

Comparison of the effects circuit training on some indicators of the purine nucleotide cycle in wrestlers

Short title: Wrestling training and the purine nucleotide cycle.

Abstract

Research shows that one component affecting systems and energy sources is the intensity of activity, which can influence the sources of adenosine triphosphate (ATP) regeneration. This research aimed to compare two different methods of wrestling training in terms of metabolic efficiency and effect on the indicators of the nucleotide purine cycle.

Considering body weight, 21 athletes were divided into two groups of circuit exercises based on wrestling techniques (n=10) and the group of prevalent wrestling exercises (n=11). After conducting physical and physiological tests, blood sampling was taken before and after eight weeks at rest to measure hypoxanthine, hypoxanthine-guanine-phosphoribosyl-transferase, lactate, uric acid, xanthine, and Keratin-kinase. For eight weeks, the athletes practiced six wrestling techniques in a circuit and prevalent wrestling exercises. Independent t-test, analysis of variance with repeated measures (ANOVA), analysis of covariance (MANCOVA), and post hoc LSD test was used for data analysis.

The results showed that there was no significant difference in the levels of hypoxanthine-guanine phosphoribosyl-transferase (P=0.31), hypoxanthine (P=0.66), xanthine (P=0.62), plasma uric acid (P=0.1), lactate (P=0.3) and Keratin kinase (P=0.47) between the two groups. However, in the intra-group analysis, hypoxanthine values decreased significantly after eight weeks of circuit exercises based on wrestling techniques (P = 0.0001) and prevalent wrestling exercises (P = 0.0001).

According to the results wrestling exercises effectively change the metabolism of purines. Furthermore, the comparison of the two methods of wrestling training demonstrated that there were no differences between them, and they affect the indicators of the nucleotide purine cycle to the same extent.

Keywords: *Circuit training, Purine Metabolism, Hypoxanthine, HGPRT.*

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Introduction

It is implied that the capacity of muscles in biochemical conditions, in order to maintain maximum power, is caused by the capacity to tolerate anaerobic glycolysis, metabolic acids buffer, and aerobic fuel metabolism (1). Since the mitochondrial activity cannot provide enough energy requirements (2). Energy plays a central role in the body's metabolic system. The main pathways of cell metabolism include adenosine triphosphate (ATP) and ATP-related cofactors (3). Studies show that the intensity of activity is considered one of the factors affecting systems and energy sources and can influence the sources of adenosine triphosphate regeneration (4, 5). Muscles are assumed to benefit from a large reserve of ATP resources used as fuel for cellular activities. However, this idea is not correct. The total amount of ATP stored inside the cells of the body is very low (about 8 mmol/kg wet weight of muscle). Due to the limited amount of ATP, there must be metabolic pathways for ATP production, and in other words, ATP cell charging takes place. Muscle tissue is unique and can change its metabolic rate more than other tissues according to different needs (6). It has also been shown that despite the short time of anaerobic exercises, the effect of these kinds of activities on the metabolism of purines is significant (7).

So as to control intense and short-term sports activities, ATP breakdown exceeds its synthesis, and the dominant energy supply system of these activities becomes the anaerobic system, so common physiological and biochemical indicators are not able to determine the state of exercise, especially in young athletes. Based on the study of Zielinski (2011), it was found that some indicators of the purine nucleotide cycle, such as hypoxanthine concentration and the activity of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) in plasma, can be an appreciate instrument used to acute control of the state of training (examination of adaptation due to training and overtraining control). Cells rely on other mechanisms to provide ATP to support cellular activity, including storing energy in more complex molecules such as glycogen and triglycerides and having a control system sensitive to the rapid increase in body metabolism at the time of energy demand (ATP). One of the methods that conserve energy sources is the purine nucleotide cycle. Accordingly, during intense training, the amount of ATP consumption in skeletal muscles is higher than its production, leading to the accumulation of adenosine diphosphate (ADP) and adenosine monophosphate (AMP). To avoid the high accumulation of AMP in the cell (4), deamination of inosine monophosphate (IMP) (in skeletal muscles) or phosphorylation of adenosine (in cardiac muscles) occurs (8). Therefore, the concentration of

