1 ± 4.2 beat·min\(^{-1}\), and Hb levels of 16.3 ± 0.6 g/dl at rest and of 17.1 ± 0.9 g/dl postexercise at the beginning of the study. The aim of the research and testing methodology was explained to all subjects who gave their informed consent before their inclusion in the study. The project has been approved by the 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.

Study design. The study procedure was adapted to the annual plan of examined athletes. A four-time measurement of several physiological and biochemical variables was administered in four characteristic training phases of an annual cycle. The 1-yr training cycle consisted of three main phases: preparatory, competitive, and transition phase (6). Four consecutive examinations were carried out at following time points of the 1-yr cycle: first examination, 17–18th of December, the end of the general subphase of the preparatory phase; second examination, 5–7th of March, the specific subphase of the preparatory phase; third examination, 28–29th of May, the beginning of the competition phase; and fourth examination, 21–22nd of October, transition phase. The same parameters were measured in both sprinters and triathletes at the same time points. Biochemical variables were measured at rest as well as after exercise. The study was performed within a standard training regime. It was not possible to ensure a full recovery (inactivity) before the exercise test, but each test was preceded by 2–3 days of light- to moderate-intensity training sessions. Performance variables were monitored only during exercise. 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 a light breakfast was consumed (bread and butter, water, without coffee or tea).

Respiratory parameters. To determine the maximal oxygen uptake (\(\dot{V}_\text{O}_{2\max}\)), an incremental running treadmill test (Woodway ES1, Waukesha, WI) was administered. The initial speed was set at 10 km/h and was then progressively increased by 2 km/h every 3 min until volitional exhaustion. Respiratory parameters (ventilation, oxygen uptake, and carbon dioxide production) were followed continuously by means of CPX-D computer system (Medical Graphics, St. Louis, MO). Heart rate was recorded every 5 s with Polar Accurex Plus device (Polar Elektro, Finland).

Lactic acid. Capillary blood samples were obtained from fingertip at rest and 3 min after exercise. Maximal lactic acid concentration (L\(_{\text{Amax}}\)) was assayed enzymatically by means of a spectrophotometric Warburg method (11) based on the increase in absorbance of NADH at wavelength 365 nm (Marcel media spectrophotometer; Zielonka, Poland).

Hx, X, UA, HGPRT. Venous blood samples (5 ml) were taken from an antecubital vein at rest, immediately before, and 5 min after exercise, to obtain plasma and red blood cells. The plasma obtained was deproteinized with 1.2 mol/l HClO\(_4\) and centrifuged. An acid supernatant was neutralized with 1 mol/l K\(_2\)CO\(_3\), centrifuged, and stored at −80°C before analysis. Blood cells were bathed three times in an isotonic salt solution (0.9% NaCl) and then hemolyzed with a hypotonic solution of Tris buffer (hydroxyethylammoniummethane; 10

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mmol/l, pH 7.4). The prepared hemolysate was stored at −20°C before analysis for HGPRT activity.

Hx, X, and UA were assayed in a neutralized plasma extract by means of HPLC method according to the methodology of Wung and Howell (44) modified by Banaszak (3). Hewlett-Packard 1050 apparatus with an UV detector (Ramsey, NY) 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, IL). The carrier phase was a buffer consisting of 1% methanol and 4% 100 mmol/l KH2PO4 (potassium phosphate), pH 5.8. The flow rate was 1.0 ml/min. 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 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.

The red blood cell HGPRT activity was determined with a method elaborated by Stolk et al. (38), modified by Banaszak (3). The incubation buffer consisted of 50 mmol/l Tris pH 7.4, 10 mmol/l PRPP, 7 mmol/l NaF, and 5 mmol/l MgCl2. The preincubation of hemolyzate (100 µl) and buffer lasted for 5 min in a 37°C water bath. The incubation started after the addition of 100 µl of 2.0 mmol/l Hx solution into a preincubation mixture to initiate the enzymatic reaction. Immediately after Hx was added, 100 µl of incubation mixture were taken and added to 100 µl 1.2 mol/l 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 HClO4. Acid extracts were neutralized with 1 mol/l K2CO3 and then used for HGPRT activity measurement by means of the HPLC method. The buffer used as carrier phase consisted of 100 mmol/l KH2PO4 with the addition of tetrabutylammonium sulphate with a final concentration of 5 mmol/l and pH 3.1. Separation was conducted at flow rate of 1.2 ml/min. The mobile phase consisted of 15% methanol and 50% buffer pH 3.1. An ODS Hypersil 150 mm × 4.6 mm column and ODS Hypersil 20 mm × 4 mm × 5 µm precolumn manufactured by Alltech (Mannheim, Germany) were used within the total exercise time in actual proportions specific for examined sprint- and endurance-trained athletes. The main differences were observed in proportion of the anaerobic nonlactacid exercise zone that was used by sprinters to a much larger extent (2.2–4.2% of total load) than by triathletes. In triathletes, the use of the anaerobic nonlactacid zone was virtually not observed and the anaerobic lactacid zone contributed to <2% of total load (vs. 3.9–5.9% in sprinters). Moreover, aerobic stimulation and mixed aerobic-anaerobic zones contributed up to 36% of total exercise time in triathletes vs. maximum 9% in sprinters, except for the general preparation phase (15 and 35%, respectively). In both groups of athletes, the aerobic compensation zone constituted the basis that made up 56.6–91.5% total net time in sprinters and 62.1–85.5% in triathletes, depending on training phase.

