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Full Length Research Paper

Xanthine oxidase and uric acid response to a 6-week pre-season training programme in male athletes

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This study was carried out to determine the influence of a 6-week pre- season exercise programme including aerobic and anaerobic loads on xanthine oxidase and uric acid levels of male athletes. Fifty voluntary subjects (the average age is 23 ± 5) participated in this study as study a group. The control group included 30 healthy resting male volunteers with the average age of 23 ± 6. For 6 weeks, the athletes participated in pre-season training programme private to their sports branches 5 times a week. The programme included both aerobic and anaerobic loads. The venous blood samples were taken from all athletes before and after a six-week program and xanthine oxidase and uric acid levels were measured. The paired and independent t tests were used for comparisons. The mean xanthine oxidase and uric acid levels of the control group were 2.86 ± 0.45 U/grHb and 4.85 ± 0.43 mg/dL, respectively. For the exercise group, the mean XO and UA levels were 3.01 ± 0.39 U/grHb and 5.33 ± 0.69 mg/dL as pre-test, and 4.38 ± 0.77 U/grHb and 8.39 ± 0.33 mg/dL as post-test, respectively. There were statistically significant differences between the groups ($p < 0.05$, $p < 0.001$) and within the study group ($p < 0.01$). There was a significant difference in the xanthine oxidase and uric acid levels of the athletes in the exercise group before and after training programme consisting of aerobic and anaerobic loads. Moreover, similar difference was seen between exercise and control groups. Thus it can be concluded that exercise has an effect on xantine oxidase and uric acid levels.

Key words: Xanthine oxidase, uric acid, preparatory, training, athlete.

INTRODUCTION

It is well known that there can be some changes in blood parameters depending on exercise (Sonmez, 2002). The increased metabolic activity during exercise increases the expenditure of ATP and induces in serum lipids, electro-lytes and some enyzmes (Cannon et al., 1990). Xanthine oxidase (XO) is an important enzyme in this process (Herken et al., 2001; Vina et al, 2000).

With intensive exercise, certain regions of the body can face temporary periods of hypoxia and ischemia-like conditions. In this state, xanthine dehydrogenase (an enzyme that functions by reducing NAD+) can be converted to XO, which functions by reducing molecular oxygen. ATP catabolism results in production of hypoxan-thine, thereby producing substrate for xanthine oxidase. When oxygen is reintroduced (reperfusion) XO produces superoxide and hydrogen peroxide as by-products, contributing to overall oxidant and free radical formation (Sachdev and Davies, 2008).

Sufficient oxygen supply ensures that ATP is replenished primarily via mitochondrial oxidative phosphorylation and xanthine are converted to uric acid (UA) by XO (Sahlin et al., 1991). This situation may apply theoretically to ischemic muscle contraction, isometric exercise, sprinting, exercising in a hypoxic environment. Exercise may produce a cellular environment in favor of activating the XO pathway (Hellsten, 1994). Hypoxan-thine was reported to accumulate after exercise, and UA concentration was shown to increase in both contracting muscle and in the plasma, suggesting that XO was activated (Norman et al., 1987; Hellsten-Westing,1993).

Blood hypoxanthine and xanthine concentrations increased dramatically in human subjects after exercise (Sahlin et al., 1991; Hellsten et al., 2003) . Furthermore, XO activity was increased in the plasma of rats (Radak et al., 1995) and horses (Rasanen et al., 1996) after running to exhaustion. In Rasanen's study UA concentration

increased exponentially with workload indicating a rapid degradation of purine products.

Heunks et al. (1999) concluded that strenuous exercise results in blood glutathione oxidation and lipid peroxidation. This can be inhibited by treatment with allopurinol, indicating that XO is an important source for free radical generation during exercise. In a study, the level of XO has been found elevated in injured human skeletal muscle, after five bouts of strenuous one- legged eccentric exercise. The number of XO structures in the exercised muscle was up to eightfold higher than control from day 1 to 4 after exercise. The increase was attributed to an enhanced expression of XO in microvascular endothelial cells and an invasion of leucocytes containing xanthine oxidase (Hellsten et al., 1997).

Long duration and exhaustive exercise can overcome our capacity for detoxifying reactive oxygen species and free radicals and cause damage (Ashton et al., 1998; Bailey et al., 2007). In many studies, it has been stated that exercise of sufficient intensity and duration increases the level of XO and UA. These studies have used a type of exercise with either aerobic or anaerobic load. (Bloomer and Goldfarb, 2004; Finaud et al., 2006; Vollaard et al., 2005). Several studies have investigated both aerobic and anaerobic exercise bouts (Bloomer et al., 2005; Magalhaes et al., 2007; Vincent et al., 2004; Bloomer and Smith, 2009) and that means still little is known.