IMP in skeletal muscles increases and may undergo further changes, resulting in inosine (9). Inosine is broken down into hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine can be removed from the muscle and cause a decrease in adenine nucleotide sources, or it can be recovered inside the muscle by the HGPRT enzyme (10, 11). Hypoxanthine is also considered an indicator of training intensity (12). It is also useful for controlling the metabolic stress of muscle tissue during training or recovery since it is directly correlated with the amount of ATP inside the cell and can be a good indicator of muscle fatigue (13). Ghanbari-Niaki et al. (2008) demonstrated that the increase in blood Glucose and the decrease in plasma ATP and Keratin caused by intense exercise is a reflection of the compensatory process against energy depletion, which is confirmed by the decrease in anaerobic power (14).

Studies conducted on the changes in purine metabolism during short-term periods (six to seven weeks) of speed training showed a decrease in muscle adenine nucleotide loss (15). Zielinski et al. (2009) investigated the effect of endurance training on changes in purine metabolism in endurance runners during the annual training cycle. The results showed that the activity of HGPRT is the highest in the competition phase and the lowest in the transfer phase, and hypoxanthine levels were the lowest in the competition phase and the highest in the transition phase (9). Zielinski et al. (2011) also investigated the effect of overload in a one-year training cycle on the metabolism of purines in semi-endurance runners. This study showed that hypoxanthine significantly decreased, and HGPRT activity increased significantly after training. Uric acid also increased (7).

On the other hand, the intensity and duration of exercise can also affect plasma purine accumulation during recovery (5). Frequency and number of training sessions can also affect the accumulation of purines (15). Considering that wrestling is a power sport and the duration of a wrestling match is short, and the anaerobic energy system is important to meet muscle needs, it seems that various exercises for preparing for wrestling can affect the metabolism of purines. Moreover, hypoxanthine is a strong predictor of performance in trained athletes, and its ability to predict is very high regardless of the type of sport. It can include power and speed sports to endurance ones (16). However, no study of the metabolism of purines in power-strength sports, including wrestling, was found. It should be mentioned that the theory of Zielinski et al. regarding the effect of the depletion of energy resources in weight-related sports, which is sometimes associated with weight loss, is inconsistent and has not been tested. Therefore, the reason for this research design is to test Zielinski's theory and increase knowledge in this type of sports and whether it is possible to consider hypoxanthine and HGPRT as indicators to

check the adaptation caused by training and overtraining control in this kind of sports. Finally, this research aimed to investigate and compare eight weeks of circuit training based on wrestling techniques and prevalent wrestling exercises and possible changes in hypoxanthine, HGPRT, xanthine, uric acid, lactate, and Keratin kinase levels in wrestlers and answer the question of whether changes in HGPRT, which is an intensity index introduced by Zielinski, can represent the adaptation caused by exercise and the training status of wrestlers or not?

Material and Method

The statistical population of this research consisted of trained wrestlers in Neyshabur city. 30 wrestlers volunteered to participate in the research. The subjects were divided into two groups of circuit exercises based on wrestling techniques (15 people) and the group of prevalent wrestling exercises (15 people). During the research, 9 subjects refused to participate due to personal reasons and injuries. The subjects were selected purposefully. To estimate the sample size of the research, G*power software was used with an alpha of 0.05 and statistical power of 0.80, and the sample size was calculated for each group of 10 individuals.