Statistical analyses. Calculations were performed using STATISTICA 9.0 software (StatSoft, Tulsa, OK). Descriptive data were expressed as means ± SD. Data collected during initial and follow-up testing were used to determine longitudinal changes in somatic, cardiorespiratory, and biochemical variables. A multiple ANOVA/MANOVA for repeated measures was used to follow the time course of measured variables. The statistical power of ANOVA/MANOVA at α = 0.05 ranged between 0.80 and 1.00 in the majority of variables, except for X and some exercise parameters where the statistical power of 0.58–1.00 was shown. Only large effect size was obtained for all variables ranging between 0.22 and 0.97. A Scheffé’s post hoc test was performed to assess the significance of differences between consecutive examinations and between groups at the same examination session. The t-test procedure was employed to compare the differences between pre- and postexercise values in the same training subphase. Significance level was set at P < 0.05.

RESULTS

Descriptive data. Sprinters and triathletes did not differ significantly with regard to age, height, weight, and body mass index (BMI) in any examination (Table 2). In triathletes, a visible increase in both body weight and BMI by 3.5% was observed between competition and transition period (P = 0.003). The level of Hb was significantly higher in triathletes than in sprinters in all training phases, and the difference reached from 9.1% in the general preparation (P = 0.000) to 10.9% in the transition period (P = 0.000). No significant Hb changes between four examination were found in either group.

Table 1. Typical structure of training loads in tested sprinters and triathletes between successive examinations in a 1-yr cycle

<table>
<thead>
<tr>
<th>Preparation</th>
<th>First Examination</th>
<th>Second Examination</th>
<th>Third Examination</th>
<th>Fourth Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprinters</td>
<td>Triathletes</td>
<td>Sprinters</td>
<td>Triathletes</td>
<td>Sprinters</td>
</tr>
<tr>
<td>Training sessions, no.</td>
<td>71</td>
<td>104</td>
<td>57</td>
<td>106</td>
</tr>
<tr>
<td>Competitions, no.</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Net exercise time, h</td>
<td>57.9</td>
<td>137.6</td>
<td>45.7</td>
<td>164.3</td>
</tr>
<tr>
<td>Per one training session</td>
<td>0.8</td>
<td>1.3</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Exercise zones, %/total time</td>
<td>56.6</td>
<td>85.5</td>
<td>87.0</td>
<td>70.4</td>
</tr>
<tr>
<td>Aerobic compensation</td>
<td>13.5</td>
<td>11.0</td>
<td>0</td>
<td>17.3</td>
</tr>
<tr>
<td>Aerobic stimulation</td>
<td>21.1</td>
<td>3.5</td>
<td>4.1</td>
<td>11.4</td>
</tr>
<tr>
<td>Aerobic-anaerobic</td>
<td>4.6</td>
<td>0</td>
<td>6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Anaerobic lactacid</td>
<td>4.2</td>
<td>0</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Anaerobic nonlactacid</td>
<td>4.2</td>
<td>0</td>
<td>3.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Data encompass the period between the beginning of the training cycle and the first examination. †Data encompass the period between the preceding and the present examination.
Cardiorespiratory characteristics. Considerable between-group differences were shown for maximal exercise and ventilatory threshold data (Table 2). Sprinters presented a lower level of all exercise variables measured during the treadmill test, especially in the specific preparation and the competition period. Percentage differences, depending on the training phase, ranged as follows: 15.6–18.5% for \( V\dot{O}_{2\text{max}} \) (\( P < 0.000 \)), 21.4–41.4% for \( HR_{\text{max}} \) (2nd and 4th examination, \( P = 0.035 \) and \( P = 0.032 \), respectively), 17.4–18.6% for \( \text{LA}_{\text{max}} \) (\( P = 0.000 – 0.058 \)), 33.1–40.4% for maximal distance (\( P = 0.000 \)), 9.9–20.5% for \( V\dot{O}_{2\text{T}} \) (\( P = 0.000 – 0.024 \)), 0.6–3.9% for \( HR_{\text{T}} \) (2nd and 3rd examination, \( P = 0.017 \) and \( P = 0.032 \), respectively), and 9.4–27.1% for \( t_{\text{VT}} \) (2nd, 3rd, and 4th examination, \( P = 0.000 \), \( P = 0.003 \), and \( P = 0.029 \), respectively). The within-group changes in exercise parameters were also substantial. As expected, both sprinters and triathletes reached their maximum cardiorespiratory capacity in the competition phase and their minimum level in the transition phase. In sprinters, \( HR_{\text{max}} \) and \( \text{LA}_{\text{max}} \) values were relatively invariable in consecutive examinations.

