Effect of Six Sessions of High Intensity Interval Training on Levels of Hypoxanthine, Xanthine, Hypoxanthine-Guanine phosphoribosyltransferase (HGPRT) and Serum Uric Acid in young College men

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ABSTRACT: Introduction and objectives: long-term sport and physical activity results in compatibility in maintaining purine derivatives but the compatibility achieved within a few sessions has not been well investigated. This study aimed to investigate the effect of a 30-seconds high intensity interval training on Hypoxanthine, xanthine, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and serum uric acid in young college men. Methods: In this study, 18 untrained healthy men were divided into two control and training groups after homogenization based on their personal characteristics. Training included six sessions (every other day for two weeks) with different intervals (4, 7, 6, 6, 5 & 4, respectively) with a fixed four-minute rest between each interval, and with a constant load of .6 on the cycle-ergometer. Blood samples were taken before and 48 hours after the last training session, and were used to analyze hypoxanthine, xanthine, uric acid, and serum HGPRT. Statistical analysis was performed using analysis of covariance (ANCOVA). Results: The results showed that high-intensity interval training for two weeks did not cause significant changes in serum HGPRT (P = .73); likewise, the increase in serum hypoxanthine (P = .170) and serum xanthine (P = .170) was not statistically significant but a significant reduction was observed in serum uric acid (P = .025). Discussion and conclusion: The results of this study indicated that two-week HIIT training is likely to enhance athletic performance and recovery of purine nucleotide cycle.

KEY WORDS: high-intensity interval training, HGPRT, hypoxanthine, xanthine, uric acid

INTRODUCTION
In recent years, many protocols have been investigated to improve performance of training activities. One of these protocols is high intensity interval training (HIIT) which is very popular among sport scientists and researchers due to time constraints and busy nature of modern societies. In this type of training, within less time and with higher intensity exercise, one can achieve more health and fitness advantages compared to low-intensity continuous training [1]. Although there is no general definition of HIIT, HIIT sessions typically refer to short training sessions with intensity above 90% and VO2 peak. Given the intensity of training, one HIIT may take from a few seconds to
several minutes in which the intervals are separated by a few minutes of rest or low intensity activity [2].

These trainings result in greater stimulation of physiological changes compared with low- to moderate-intensity trainings [3, 4]. Research shows that HIIT workouts for about 5.2 hours can cause physiological changes or similar benefits as much as 10.5 hours of endurance training [1]. HIIT with monotonous low-intensity exercise can bring about higher energy costs for the body. Although higher intensity training raises the consumption of carbohydrates, the evidence shows that the muscle compatibility produced by HIIT causes higher fat oxidation in people who are adopted to this type of training [5, 6]. In addition, HIIT training reduces the resistin gene expression in visceral adipose tissue [7].

High levels of uric acid and hypoxanthine in recovery period of HIIT represent the further destruction of ATP and higher purine nucleotide catabolism compared to endurance training and higher depletion of purines in the urine, and loss of purines requires them to be reconstructed by reproduction through the de novo pathway [8]. Loss of purine compounds lead to more negative energy and perhaps higher energy costs in comparison with continuous low intensity exercises [8]. Therefore, it can be concluded that HIIT in a lower …. results in more energy consumption and, in total, less fat tissue in participants of this type of exercises [8].

In a steady state, level of hydrolysis and ATP reconstruction are equal. physical activity increases ATP breakdown so, its higher degradation compared to its restructuring leads to production of inosine monophosphate IMP and ammonia in skeletal muscles [9, 10], and IMP may be revived in form of AMP. This recovery occurs at intervals between exercise activities with different intensities [11] and inosine breaks down to hypoxanthine and this happens by purine nucleotide phosphorylase [12]. Both inosine and hypoxanthine shed into the blood, and thereby, reduce the intramuscular adenine nucleotide reservoirs [13]. IMP of a purine nucleotides cannot pass the sarcolemma and its intramuscular reserves can be restored through the recovery path or by reproduction through the de novo pathway [14]. The reproduction path is of high importance because of reproduction of IMP, 6 high energy phosphate is required; therefore, it is not affordable for the body to reconstruct it. As a result, the recovery path of purine nucleotides is of particular importance for the body [15].