The research method was quasi-experimental. Subjects signed a written consent to participate in the research. The entry criteria included perfect health, no history of illness, and no smoking, and the subjects had at least three years of regular wrestling practice. Exclusion criteria included smoking or drug use (17), history of heart disease, kidney disease, or type one or two diabetes (18). These cases were checked through a questionnaire. One week before the start of the research, according to the research project schedule, anthropometric measurements, including height, weight, fat percentage, and body mass index, were measured. The three-point method of Jackson and Pollack was used to calculate the subcutaneous fat, and the fat was measured at three points of the chest, abdomen, and right thigh by a caliper. Body density was calculated with Jackson and Pollock's subcutaneous fat equation (19) and then converted to body fat percentage with the Siri equation (20).

The number of circuit training sessions based on wrestling techniques was held in the first four weeks, four sessions per week, five sessions in the next two weeks, and six sessions per week in the last two weeks. The duration of sports training was eight weeks. Each exercise session consisted of 15 to 20 minutes of general and specific warm-up and then performed the exercises according to the planned program progressively and lasted between 22 and 57 minutes. At the end of each session, the subjects cooled down for 10 minutes. In each session, wrestling techniques were performed in six or eight stations. Each station was practiced for 5, 10, 25, and 30 seconds in one session and 15 and 20 seconds in the next

session. During the exercises and the rest periods, the heart rate of the subjects was controlled with a polar heart rate sensor, which varied between 175 and 210 beats per minute.

In each session, the circuit exercises were performed so that the subjects performed six wrestling techniques, including Flying mare, over-under, Fireman's carry from inside, front head lock, Rear takedown with single leg tackle, and buttocks at maximum speed and certain sets. In terms of time, the exercises of the prevalent training group were the same as the circuit training group based on wrestling techniques, but the only difference was that the prevalent training was done in pairs. In the sessions where the duration of the exercises was less, resistance and aerobic exercises were performed the same as in the circuit exercise group. Aerobic training included 12 minutes of running two to three sessions a week.

The subjects were asked not to do any exercises 48 hours before the pre-test blood sampling. On the blood sampling day, subjects availed themselves at the wrestling club. Subjects ate a standard breakfast of about 500 kilocalories (some bread, four dates, two walnuts, 40 grams of cheese, and some vegetables) around seven A.M. Then, at around 9.15 A.M., 10 ml of blood was taken from the vein of each subject's hand while resting; the blood samples were transferred to test tubes containing EDTA, and after, centrifugation and plasma separation was maintained at -80 degrees centigrade. The subjects did the relevant exercises for eight weeks according to the training program. The second stage of blood sampling was also done in a resting state, i.e., having no physical activity for 48 hours before sampling. Then the plasma samples were transferred to a specialized laboratory to determine the concentration of hypoxanthine, HGPRT, uric acid, xanthine, Kerating kinase, and lactic acid.

To measure hypoxanthine, the kit of Sigma Company, made in Germany, was used with a photometric reading method by a device with a sensitivity degree of 2.6 ng/ml and a coefficient of intragroup variation of 10% and for the measurement of xanthine, the kit of Sigma Company, made in Germany, was used with a photometric reading method by a device with a sensitivity degree of 2.6 ng/ml and a coefficient of intragroup variation of 10%. In order to measure HGPRT, the ELISA method and the Casabio kit with a sensitivity degree of 0.039 ng/ml and coefficient of intragroup variation of 8%, made in China, were used. To measure lactate, the calorimetric method and the Greiner kit made in Germany were used with a sensitivity level of 0.11 mg / dL and a coefficient of intra-group variation of 0.7%. Uric acid was measured using the sandwich ELISA method, Demeditec Company kit made in Germany with a sensitivity degree of 1.76 international microunits per milliliter and a coefficient of intragroup variation of 2.6% was used, and to measure Keratin Kinase, the photometric method and the Pishtaz Teb Company made in Iran was used with a

sensitivity degree of 2 international units per liter and a coefficient of intra-group variation of 1.6%.

Descriptive statistics were used to calculate central tendency and dispersion index. Shapiro-Wilk test was also used to check the normality of data distribution. Independent t-test, ANOVA, MANCOVA, and LSD post hoc tests were used to investigate the difference between independent and dependent variables in the studied groups. All statistical operations were performed according to the specific research objectives by SPSS software, and the significance level of hypothesis testing was considered $P \leq 0.05$.