Hx. The concentration of plasma Hx changed significantly in the 1-yr training cycle in both sprinters and triathletes in resting conditions as well as after maximal exercise as shown in Fig. 1A. The level of Hx was considerably lower in sprinters in each examination. In sprinters, postexercise Hx concentration decreased between the general and the specific training period from 14.8 to 10.0 \( \mu \text{mol/l} \) (by 32.4%; \( P = 0.000 \)) and then between the specific and the competition phase to 8.1 \( \mu \text{mol/l} \) (by 19.0%, \( P = 0.000 \)). In total, a decline of 45.3% was noticed in relation to initial value in the competition phase. In the transition period, postexercise Hx concentration increased rapidly in sprinters and achieved the highest level of 18.0 \( \mu \text{mol/l} \), 122.2% higher compared with the previous examination (\( P = 0.000 \)). The resting values changed significantly in the first part of the training cycle, decreasing by 9.7% (from 3.1 to 2.8 \( \mu \text{mol/l} \)) between the general and the specific phase (\( P = 0.000 \)), and then increasing by 23.1% (from 2.6 to 3.2 \( \mu \text{mol/l} \); \( P = 0.000 \)) between the competition and the transition phase.

Triathletes presented a similar pattern of changes; however, a much higher Hx concentration and smaller magnitude of change were found in these athletes. They started in the general preparation with an average postexercise Hx value of 21.1 \( \mu \text{mol/l} \), which decreased to 18.8 \( \mu \text{mol/l} \) (by 10.9%, \( P = 0.025 \)) in the specific preparation and finally reduced to 14.1 \( \mu \text{mol/l} \) in the competition phase, 25.0% lower than in the previous examination and 33.2% lower than at the first examination (\( P = 0.000 \)). In the transition period, Hx concentration ex-

### Table 2. *Somatic and exercise characteristics in four consecutive examinations in sprinters and triathletes*

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>( V\dot{O}_{2\text{max}} ), ml·kg⁻¹·min⁻¹</th>
<th>( \text{HR}_{\text{max}} ), beats/min</th>
<th>( \text{LA}_{\text{max}} ), mmol/l</th>
<th>( \text{Distance}_{\text{max}} ), m</th>
<th>( V\dot{O}_{2\text{maxVT}} ), ml·kg⁻¹·min⁻¹</th>
<th>( \text{HR}_{\text{VT}} ), beats/min</th>
<th>( t_{\text{VT}} ), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>T</td>
<td>S</td>
<td>T</td>
<td>S</td>
<td>T</td>
<td>S</td>
<td>T</td>
<td>S</td>
<td>T</td>
<td>S</td>
</tr>
<tr>
<td>22.9 ± 3.2</td>
<td>22.8 ± 2.1</td>
<td>—</td>
<td>—</td>
<td>182.8 ± 8.2</td>
<td>181.4 ± 3.3</td>
<td>77.6 ± 8.7</td>
<td>77.8 ± 3.7</td>
<td>52.3 ± 4.5</td>
<td>64.2 ± 6.5</td>
<td>188 ± 6</td>
</tr>
<tr>
<td>182.8 ± 8.2</td>
<td>181.4 ± 3.3</td>
<td>—</td>
<td>—</td>
<td>182.8 ± 8.2</td>
<td>181.4 ± 3.3</td>
<td>77.6 ± 8.7</td>
<td>77.8 ± 3.7</td>
<td>52.3 ± 4.5</td>
<td>64.2 ± 6.5</td>
<td>188 ± 6</td>
</tr>
<tr>
<td>77.6 ± 8.7</td>
<td>77.8 ± 3.7</td>
<td>77.9 ± 8.3</td>
<td>75.2 ± 3.8</td>
<td>77.9 ± 8.3</td>
<td>74.8 ± 3.0</td>
<td>77.8 ± 7.7</td>
<td>77.4 ± 4.8</td>
<td>14.9 ± 0.5</td>
<td>16.4 ± 0.6</td>
<td>149 ± 5.0</td>
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<tr>
<td>23.2 ± 1.8</td>
<td>23.4 ± 1.4</td>
<td>23.3 ± 1.7</td>
<td>22.9 ± 1.1</td>
<td>23.3 ± 1.7</td>
<td>22.7 ± 0.9</td>
<td>14.9 ± 1.2</td>
<td>16.4 ± 1.0</td>
<td>55.7 ± 4.4</td>
<td>68.2 ± 6.6</td>
<td>189 ± 5.0</td>
</tr>
<tr>
<td>105 ± 10.9</td>
<td>106 ± 1.0</td>
<td>106 ± 1.0</td>
<td>107 ± 1.0</td>
<td>106 ± 1.0</td>
<td>107 ± 1.0</td>
<td>108 ± 1.0</td>
<td>108 ± 1.0</td>
<td>187 ± 4.4</td>
<td>191 ± 8.1</td>
<td>195 ± 5.5</td>
</tr>
<tr>
<td>5 ± 0.5</td>
<td>9.1 ± 1.4</td>
<td>8.0 ± 1.7</td>
<td>9.8 ± 1.4</td>
<td>8.3 ± 0.5</td>
<td>10.2 ± 0.9</td>
<td>7.6 ± 0.9</td>
<td>9.2 ± 1.8</td>
<td>461 ± 5.0</td>
<td>537 ± 5.1</td>
<td>466 ± 7.5</td>
</tr>
<tr>
<td>5 ± 0.5</td>
<td>9.1 ± 1.4</td>
<td>8.0 ± 1.7</td>
<td>9.8 ± 1.4</td>
<td>8.3 ± 0.5</td>
<td>10.2 ± 0.9</td>
<td>7.6 ± 0.9</td>
<td>9.2 ± 1.8</td>
<td>461 ± 5.0</td>
<td>537 ± 5.1</td>
<td>466 ± 7.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. S, sprinters; T, triathletes; BMI, body mass index; \( \text{HR}_{\text{max}} \), resting hemoglobin concentration; \( V\dot{O}_{2\text{max}} \), maximal oxygen uptake; \( \text{HR}_{\text{max}} \), heart rate at the end of the test; \( \text{Distance}_{\text{max}} \), distance covered in the test; \( V\dot{O}_{2\text{maxVT}} \), oxygen uptake at ventilatory threshold; \( \text{HR}_{\text{VT}} \), heart rate at ventilatory threshold; \( t_{\text{VT}} \), time to ventilatory threshold. *Multiple ANOVA. †Significant differences between pre- and postexercise values at the same time point.
ceed the initial value, reaching 24.9 μmol/l (increase by 76.6% since competition period, \( P = 0.000 \)). Resting values were relatively stable in triathletes during the main part of the training cycle. A significant increase was noticed only between the competition and the transition phase from 3.5 to 4.3 μmol/l (by 22.9%, \( P = 0.037 \)).