Purine nucleotides and their metabolic products are involved in biological processes of most organisms [16]. Purine metabolism involves production and degradation of purine nucleotides that have adenine and guanine determined by that of adenylate and guanylate reserves [17]. The concept of energy charge is proposed by Atkinson (1968) which is an indicator of the total amount of adenine nucleotides reservoir in ATP, ADP and AMP cells, and its value is between 0 and 1 [18]. Adenylate energy charge (AEC) value in different organs of the organisms is between .75 and .95, for example, in human body it is between .6 and .8 in liver, between .7 and .9 in the heart, and between .85 and .95 in brain, erythrocytes and skeletal muscle [19]. AEC values are the highest in skeletal muscle than in other organs, and values less than .5 are indication of the collapse of the cell or cell death [18]; hence, it is important to maintain cellular energy charge. Cell biosynthesize the nucleotides in two pathways: de novo and salvage pathways. In de novo pathways, the nucleotide bases are assembled from simpler compounds such as CO2, glutamine and glycine which are involved in the production of de novo nucleotides [20, 21]. Salvage reactions convert purine derivative to mono nucleotides and this makes the intracellular energy expenditure more efficient. Of the adenine purine nucleotides, hypoxanthine is the final compound of the cycle which can return to the path, and the return to adenine reserves is done by hypoxanthine-guanine phosphoribosyltransferase (HGPRT) enzyme of the facial muscle [14, 22]. Therefore, the exit of purine bases is an indication of the loss of purine nucleotides precursors in muscles, and complete reconstruction of ATP depends on energy recharge [10].

The duration and intensity indicate the designing of optimal compatibility training [23]. Accordingly, to determine the intensity of the activities different criteria including maximum heart rate, maximum oxygen consumption and lactate and lactic acid concentrations are used [24]. Classic metabolic and cardiovascular indices have some limitations for controlling the workouts of trained and elite athletes and there is no clear link between some of these indices and athletic performance [25-28]. Therefore, it is suggested that some indices of purine nucleotide cycle such as concentrations of hypoxanthine, xanthine, uric acid, and plasma HGPRT activity that may be more accurate for controlling the training status be used. According to recent research, hypoxanthine can be considered as an indicator of training intensity [25-28].

According to available studies, it seems that most previously conducted studies have more focused on no extreme and endurance activities, and that recent studies show beneficial effects of HIIT training on athletic performance and health factors. With the noticed advantages in mind, and lack of a study that has exclusively investigated the purine nucleotide recycle during a training period, the current study was
HIIT TRAINING ON PURINE NUCLEOTIDE CYCLE VARIABLES

METHODS

Sample and population: the present study had a quasi-experimental design and conducted with humans in two research groups. The participants of the present study were selected from students at Martyr Navab Safavi, Mazandaran University, after a call and being informed about the conditions and details of the study. The 18 eligible individuals (non-addiction to drugs and alcohol, non-athletes with no history of regular exercise for at least 6 months, no history of renal, hepatic, cardiovascular diseases, diabetes, and no injury or physical problem) were randomly divided into two groups: control (n = 9) and experimental (n = 9).

Table 1. Individual characteristics of the experimental and control groups (values are shown as mean ± standard deviation).

<table>
<thead>
<tr>
<th>Variable group</th>
<th>No</th>
<th>Age (year)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>23.4 ± 21.91</td>
<td>178.18</td>
<td>9.40 ± 69.91</td>
<td>2.96 ± 22.06</td>
</tr>
<tr>
<td>HIIT</td>
<td>9</td>
<td>1.72 ± 21.18</td>
<td>175.50</td>
<td>8.22 ± 67.36</td>
<td>2.90 ± 21.82</td>
</tr>
</tbody>
</table>

All the participants received a 2-week nutrition record. Also, before the start of the experiment, the participants were reminded not to change their diet and merely use the Mazandaran University self-food and not to change their daily activity during the exercises. They were also informed about as the necessity and importance of the research.