Results

Before the start of wrestling training, the subjects' average age, weight, height, body mass index, and training history were calculated using descriptive statistics. The results showed that there was no significant difference between the two groups before the start of wrestling training (Table 1).

The results showed no significant difference between circuit exercises based on wrestling techniques and prevalent wrestling exercises in any of the measurement stages in plasma HGPRT values ($P=0.31$). Furthermore, in the intra-group examination of the subjects, it was found that the wrestling exercises of the circuit training group based on wrestling techniques ($P=0.1$) and the prevalent wrestling training group ($P=0.07$) during eight weeks had no significant effect on the HGPRT values. It is also demonstrated that after eight weeks of wrestling training, there was no significant difference in the amounts of hypoxanthine ($P=0.66$), xanthine ($P=0.62$), and plasma uric acid ($P=0.1$), lactate ($P=0.3$) and Keratin kinase ($P=0.47$) between the two groups. In addition, in the intra-group analysis, there was a significant decrease in hypoxanthine values after eight weeks of circuit exercises based on wrestling techniques ($P=0.0001$) and prevalent wrestling exercises ($P=0.0001$) (Table 2).

Discussion

This research showed no difference in plasma hypoxanthine values between circuit exercises based on wrestling techniques and prevalent wrestling exercises in any of the measurement stages. In addition, after eight weeks of circuit exercises based on wrestling techniques and prevalent wrestling exercises, a significant decrease in plasma hypoxanthine values during the resting time was observed. It has been found that the amount of hypoxanthine released from the muscle increases immediately after exercise and decreases during rest (21). During exercise, the main source of plasma hypoxanthine is skeletal muscles (22). In line with the results of this research, Zielinski et al. (2012) investigated the adaptation caused by training in sprinters and triathletes in an annual training cycle on purine metabolism. During the general physical fitness stage and competitions, they observed a decrease in hypoxanthine in both groups. They stated a decrease in the

release of hypoxanthine from the muscle and an increase in its reamination to IMP or a decrease in the production of hypoxanthine as the reason for this observation (23). Usually, the concentration of hypoxanthine decreases during high-intensity exercises and increases significantly during aerobic exercises. It has been shown that the decrease in the release of purines from the skeletal muscle following speed training is due to a sharp decrease in IMP production resulting from the decrease in ATP breakdown caused by exercise in the muscle (24).

In line with the results of this study, Spencer et al. (2004) showed that the concentration of hypoxanthine in hockey players at the national level decreases significantly during seven weeks of training. They stated that the decrease in hypoxanthine might be due to a decrease in hypoxanthine production and an increase in its recycling in the muscle. In addition, adaptation due to intense and speed-specific hockey training has caused the storage of adenine-nucleotide resources during intense training (25). Zielinski et al. (2009) investigated the effect of endurance training in endurance runners on the metabolism of purines in the annual training cycle. The results showed that plasma hypoxanthine levels decreased and erythrocyte HGPRT activity increased. With these changes, it can be suggested that the re-phosphorylation of hypoxanthine to intramuscular IMP has increased. However, they did not measure muscle HGPRT (9).

In this study, considering that the subjects in both groups had aerobic exercise programs in addition to specific exercises, the reason for the reduction of hypoxanthine during rest may be muscle adaptation using other energy sources such as the glycolytic pathway and aerobic phosphorylation. In other words, the mechanism of this decrease may be an improvement in the re-phosphorylation of ATP due to the anaerobic or aerobic system in exercise training (21). The reduction of plasma hypoxanthine in this study may be due to less production due to the decrease in AMP deaminase activity or the increase in HGPRT activity and conversion of hypoxanthine to IMP. The lack of difference between the two types of training in the effect on hypoxanthine is due to the similarity of the two types of training. Also, probably due to the similar training durations, the effectiveness of the two types of training was the same.