In both group of athletes, the postexercise plasma Hx levels were significantly higher than the resting concentrations. The smallest resting-postexercise differences were revealed in the competition period in both sprinters (increase by 211.5%; \( P = 0.000 \)) and triathletes (302.9%; \( P = 0.000 \)) and the largest differences in the transition period (462.5% and 479.1%, respectively; \( P = 0.000 \)).

**HGPRT.** The activity of the erythrocyte HGPRT at rest changed significantly along with consecutive training phases in both sprinters and triathletes (Fig. 1B). A higher activity was observed in sprinters than in triathletes in each examination with the largest relative difference of 8.4% (\( P = 0.000 \)) in the specific preparation phase and the smallest difference in the transition phase (4.6%, \( P = 0.007 \)). The pattern of changes was the same in both groups of athletes: HGPRT activity increased from the general preparation to the competition period and then showed a relatively rapid decrease in the transition phase. The rate of increase between the general and the specific preparation was faster in sprinters (from 62.0 to 69.0 nmol IMP·mg Hb·h\(^{-1} \)) (11.3%; \( P = 0.000 \)) than in triathletes (from 58.4 to 63.2 nmol IMP·mg Hb·h\(^{-1} \)) (8.2%; \( P = 0.000 \)). Until the next period (competition), the rate of increase was slower in both groups. The HGPRT increased by 4.1% (from 69.0 to 71.8 nmol IMP·mg Hb·h\(^{-1} \)) (\( P = 0.025 \)) in sprinters and by 5.4% (from 63.2 to 66.6 nmol IMP·mg Hb·h\(^{-1} \)) (\( P = 0.000 \)) in triathletes. Until the transition period, a pronounced decrease in HGPRT activity was found in sprinters (58.5 nmol IMP·mg Hb·h\(^{-1} \)) (18.5%; \( P = 0.000 \)) as well as in triathletes (55.8 nmol IMP·mg Hb·h\(^{-1} \)) (16.2%; \( P = 0.000 \)).

**Xanthine.** The changes in plasma X concentration were not significant during the whole training cycle in both sprinters and triathletes, neither at rest nor postexercise (Fig. 2A). The level of postexercise X was, in general, higher in triathletes than in sprinters; however, significant differences were revealed only in the general preparation and in the transition period (in both cases: 2.8 vs. 2.5 μmol/l; \( P = 0.019 \) and \( P = 0.025 \), respectively). The differences between X concentration at rest and postexercise were significant in each examination and ranged from 1.0 to 1.1 μmol/l (66.7–78.6%; \( P = 0.000 \)) in sprinters and from 1.2 to 1.3 μmol/l (75.0–86.7%; \( P = 0.000 \)) in triathletes.