Thus the purpose of this study was to determine the influence of a 6 week pre-season exercise programme including aerobic and anaerobic loads on xanthine oxidase and uric acid levels of male athletes.

METHODS

Subjects and study design

Active sportsmen were the subjects and they were informed of the purpose of the study. 50 voluntary male (18 soccer players, 12 basketball players, 12 runners, 8 wrestlers) aged between 18 – 29 participated in the study. The average age was 23 ± 5. Their sports age were between 2 – 10 years. The control group included 30 healthy sedentary male volunteers with the average age of 23 ± 6. The subjects had no major medical illnesses. No subject reported tobacco use within the 6 months prior to the study and none was taking antioxidant compounds, including vitamins and medications (anti -inflammatory agents). The study protocol was in accordance with the declaration of Helsinki and was approved by the local ethics committee. All the subjects gave their written consent prior to participating in the study after they had been informed about the design and the risks.

Exercise training

For 6 weeks, the athletes in the exercise group participated in a pre-season training programme (PTP) private to their sports branches 5 times a week. Two days a week were “sedentary” days so subjects in the exercise group were asked to avoid all physical activity. PTP included both aerobic and anaerobic exercises devoted to strength, velocity, aerobic endurance, flexibility, coordination but not technical and tactical drills.

Taking blood samples

10 ml venous blood samples were taken from all athletes in both the study and control groups, in the beginning of the study. After a six-month training program, 10 ml venous blood samples were once again taken from the subjects and put into vacutainer tubes without K_3 EDTA and anticoagulants. The blood samples were centrifugated at 3000 rpm for 10 min and insulated into plasma and sera.

XO enzyme activities were defined at hemolysis. It was frozen at -80°C for uric acid analysis in the sample sera which were insulated. Erytrocytes remaining in the tubes after insulation of the plasma were washed twice through NaCl solutions in order to obtain an erytrocyte pack. 0.2 ml was taken and combined with 1.8 ml distiled water to formulate hemolysis to analyze antioxidant enzymes and were preserved at -8°C until the analysis time. The uric acid concentration was analyzed at Olympus 2600 autoanalyzer by using a commercial kit.

Measurement of xantine oxidase activity

XO activity was determined according to that xantine added to medium as substrate was converted into XO enzyme and uric acid within the sample and that change in absorbance was recorded spectrophotometrically at 293 nm (Hashimato, 1974).

The reactives used are xantine (4 mM), uric acid standard (10 mM, 50 µ), phosphate buffer (50 mM, pH 7.5) and TCA (100%).

The samples were defrosted from -80°C. Reactives were transferred into tubes to carry out the experiment. After the TCA addition, tubes were blended thoroughly and centrifugated at 4500 rpm for 15 - 20 min. 1 ml of the clear supernatant was taken into the tube and its absorbances were read at 293 nm.

Calculation was made by using the concentration and absorbance value of the uric acid standard used.

Data and statistical analyses

The results were presented as means and standard deviations (x ± SD) . For comparisons within the same group (pre and post tests) and between the groups paired samples t test and independent samples t test were used respectively. The significance level was $p < 0.05$.

RESULTS

The mean xanthine oxidase level of the control group was 2.86 ± 0.45 U/grHb. For the exercise group the mean pre-test XO level was 3.01 ± 0.39 U/grHb. It refers to a statistically significant difference between groups for pre-test results at 0.05 level ($p < 0.05$). For the exercise group the mean post-test XO level was 4.38 ± 0.77 U/grHb. It refers to a significant difference between the experiment (post- test results) and control groups at 0.001 level ($p < 0.001$). There was also a statistically significant difference between pre and post-test XO results of the exercise group ($p < 0.05$) (Table 1).

The mean uric acid level of the control group was 4.85 ± 0.43 mg/dL. For exercise group, the mean pre-test UA level was 5.33 ± 0.69 mg/dL. It refers to a statistically significant difference between groups for pre-test results at

Table 1. Mean xanthine oxidase (XO) and uric acid (UA) scores of the study (pre and post-test) and control groups; relationship between the groups (study and control) and within the study group (pre and post test).