Training Protocol

The training protocol was adopted from Burgomaster (2005) [29] which was a 30-seconds high intensity activity on a bicycle ergometer (Lode Holland) for two weeks, three sessions per week. The training group performed their activities in the first session in four intervals which included 30 seconds of pedaling by additional load of .6 and the maximum intensity with 4 minutes of rest at each interval. The second session had five intervals which included 30 seconds of pedaling by additional load of .6 and the maximum intensity with 4 minutes of rest at each interval. In the third and fourth sessions, training included 6 intervals with 30 seconds of pedaling by additional load of .6 and the maximum intensity with 4 minutes of rest at each interval. The fifth session included 7 intervals with 30 seconds of pedaling by additional load of .6 and the maximum intensity with 4 minutes of rest at each interval. The sixth session was performed in 4 intervals with 30 seconds of pedaling by additional load of .6 and the maximum intensity with 4 minutes of rest at each interval. The duration of rest between the intervals and .6 additional load on the bicycle was fixed in all the training stages. The control group did not perform any physical activity and only followed their own daily activities.

Blood sampling

For blood sampling, prohibited items included:
1. No use of drug or supplement during the study
2. No change of diet at least 2 days before drawing blood
3. No exercise activity except study protocol and longtime walking at least 72 hours before drawing blood
4. No drink coffee, dark tea, banana and greasy food at least 24 hours before drawing blood

Blood samples were taken from brachial veins of the participants early morning after 12 hours of fasting in two stages: 48 hours before the training starts and 48 hours after the last training session, and were poured in test tubes. Then blood samples were centrifuged at 3000 rpm for 10 minute, and after that, serum was isolated and used for analysis. Serum hypoxanthine and xanthine were measured using ELISA method by the Quantitative Diagnostic Kit of SIGMA Corporation, America. Enzyme hypoxanthine guanine phosphoribosyltransferase of serum HGPRT was measured between the samples by ELISA method using the Quantitative Diagnostic Kit of CUSABIO Company (China) with sensitivity of .039 ng/ml. The serum uric acid was also measured between the samples using the biochemistry method of Bionik Company (Iran) with sensitivity of .0347 mg/dl.

Also, to examine changes in plasma volume first, the amount of hemoglobin and hematocrit was estimated before and after the training using the changes in plasma volume according to Dale and Castile (1974) equation [30].

\[
BV_a = BV_b \times (HGB_b/HGB_a)
\]

\[
RCV_a = BV_a \times HCT_a
\]

\[
PV_a = BV_a \times RCV_a
\]

\[
PV_b = [1 - (HCT_b/100)] \times 100
\]

**Statistical procedures**

All data were analyzed using SPSS software version 20. To determine whether the data is normal, the Kolmogorov-Smirnov test was used. After ensuring about the normality of data, analysis of covariance (ANCOVA) was performed to compare changes between the groups, and dependent-samples t-test was run to investigate the intra-group changes. Alpha level was set at .05.

**STATISTICAL RESULTS**

Eighteen young college men were divided into two groups: control and intense training. The Kolmogorov-Smirnov test showed that the data has a normal distribution (P > .05) and also the Levene test showed that the variances are homogenous (P > .05).

Statistical analysis did not any significant inter group changes in HGPRT serum levels (P = .73). Analysis of intragroup changes also indicated no significant differences between the pre-test and post-test values (p > .05) (Figure 2).

Statistical analysis did not show significant intergroup changes in hypoxanthine serum levels (P = .170). However, investigation of intragroup changes indicated significant differences in hypoxanthine values in the pretest-posttest (P = .037) (Figure 3).

Statistical analysis showed that there is not a significant difference between the two groups in levels of serum xanthine (P = .170). However, investigating intragroup changes indicated significant differences in the pretest-posttest values of xanthine in the training group (P = .037) (Figure 4).