**UA.** The training-related changes in UA concentration were not significant in consecutive examinations for both groups (Fig. 2B). There were observed differences between sprinters and triathletes. The latter group presented a higher level of UA in the whole training cycle. The differences in resting UA concentration were less pronounced and were significant only in the transition period (268.0 vs. 320.4 μmol/l, respectively; \( P = 0.002 \)). The divergence in postexercise values was much more clear. In triathletes, the level of UA was higher by 64.3 μmol/l (408.3 vs. 344.0 μmol/l, 15.8%; \( P = 0.011 \)) in the general preparation period and by 81.6 μmol/l (430.2 vs. 348.5 μmol/l, 19.0%; \( P = 0.000 \)) in the transition phase. In both groups of athletes, postexercise UA concentrations were always significantly higher than resting values and the range of differences was 30.5–80.6 μmol/l (10.2–30.2%; \( P = 0.000 \)) in sprinters and 70.0–115.5 μmol/l (22.2–39.4%; \( P = 0.000 \)) in triathletes.

**DISCUSSION**

In the present study, we demonstrated a considerably lower resting and postexercise plasma concentration of Hx in highly trained sprinters than in triathletes in four characteristic training phases of the 1-yr cycle. In both groups, a significant decrease in plasma Hx concentration in the competition phase and a considerable increase in the transition phase were observed suggesting reduced Hx release from muscle into plasma. It was also found that the resting erythrocyte HGPRT activity increases in the competition period, when athletes incorporate more high-intensity exercise in their training, and declines in the transition (detraining) phase. Sprinters showed higher HGPRT activity in all examinations.

Our study provides evidence that high-intensity exercise during long training periods induces an increase in erythrocyte HGPRT activity and, concurrently, a decrease in both resting and postexercise plasma Hx concentration. We assume that these changes mirror muscle adaptations leading to the reduction of AdN loss with intense exercise. A lower purine concentration in the competition period suggests that the specific training enabled the subjects to do high-intensity exercise at a reduced purine efflux from the muscle. Such an adaptation is of...
great practical value for athletes who often perform high-intensity exercise. Our findings may indicate a more economical use of energy resources as a result of sprint training. In support of this, Stathis et al. (37) demonstrated an increase in performance with a lower posttraining resting ATP and total AdN contents and the reduction in the magnitude of ATP depletion during a 30-s sprint, which indicates a better restorative capacity of the muscle.

Our study also showed that the “classical” cardiorespiratory and biochemical exercise parameters (oxygen uptake, heart rate, and lactic acid concentration) may be inadequate for training control in highly trained athletes. First of all, in sprinters, these variables do not describe the essence of training that is aimed at an effective performance in explosive exercise (sprint run); however, they are of some diagnostic value in the general preparation phase. In highly trained triathletes, the threshold and maximal cardiorespiratory parameters did not change significantly between main training phases. Admittedly, a significant change in \( V_{O2\max} \), distance covered in the test, and anaerobic threshold parameters was revealed in the transition phase in both groups of athletes; however, it is of little importance for training control because of the termination of the training cycle and beginning of the detraining phase. Hence, the use of exercise indicators based on anaerobic metabolism seems to be essential in highly trained athletes regardless of their specialization. We suggest that plasma Hx concentration and HGPRT activity could be adequate tools for more precise training control.

Hx. The trends in the change of Hx concentration in our 1-yr training study are similar to those observed in habitually active males (18, 21, 37) and in female field hockey players (35) after a short (7-wk) sprint training cycle. Analogous changes were also observed after a short-duration endurance training in men (1) and during a year-long cycle in long-distance (47) and middle-distance (46) runners. In general, the Hx concentration decreases during periods of high-intensity training and increases markedly in training phases with less stressed anaerobic content. As revealed in our study, the resting and postexercise plasma Hx concentrations are lower in sprint-trained than in endurance-trained athletes due to a significantly lower proportion of anaerobic training loads in each training phase in the latter ones. In addition, in sprinters, a significant decrease in Hx concentration occurred as early as after the first training period (general preparation) whereas the analogous metabolic response was less pronounced in triathletes and nonexistent in long- and middle-distance runners (46, 47).

There are suggested mechanisms described in the literature that could explain the above phenomenon. It is known that the muscle IMP level is higher immediately after exercise cessation compared with preexercise conditions and the muscle inosine content increases after 3–10 min of rest (15, 37, 45). The release of inosine and Hx from the muscle is very slight (virtually negligible) at rest and it intensifies after a high-intensity short-duration exercise (12, 15, 37, 47). This indicates that the intramuscular purine salvage and Hx efflux from the muscle (10) counterbalance the rate of Hx production (15). In the study of Stathis et al. (37), the skeletal muscle content of inosine decreased in immediate 3 min recovery period after a 30 s sprint posttraining. It was also shown that the plasma concentrations of endogenous purines (inosine, Hx, and UA) as well as the excretion of the urine pool with urine were reduced during recovery after a 30-s maximal sprint exercise in trained individuals (36). These data suggest that the sprint training brings about a considerable reduction of muscle purine loss after intense exercise. Presumably, such an adaptation lessens the necessity to replace purine nucleotide with the energy-consuming de novo synthesis.