The present study's results showed no difference between the plasma HGPRT values in the two groups. Also, in the intra-group analysis, there was no change in HGPRT values after eight weeks of wrestling training. Zielinski et al. (2012) compared the effect of training loads on the metabolic response of purine derivatives in elite sprinters and endurance triathletes in an annual training cycle. The results demonstrated that the activity of HGPRT in sprinters is higher than in triathletes, and

its activity increased in the competition phase and decreased in the transition phase (23).

In the present study, there was no change in the level of HGPRT in plasma. However, the circuit training program based on wrestling techniques and common wrestling training significantly reduced the amount of hypoxanthine during rest time. In this study, muscle, and erythrocyte HGPRT were not measured, and only its plasma level was investigated. Nevertheless, since the level of plasma hypoxanthine during rest was significantly reduced, erythrocyte and muscle HGPRT activity has probably increased due to the exercise program and conversion of hypoxanthine to IMP. The increase in muscle HGPRT activity can be the reason for the decrease in plasma hypoxanthine.

The results of the present study showed that there was no significant difference between the plasma uric acid levels in the two groups. Zielinski et al. (2012) compared the effect of training load on uric acid response in elite sprinters and endurance triathletes in an annual training cycle. The results showed that uric acid levels were not different in sprinters and triathletes (23, 27). Zielinski et al. (2013) investigated the changes in uric acid levels during the annual exercise cycle in elite, amateur, and recreational runners. They did not observe significant changes in uric acid levels (26, 27). Lombardi et al. (2010) did not observe a significant change in uric acid levels during different training phases of alpine skiers for four years. They concluded that uric acid is not a useful indicator for determining high physical demands or overtraining, although its role as a strong antioxidant is very important (28). Trained muscles may remove the uric acid in the blood to replace urate reserves and be used as a free radical neutralizer, or it may be oxidized to allantoin during exercise (29). In another study in 2009, Zielinski et al. did not observe a significant change in plasma uric acid levels after endurance training in runners. They stated that this was a lower release of hypoxanthine from the muscle. The lack of change in uric acid after eight weeks of wrestling training in the present study may be due to the significant decrease in plasma hypoxanthine levels at rest time as a result of less hypoxanthine release from the muscle and its conversion to IMP (9). Trained muscles may also take it to replace urate reserves or oxidize to allantoin during exercise (30).

The research showed that plasma xanthine values did not differ between the two groups. Zielinski et al. (2009) investigated the effect of endurance training on changes in purine metabolism. The results showed that the amount of xanthine in the specific physical fitness stage of annual training is the lowest, and the amount of uric acid is the highest. The activity of the xanthine-oxidase enzyme probably has reduced the amount of xanthine and converted it into uric acid. They mentioned that it seems that in athletes, the most important factor affecting metabolic

changes is the specific program and the structured training load (intensity, volume, and frequency) based on the purpose of different stages of training, which can be the reason for the changes in this index (9). In the present study, there were no differences between the two groups after eight weeks, and only the amount of xanthine within the group decreased, which may be due to the exercise adaptation and the reduction of hypoxanthine release from the muscle. It seems that the similarity of the two training methods is the lack of difference between the two training methods in affecting the xanthine plasma values at rest time.