Statistical analysis indicated significant differences in serum uric acid levels between the two groups (P = .025). Similarly, the intragroup analysis showed that there is a significant difference in uric acid in the training group between the pre-test and post-test (P = .048).

Results of intergroup analysis of plasma volume did not show significant differences between the two groups (P = .454). The intragroup investigation of the pre-test and post-test changes did not show any significant differences in each group (P = .762) (Figure 6).

![Figure 2. Mean and SD of HGPRT levels in the TG and CG.](image-url)
Figure 3. Mean and SD of hypoxanthine levels in the TG and CG. * Marks a significant difference compared to the pretest

Figure 4. Mean and SD of xanthine levels in the TG and CG. * Marks a significant difference compared to the pretest
Figure 5. Mean and SD of uric acid levels in the TG and CG. @ Marks a significant difference compared to the CG (intergroup difference)*

Marks a significant difference compared to the pretest

Figure 6. Mean and SD of plasma volume in the TG and CG.
DISCUSSION

The aim of the present study was to evaluate the effect of six sessions of 30-second HIIT on some variables such as the purine nucleotide cycle. Our results showed that this type of training did not result in a significant increase in resting serum and serum xanthine. Also, a significant reduction in resting uric acid was reported. One of the most important findings of this study was a considerable though insignificant, increase in serum HGPRT occurred in this short-term two-week training. It is noteworthy that changes in plasma volume was not significant in this short period study.

Higher activity of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) enzyme can indicate the elite level and higher anaerobic energy systems of athletes [25, 31]. Inosine monophosphate HGPRT activity results in rephosphorylation of hypoxanthine to inosine monophosphate and guarantees the maintenance of IMP. Since IMP cannot pass the sarcolemma skeletal muscle, maintaining IMP for rephosphorylation pathway of adenine nucleotides is of particular importance. This value can be maintained by HGPRT activity, and in this pathway HGPRT along with phosphoribosyltransferase pyrophosphate (PRPP) can convert hypoxanthine to the IMP and prevent the loss of cell purine bases at the cell surface, and thereby, there is less need for reconstruction of ATP from the de novo pathway. In fact, it is suggested that higher HGPRT activity after high intensity training which the dominant share of the anaerobic energy is an indication of a better recovery pathway for reconstruction of adenine purine nucleotides [25]. It is probable that more cytosolic energy supply leads to better compatibility in the recovery pathway. In this line, the results of the present study in which a 20% increase in serum HGPRT activity was reported is consistent with Zelinsky et al.’s study [26, 28, 31]. They showed that in the four-season training in a yearly cycle [32] in the match season where the training system moves towards the anaerobic training, the activity of this enzyme is high. Zelinsky (2011) [28] also indicated that the sprinters had higher HGPRT activity compared to the triple runners; therefore, it can be concluded that when the anaerobic energy system is dominant, similar to what occurred in the 30-second HIIT, HGPRT enzyme activity is high and the body is more economical in terms of energy.

Hypoxanthine concentration depends on the intensity of exercise [33] and after intense strenuous exercise it can speed up to 40 times of its resting values [34]. Hypoxanthine is the end product in adenine purine recovery path so that if in the course of oxidation by the xanthine enzyme, the oxidase converts to xanthine, purine will be lost, and consequently, again with xanthine, oxidase activity converts to uric acid and is excreted from the body. Therefore, the hypoxanthine value is important and can be considered as a severity index [25]. There is a correlation between an increase in hypoxanthine and decrease in blood PH, and it has been proved that there is a critical point in 107-115% of the maximum oxygen consumption of a critical point for hypoxanthine [12]. When exercise is associated with more harm such as exercises performed by eccentric contraction, the amount of xanthine oxidase is still high to 96 hours after the exercise [13] and finally, xanthine and uric acid levels are higher than their resting levels. On the other hand, since hypoxanthine represents ATP depletion, it can be considered as an indicator of discharge and cellular metabolic stress [35]. In fact, the higher HGPRT activity represents using the maximum capacity of purines and it can be predicted that if the activity of this enzyme is high, it keeps the hypoxanthine level low, and cell will be capable of returning purine bases in rephosphorylation pathway. In the present study, it was shown that hypoxanthine activity is still high after 48 hours but the increase was not significant, and this can corroborate with Gerber's (2014) [8] study who indicated that high intensity workouts lead to more purine nucleotides depletion and ultimately higher energy metabolism compared to low-intensity exercises. Also, Stathis (1994) [36] showed that resting ATP even after 72 hours after the last exercise session does not return to its resting levels before the exercise and it can be the reason for the imbalance between the destruction and reconstruction of ATP. As a result, in the present study where high hypoxanthine can be the reflection of ATP depletion, it can be inferred that still the ATP levels are not repaired. This can be important for coaches similar to what is important in tapering and the resting before the match in terms of intensity and duration of the interval between the last pre-match training session and the match day or the peak of the athletic performance.