In our study, we demonstrated that the training mode of sprinters reduces the resting and postexercise plasma purine concentration to a greater extent than it is observed in endurance-trained athletes. The training phases characterized by a high amount of repeated sprint exercise led to a decreased plasma Hx concentration. A tendency was also observed, however, much weaker pronounced, towards the decrease in plasma UA concentration after the test until exhaustion. In the general preparation phase, athletes were exposed first and foremost to endurance aerobic exercise whereas it is anaerobic training load that is most effective in decreasing postexercise plasma Hx concentration as demonstrated by Zieliński et al. (46). We did not notice any significant decline in plasma purine concentration after exercise in middle- and long-distance runners during the general preparation period. A significant decrease was visible only in the competition phase after the training volume was reduced and its intensity increased (46, 47). However, in triathletes a significant Hx decrease was observed also in the specific preparation period. The reason may be another structure of training loads, especially much higher volume of training (number of sessions and net total time) in aerobic-anerobic energetic zone that could affect earlier postexercise changes in Hx.

Therefore, we presume that the repeated anaerobic exercise based on short bouts of sprint running, predominant in sprinters, accounts for the decrease in postexercise purine concentration to a great extent. The changes in plasma purine concentration are supposed to be affected by the efflux of Hx from the muscle after exercise. The lowered plasma purine accumulation could result from the increase in phosphofructokinase and HGPRT activity and from the decrease in AMPd activity in the muscle as well as from the lack of purine nucleotide phosphorylase changes, as demonstrated in the study of Hellsten-Westling et al. (18) based on a 6-wk sprint training. Since phosphofructokinase is an important regulator of glycolysis, its raised posttraining activity may be the cause for greater contribution of glycolytic energy sources and for lesser demand for energy gained from AdN degradation. Furthermore, the reduced activity of AMPd could slow down the AMP deamination to IMP. Since HGPRT catalyzes the rephosphorylation of Hx to IMP, its intensified activity may be responsible for intramuscular Hx metabolism via this pathway and, consequently, may reduce the efflux of Hx from the muscle. Thus the training based on high-intensity intermittent exercise results in the increase of HGPRT activity and the decrease in AMPd activity in the skeletal muscle. After a period of sprint training, the postexercise concentration of plasma purines declines. These changes reflect the muscle adaptation limiting the loss of AdN during high-intensity exercise (18).
It is to stress that lower plasma Hx concentration is related to reduced resting muscle ATP and total AdN content after sprint training (18, 37). Stathis et al. (37) demonstrated that a reduction in purine efflux following sprint training is due to the reduced production of IMP, the result of a reduced ATP degradation in the muscle with exercise. The reduced muscle ATP may also be due to a lack of adequate recovery time for de novo synthesis in the previously active muscle, which has been shown to be recovered after 72 h following the last training bout (18). In our study, the level of ATP or AdN is not known. The exercise test was performed in periods of a standard training regime but preceded by less intensive training days; thus a considerable depletion of ATP pool is not supposed to occur for this reason.

The effect of training on plasma Hx accumulation is also connected with the recruitment of muscle fibers. In rats, the increase in Hx production during a maximal-intensity exercise is evoked by the prevailing recruitment of type IIX fibers (fast-twitch, glycolytic, and typical for sprinters) characterized by high activity of AMPd whereas during a prolonged exercise, Hx is produced mainly by type IIA fibers (fast-twitch, oxidative-glycolytic; Refs. 9, 28). In humans, extreme differences in ATP degradation and IMP formation in type I and II muscle fibers with intense exercise were demonstrated (22). The type IIX fibers, apart from a very active glycolytic pathway, draw a substantial portion of energy from mitochondrial (oxidative) sources. This affects the ultimate production of Hx that effluxes the muscle into the plasma and influences the potential drive for subsequent resynthesis. Different training modes bring about recruitment of different muscle fiber types and different adaptation changes to secure adequate energy sources. The altered recruitment patterns in the more stressful periods of training may be the cause of differences in the metabolic environments of the muscle and blood system between sprint- and endurance-trained athletes.

However, it is not clear whether the decreased Hx release from muscle can be explained by the intensified conversion of Hx to IMP or by the decrease in Hx formation, as Hellsten-Westing et al. (18) noticed. The first possibility, reducing the nucleotide loss, could be considered as an advantageous adaptation of muscles being subject to metabolic stress. The reduction of Hx release may be also caused by the decrease in AdN degradation, which, in turn, could be achieved by the increase of muscle ability to ATP resynthesis in the process of anaerobic glycolysis. In this way, the balance between ATP consumption and resynthesis at high-intensity exercise would be redressed. Considerable decrease in the activity of AMPd could also inhibit the rate of AMP deamination and the rate of IMP degradation to purine at the same time. In our study, the reduced Hx release from the muscle could be explained by intensified Hx reamination to IMP or by reduced Hx production. The higher muscle HGPRT activity after training suggests an increased rephosphorylation of intracellular Hx (18).