	CG (Mean ± SD)	EG (Pre-test mean ± SD)	EG (Post-test mean ± SD)	p value
XO (U/grHb)	2.86 ± 0.45	3.01 ± 0.39	-	[a] < 0.05
XO (U/grHb)	2.86 ± 0.45	-	4.38 ± 0.77	[a] < 0.001
XO (U/grHb)	-	3.01 ± 0.39	4.38 ± 0.77	[b] < 0.01
UA (mg/dL)	4.85 ± 0.43	5.33 ± 0.69	-	[a] < 0.05
UA (mg/dL)	4.85 ± 0.43	-	8.39 ± 0.33	[a] < 0.001
UA (mg/dL)	-	5.33 ± 0.69	8.39 ± 0.33	[b] < 0.01

SD, Standard deviation; EG, exercise group; CG, control group. [a] Independent samples t test, [b] Paired samples t test.

0.05 level ($p < 0.05$). For exercise group the mean post-test UA level was 8.39 ± 0.33 mg/dL. It means there is a significant difference between the experiment (post test results) and control groups at 0.05 level ($p < 0.001$). There was also a statistically significant difference between pre- and post-test UA results of the exercise group ($p < 0.05$) (Table 1).

DISCUSSION

This study was conducted to describe the effects of six-week pre-season training program in which male athletes attended aerobic and anaerobic exercises on XO and UA levels. Significant differences were found in the comparisons of the control grup level and pre and post test levels of the exrcise group. There was also significant differences between pre and post-test XO and UA levels of the exercise group. It is noteworthy that the XO and UA levels of the experiment group were significantly different from those of the control group even before the start of training. This difference may be creditted to the fact that the experiment group consists of active athletes regularly working out and that the XO and UA levels of the group were higher due to the exercise effect. The difference between two groups also remained after the measurements following the training. Moreover, this significant difference was also observed in the pre and post-test comparison of the experiment group. The significant increase in the XO and UA levels of the experiment group before and after the training program consisting of aerobic and anaerobic exercises has heavily proven that exercise has an effect on XO and UA levels. The present results were consistent with the previous reports.

XO is a relevant source of free radicals during aerobic exercise (Gomez-Cabrera, 2006). Many studies have indicated that XO is responsible for exhaustive exercise-induced oxidative stress in several tissues including muscle, liver and heart. During exercise, as Vina et al. (2000) stated that XO activity was significantly increased in the circulation and tissues. In the study of Hellsten et al. (2003), the effect of 7 days of strenuous exercise on the quantity of xanthine oxidase in muscle was investigated. After the week, the number of xanthine oxidase immunoreactive cells were higher in the muscle. Tidball (2005) showed that neutrophils rapidly promoted muscle damage after exercise. Radak et al. (1995) found that XO has been implicated also in free radical production during anaerobic exercise. Gomez-Cabrera et al. (2006) found that XO was also involved in free radical generation during resistance exercise in weightlifters. Moreover, similar findings were provided in runners (Robertsen et al., 1991), sprinters (Tauler et al., 1999), professional cyclists (Mena et al., 1991; Gomez-Cabrera et al., 2006), marathon runners (Gomez- Cabrera et al., 2006), and ultra marathon runners (Padak et al., 2000).

UA is an end product of purine metabolism and has been suggested to function as the most important anti-oxidant molecule (Halliwell and Gutteridge, 1989; Battino et al., 2002; Palmer et al., 2003; Sureda et al., 2007). In this study, UA increased in response to exercise and it is consistent with the findings of others (Liu et al., 1999; Hellsten, 2000; Gonzales et al., 2008). In others, during intensive exercises such as shortdistance running (Westing,1989) ultramarathon race (Mastaloudis et al., 2001) and mountain cycling (Aguilo et al., 2005) UA level increased as well. The exercise-induced increase in UA maybe due to enhanced purine oxidation and subsequent formation of uric acid (Hellsten et al., 1997; Hellsten, 2000).

Gomez-Cabrera et al. (2006) described and summarized physical exercise as a double-edged sword: when practiced strenuously it causes oxidative stress and cell damage; in this case antioxidants should be given. But when practiced in moderation, it increases the expression of antioxidant enzymes and thus should be considered an antioxidant.

Conclusıon

Significant differences were found in this study between the control and experiment group. Although this study indicated that the XO and UA levels increased significantly after a 6-week PTP, the results should not be generalized. The lack of female athletes was a limitation for this study and there was no chance to compare the

athletes considering sex. Moreover, the type of exercise used in PTP was both aerobic and anaerobic. Because of this reason, it is unable to say which exercise XO and UA levels change was not possible. Yet, this study may give an idea for future research which will be carried on the same subject. Further research is needed.

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