Purines convert to xanthine on an irreversible pathway so, in addition to the conversion of hypoxanthine to xanthine that occurs in the oxidation pathway, there are also other ways that convert hypoxanthine to xanthine; therefore, xanthine can be considered as the intersection of adenine and guanine purine nucleotides. Guanine is converted to xanthine by the guanine deaminase enzyme and also the monophosphate xanthine produced from IMP can convert to xanthine, and ultimately excreted from the body by uric acid [37]. It was shown in the present study that the insignificant increase in xanthine occurs after training and it may be a reflection of energy discharge and adenine and guanine purine nucleotides. This increase shows the loss of purine base and higher energy depletion compared to the control group as well as the metabolism of high intensity activities [8] and, of course, it can be concluded that reconstruction of energy reserves requires more time. Also, significant reduction in uric acid within 48 hours after the 30-second intense activity observed in this study is consistent with Bije and Jafari's (1391) [38] study which indicated a reduction in plasma uric acid in after three months of aerobic exercise. The findings of the present study, however, contradict the results found by Shemshak et al. (1386) [39] and Degoota et al. (2003) [40] which showed an increase in uric acid after a period of training. The difference between serum uric acid values and other purine nucleotide variables observed in previous studies can be classified in several categories:

1. Time of blood sampling: time of blood sampling after exercise is one of the most important differences observed in the studies and thus is of high importance to see whether there has been sufficient
time for recovery in adaptations and whether the acute effects of the last training session can be observed in participants. For example, some variables such as hypoxanthine immediately increase slightly but in 30 and 60 minutes of recovery reach their highest level and after that get close to their resting values [41]. Similarly, other variables such as xanthine oxidase that produce xanthine and uric acid are still high up to 96 hours after the exercise [41].

2. Exercise intensity: the intensity of the exercise is another difference among the studies. The more the exercises are intense, the higher ATP breakdown and purine excretion will be [8].

3. Type of activity: another factor contributing to differences in values of purine nucleotide can be attributed to the type of activity. It appears logical that in activities in which all the muscles are involved, higher levels of purine nucleotides in blood be reported compared to exercises in which only part of the muscles are performing the activity similar to the training performed in this study on bicycle ergometer. Also, if activities are performed with more damage like in eccentric exercises compared with exercises that are performed with less damage [13]. damage nucleotide markers for example uric acid, xanthine and xanthine oxidase. Ultimately, a connection can be assumed between inflammation and damage and some purine nucleotide indicators.

4. Participants: the last difference among the studies is related to the participants who are determinants of the purine nucleotide levels depending on the elite level and energy system so that, as reported by Zelinsky, elite runners had lower levels of resting uric acid and higher resting HGPRT activity compared to recreational and amateur runners. Likewise, sprinters have higher HGPRT activity compared to triple runners.

Overall, this type of HIIT on cycle ergometer increased hypoxanthine, xanthine and serum HGPRT and reduced levels of serum uric acid during sixth sessions. Hence, it can be concluded that 2 weeks of high intensity interval training can cause compatibility for improving athletic performance and recovery pathway of purine nucleotide cycle.

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