An interesting evidence for training adaptation is the significant decrease of resting plasma Hx concentration in the periods of specific preparation and competition in our sprinters. An inverse phenomenon, i.e., the increase of Hx concentration, was observed during the periods of detraining (transition phase) in this and our previous studies (46, 47) on middle- and long-distance runners. Similarly, the reduced pre- and post-training resting level of plasma Hx concentrations after sprint training was reported earlier by Stathis et al. (37) and interpreted as the result of diminished purine efflux from the muscle to the blood and/or better ability to remove plasma Hx after training. Hellsten-Westing et al. (21) demonstrated an increased maximal HGPRT activity in the skeletal muscle after training that may support the decreased efflux of Hx into the blood. They also found that posttraining plasma concentrations of Hx and UA were reduced during the recovery after a set of 2-min exercises at various intensities. However, when resting parameters are analyzed, it is necessary to take into account some limitations that we addressed in our earlier study (47). They are related to the length and contents of a training period. Purine metabolites in the plasma at rest are in a constant state of flux because of purines are produced, entering the blood from the muscle and being excreted. In the research of Stathis et al. (36), pre- and posttraining Hx concentrations did not differ significantly. Probably, the adaptation processes could not develop due to a very short-term training cycle (1 wk). In contrast, the study of Spencer et al. (35) revealed a considerable decrease of resting Hx concentration after training lasting for 7 wk. This suggests that the longer the specific training period the more pronounced is the adaptation change. Changes in resting concentrations occur only after a longer period of training. This view is supported by this study in which we consider a long-term training cycle in competitive athletes with examinations made at intervals of 3–5 mo.

Our data suggest that plasma Hx concentration may be a measure of training status. This is reinforced by the lack of changes in parameters such as LA concentrations, \( V_{\text{O}2\text{max}} \), and \( V_{\text{O}2\text{VT}} \) that are of limited diagnostic value in highly trained athletes. Hx may be used as a marker for tracking anaerobic metabolic changes. We suggest that the currently used concepts of anaerobic threshold and training recommendations derived from them should be analyzed in the context of purine metabolism. It seems that there exists the possibility to obtain diagnostic data similar or equal to muscle biopsy, a method of limited application due to invasive procedure and ethical problems (allowed only for medical purposes or forbidden in some countries). The change in Hx level is a good explanation for IMP degradation and thus for purine resynthesis degraded during exercise. We suggest the use of plasma Hx concentration as a marker of AdN degradation in the muscle and an indicator of exercise- and training-induced energetic stress. The application of plasma Hx concentration to detect the state of overtraining also seems to be worth consideration.

HGPRT. HGPRT is a key enzyme of purine resynthesis in human skeletal muscle both through salvage and de novo pathway. Its muscle activity ranges from 70 to 160 \( \text{nmol} \cdot \text{mg}^{-1} \cdot \text{h}^{-1} \) (8, 24, 42). The purine salvage pathway via IMP resynthesis from Hx is limited by the muscle purine loss. It is estimated that HGPRT accounts for ~75% intramuscular Hx recovery at rest (10). In this study, we demonstrated significant changes in erythrocyte HGPRT activity in sprinters and triathletes during a 1-yr training cycle. The results are convergent with those obtained by Hellsten-Westing et al. (18, 21) in a short-duration sprint training and with our previous longitudinal studies (46, 47). In the same training periods, the examined groups of sprinters and triathletes presented different HGPRT activity that was significantly lower in the latter subjects. However, the pattern of changes was the same for both sprint- and endurance-trained individuals, that is, the
highest pre- and postexercise activity of HGPRT was observed during the competition period and the reduced activity was found in the transition phase. This confirmed the results of our earlier studies based on analogous training periods in middle- and long-distance runners (46, 47). Until now, factors affecting HGPRT activity in the skeletal muscle have not been recognized. Studies on purine salvage pathways were conducted with the use of rodent heart (14) and liver (23). It was revealed that the regulation of purine salvage depends on the levels of PRPP and ribose 5’-phosphate (23).

We speculate that in our study the repurification of Hx to IMP due to intramuscular HGPRT activity could be more intensified in sprinters than in triathletes, which was indicated by lower plasma Hx concentration and raised erythrocyte HGPRT activity in the competition period. Metabolic changes of this kind may be evidence for an advantageous muscle adaptation to metabolic stress and for limiting the energy-consuming de novo pathway. This also may indicate that sprint training induces adaptations in purine salvage in the muscle in a more economic way than endurance training. Definitive conclusions on this matter are not possible because of the lack of muscle biopsy in our study. All considerations based on red blood cells are of limited value; however, erythrocyte may be recognized as a suitable model reflecting muscle metabolism to a great extent.

The biosynthesis de novo is a crucial source producing and restoring the muscle pool of AdN. Endurance training, to which the muscle is exposed, does not entail the increase of de novo synthesis (40). However, these results may be affected by limitation in providing substrates. Until now, no data concerning de novo synthesis in human muscle are available.

X. The concentration of X was higher in triathletes and significantly different from sprinters’ values in the phases of general preparation and transition. This picture resembles the differences in Hx concentration and is related to different contribution of high-intensity exercise in sprinters and triathletes. Resting X concentration did not change significantly in both sprinters and triathletes. Similar X concentrations were reported in marathon runners (5), in men practicing leisure sports (32), and in highly trained middle-distance runners in a 1-yr cycle (46). However, in long-distance runners the concentration of plasma X showed significant changes between the periods of specific preparation and competition (47). The changes in the resting-postexercise value were not significant in our study, which is in line with our earlier studies (46, 47).