The results of this research showed that there was no difference between the two groups in any of the stages of plasma lactate measurement. In the intra-group comparison of the subjects, it was found that lactate values decreased significantly after eight weeks in the prevalent wrestling training group and the circuit training group. Zielinski et al. (2011) investigated the effect of changes in training load and subsequent non-training on lactate levels in semi-endurance runners in an annual training cycle. The results showed that lactate levels at rest did not change significantly during the annual training cycle (7). Zielinski et al. (2012) compared the effect of training load on lactate levels in elite sprinters and endurance triathletes. The results showed that the changes in plasma lactate during the resting time in the two groups were not different. Also, in the group of triathletes, there was a significant difference in lactate levels at different stages of the training cycle (23). The mechanisms responsible for lactate reduction are still not fully understood. It has been found that lactate removal from blood (metabolic clearance rate) increases in trained rats through gluconeogenesis and lactate oxidation. Active muscles seem responsible for changes in lactate oxidation during exercise (29). In general, increasing the amount of lactate removal from the blood and increasing its oxidation due to adaptation to circuit exercises based on wrestling techniques and prevalent wrestling and reducing its production can be one of the possible reasons for the decrease in lactate concentration observed in this research. It is also possible that improving cardiovascular endurance and the aerobic system also played a role in reducing lactate. In addition, the decrease in lactate and pH is probably associated with the decrease in the concentration of 2 and 3 diphosphoglycerate and the concentration of ADP, allosteric inhibitors of PRPP activity. As a result of PRPP synthesis, IMP substrate increases and leads to a decrease in lactate and hypoxanthine at rest. It seems that the similarity of the two training methods is the lack of different two training methods in affecting the plasma lactate values at rest time.

The results of the present study showed that there was no difference in Keratin kinase levels between the two groups. It may not have been established that the appropriate intra-muscular adaptation to change the Keratin kinase values during

the resting time of the wrestlers in the circuit training program is based on wrestling techniques and prevalent wrestling training. Certainly, in some cases, there is no need to change; the same previous function of the enzyme is adequate, and there is no need for adaptation.

Conclusion

In general, according to the results of this research, strength training and training that use the anaerobic energy system effectively change the metabolism of purines and influence the production of the resulting metabolites. It was also found that the two methods of wrestling training are not different, and both methods affect the metabolism of purines to the same extent. In addition, the comparison of the two methods of wrestling training showed that these two exercises, due to the similar training durations, did not differ from each other, and they affect the indicators of the purine nucleotide cycle to the same extent.

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Conflict of interest

No conflict of interest has been declared by the authors.

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Table1. Mean and standard deviation of anthropometric indices and the subjects' training history

	Circuit training group based on wrestling techniques	Prevalent wrestling practice group	P value
Age (years)	17.5±2.41	16.81±1.83	0.47
Height (cm)	171.50±4.86	171.73±5.02	0.92
Weight (kg)	68.31±16.54	69.66±14.55	0.84
Body mass index (kg/m²)	23.06±4.52	23.53±4	0.80
training history (years)	3.60±1.95	3±1.54	0.44

Table2. Mean and standard deviation of biochemical indices in two groups

	Circuit training group based on wrestling techniques		P (intra-group variation)	Traditional/Prevalent wrestling training		P (intra-group variation)	P (inter-group variation)
	Pre-test	After 8 weeks		Pre-test	After 8 weeks		
Blood sampling stage							
Hypoxanthine Ng/ µl	53.20 ±10.29	*33.5 ±20.86	0.0001	45.78 ±13.04	*26.5 ±22.12	0.001	0.66
Hypoxanthine guanine phosphoribosyl transferase Ng/dl	0.34 ±0.03	0.37 ±0.06	0.10	0.37 ±0.04	0.43 ±0.07	0.07	0.31
Uric acid Ng/dl	5.95 ±0.86	5.77 ±0.89	0.66	6.28 ±1.33	6.79 ±1.45	0.1	0.1
Creatine kinase IU/L	32.80 ±10	32.50 ±8.45	0.03	34.44 ±11.37	37.66 ±8.45	0.1	0.47
Lactate Mg/dl	28.70 ±3.02	*18.5 ±5.02	0.001	35.14 ±12.20	*17.9 ±4.41	0.0	0.30
Xanthine Ng/ µl.	63.70 ±25.11	57.95 ±68.31	0.01	51.17 ±14.57	*29.6 ±24.71	0.0	0.62

*Significant difference between pre-test and after eight weeks of wrestling training.