UA. No significant changes in plasma UA concentration were found in both sprinters and triathletes. Analogous results were obtained in our earlier study on long-distance runners (47).

Blood samples were obtained 5 min after the test, and the full spectrum of postexercise changes could not be recorded. UA reaches its maximal plasma concentration relatively late after exercise as it is a final metabolite of AdN degradation accompanied by slow-acting enzymes. The delay in plasma accumulation probably results from the activity of X oxidase that is relatively less active in skeletal muscle compared with other tissues, especially to liver (43). Therefore, UA production is initiated mainly after Hx is released from the muscle into the blood. The most intensified Hx degradation to UA occurs in the liver (20). Because of the delay in UA formation, its application as a parameter of direct training control is not possible; however, the role of UA as a powerful antioxidant seems to be unquestionable. UA may be extracted from blood by exercised muscle to replenish the muscle urate stores, used as a free radical scavenger, and oxidized to allantoin during exercise (16, 17). The lack of resting changes in plasma UA concentration was also demonstrated by Lombardi et al. (25) in top level alpine skiers in a 4-yr training cycle. They also concluded that UA is not suitable for the assessment of training status, in line with our results.

Limitations. A single blood sampling point at 5 min of recovery may be considered as the potential limitation of our study. It is known that Hx concentration reaches maximum value after 10–20 min (13, 19, 31) and UA concentration even later (36, 37). Certainly, further postexercise samples would provide more precise information for our analysis. Nevertheless, our data also seem to be valuable in the practical context of the sport training. As revealed in earlier studies, the procedure of sampling similar to ours enables to assess differences in metabolic response depending on sport specialization and exercise intensity (4, 5, 30).

Paradoxically, the structure of training loads in sprinters encompasses mostly aerobic exercise when summarized net exercise time is analyzed. This results from a huge amount of exercise devoted to warm-up, general fitness, and other less intensive but necessary components that are the base for specialized training. Thus the training was not a pure “sprint work.” However, the amount of high-intensity exercise was substantially higher in sprinters than in triathletes and the year-round training workload was planned and executed to adapt to high-intensity exercise. The final outcome of the training was the ability to run a very short distance at maximal speed. In this sense, we surely may call it sprint training.

Measuring HGPRT activity in the red blood cell may be considered questionable because the relation between muscle and erythrocyte metabolism is not clear. We assumed that erythrocyte can serve as a simple and easy available model that is parallel to metabolic changes in the muscle to the extent that enables drawing right conclusions. We presupposed that HGPRT reacts to training loads in a predictable way, which was actually confirmed. It is important to mention that Polish legal regulations do not permit the muscle biopsy for purposes other than strictly medical. Moreover, competitive athletes rarely consent to muscle biopsy.

Strengths. The present study has some advantages. It contains a direct comparison of two highly trained groups of athletes practicing quite opposite sport disciplines, which was not done so far. Moreover, competitive sprinters have not been the subject of any study in the context of purine metabolism as yet. The group of sprinters was homogenous, represented by individuals at national and Olympic level (one of the fastest 4 × 100-m relay teams), being led by one coach since several years and adhering to the same planned and precisely structured training schedule. Triathletes practiced at slightly lower sport intensity (4, 5, 30).

Conclusions. The long-lasting sprint training in highly trained sprinters caused a more pronounced decrease in pre-

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and postexercise plasma Hx concentration as well as a more intensified erythrocyte HGPRT activity in the periods of specific preparation and competition than did endurance training in competitive triathletes. This suggests a more effective use of anaerobic metabolic energy sources induced by sprint training characterized by high amount of exercise in the anaerobic lactacid and the nonlactacid zone. The reduction of training activity in the transition period brought about an inverse metabolic response resulting in an increase of Hx concentration and a decrease in HGPRP activity.

In our opinion, the changes in plasma Hx concentration and erythrocyte HGPRP activity are sensitive metabolic indicators of the training status. Higher resting HGPRP activity and lower Hx concentration are signs of adaptive changes of purine salvage. These parameters possibly provide indirect information about the potential energetic status of the muscle, especially in highly trained athletes in which no significant adaptation changes are detected when examined by means of commonly acknowledged biochemical and physiological parameters intended for measuring cardiorespiratory aerobic capacity, rather than anaerobic potential. The observed changes in plasma Hx concentration and erythrocyte HGPRP activity during the 1-yr training cycle and the between-group differences prove that these characteristics are particularly useful in sprint training control because of the lack of other adequate biochemical tools designed for the assessment of the effects of anaerobic exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.Z. conception and design of research; J.Z. and K.K. performed experiments; J.Z. and K.K. analyzed data; J.Z. and K.K. interpreted results of experiments; J.Z. and K.K. prepared figures; J.Z. and K.K. drafted manuscript; J.Z. and K.K. edited and revised manuscript; J.Z. and K.K. approved final version of manuscript.

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