The Effect of Eight Weeks of Testosterone Enanthate Consumption on Oxidative Indicators of Kidney Tissue in Resistance Trained Rats

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Abstract

Introduction: Taking anabolic steroids can affect the state of oxidative stress in different tissues of the body such as kidneys. This study aimed to examine the effect of 8 weeks of resistance training with testosterone enanthate consumption on tissue oxidation indicators in male rats.

Methods: In the current experimental study, 18 rats with an average weight of 250 ± 50 g were divided into 3 groups of 6 animals including: (1) control, (2) training, and (3) training + testosterone. Rats in the training and training + testosterone groups underwent resistance training for 8 weeks and 5 sessions per week. Also, rats in the training + testosterone group received 20 mg/kg of testosterone enanthate by intramuscular injection 3 days a week. The resistance training protocol consisted of climbing a 1-meter ladder 5 days a week (4 sets of 6 with a rest interval of 60 to 90 seconds) in which the weights were 60% of the rats’ body weights in the first week, and 20% of the body weight was added to the weights every week. One-way analysis of variance was used to analyze the research findings (P≤0.05).

Results: Glutathione peroxidase (GPx) level in the training + testosterone group was significantly lower than the control group (P=0.007); however, there were no significant differences in malonaldehyde (MDA) (P=0.64) and superoxide dismutase (SOD) (P=0.29) levels between the research groups.

Conclusion: It seems that testosterone consumption can be effective in increasing oxidative stress factors in the kidney tissue of resistance-trained rats.

Keywords: Oxidative stress, Testosterone enanthate, Resistance training, Kidney.

Introduction

Anabolic androgenic steroids (AAS) are testosterone-originated compounds that exhibit anabolic or anabolic effects depending on the target tissue.1 Testosterone, often referred to as the father of steroids, was first extracted in 1935 by Buttenet et al, and after that, the use of this hormone without legal prohibition became common for the treatment of specific diseases and for general and specialized strengthening. Its medicinal type, called testosterone enanthate, is a natural androgenic hormone which is widely used by athletes.2 It has been reported that 87% of these performance-enhancing drugs among men and women are testosterone enanthate and testosterone propionate, which are used by 6.4% of men and 1.6% of women.3 The use of AAS in medicine is limited, and it is only used with great caution in some disorders such as stunted growth, osteoporosis, hypogonadism and anemia, infertility, kidney disease, aplastic anemia, osteoporosis, AIDS and some cancers. AAS drugs are typically employed in higher doses and for longer periods among athletes, e.g., weightlifters, swimmers and bodybuilders to enhance muscle mass, physical function and strength.4 Such implementations can lead to side effects on various organs, including kidneys.5,6 Kantarci et al5 showed an escalation in renal volume, renal cortical thickness and blood urea nitrogen (BUN) in the groups...
using AAS compared to non-AAS groups. These results were also reported by Frati et al. AAS abuse can alter renal morphology (as shown by renal hypertrophy) and also increase oxidative stress, and collagen deposition.\(^6\,^8\) Oxidative stress is an important parameter that can damage organ, and this metabolic disorder is characterized by either a decline in the antioxidant enzyme system and an increment in reactive oxygen species.\(^7\) Oxidative stress can be driven by the humoral system controlling blood pressure, especially by the renin-angiotensin system.\(^10\) Research by de Lima et al\(^11\) showed that nandrolone consumption caused renal hypertrophy, which was associated with decreased organ function and increased collagen deposition.

Increased oxidative stress is followed by decreased superoxide dismutase (SOD) activity; also, nandrolone consumption in rats due to increased oxidative stress in renal tissue, leads to renal damage and renal fibrosis. Exercise has been reported to play an important role in the pharmacokinetics of drugs. Exercise and physical activity increase blood circulation in the liver and accelerate biotransformation and excretion of substances by kidneys.\(^12\) Ahmadi\(z\)adeh et al showed that regular exercise reduced the toxic effects of morphine on the liver and kidneys.\(^13\) Since regular exercise can possibly reduce the toxic effects of drugs, the role of exercise in reducing drug side effects is significant.\(^14\)

Research has shown that resistance training can counteract the increase in oxidative stress as a result of increased levels of free radicals.\(^15\) On the other hand, it has been observed that a training session, depending on its duration and severity, can cause different intensities of oxidative damage.\(^16\) Some reports suggest that in response to resistance training, free radicals and reactive oxygen species (ROS) are increased during training sessions.\(^17\) Riezzo et al\(^18\) showed an escalation in oxidative stress and a diminution in the ability to inhibit radicals in the kidney tissue of the steroid group along with strenuous exercise. Many athletes use high amounts of steroid use.\(^19\) There is little research on changes in renal oxidative enzymes due to the use of this steroid along with high-intensity resistance training. However, research has shown improvements in the function of antioxidant defense enzymes and overall improvement in oxidative stress due to resistance training.\(^20\) Therefore, this study aimed to examine the effect of eight weeks of resistance training alone and in combination with testosterone enanthate consumption on oxidative indicators of the kidney tissue in male rats.

Materials and Methods

In the present study, 18 eight-week male rats with an average weight of 250 ± 30 g were prepared from the Pasteur Institute (Iran) and transferred to the Sports Physiology Laboratory of Zanjan University. Rats were then maintained in a standard environment for 8 days with a 12-hour light/dark cycle, a temperature of 22±2°C, and a humidity of 45±5% for adapting to the new conditions. The rats were randomly categorized into three groups, including: (1) control (n= 6), (2) training (n= 6), and (3) training + testosterone (n= 6). Rats in the training and training + testosterone groups underwent resistance training for eight weeks and 5 sessions per week. Also, rats in the third group received 20 mg/kg of intramuscular testosterone enanthate (manufactured by Iran Hormone Company, Iran, the batch number of 0069) three days a week.\(^20\)

Training Protocol

In the present study, the resistance training protocol comprised 5 days of training per week (4 sets of 6 with a rest interval of 60 to 90 seconds) in the form of climbing a 1-meter ladder with 26 steps and tying weights to the tails of rats in which weights were 60% of the rats' body weights in the first week, and 20% of body weight was added to the weights every week; also, 2 sessions of weighing were carried out per week.\(^21\)

Biochemical Measurements

Forty-eight hours after the last training session and testosterone consumption, rats were anesthetized with xylazine and ketamine, and kidney tissue was extracted and transferred to the laboratory for measuring the variables. SOD activity was determined using the Randox Laboratories Ltd, UK kit, according to the manufacturer’s method.\(^22\) Glutathione peroxidase (GPx) activity was evaluated via the Randox Laboratories Ltd kit, the UK, according to Paglia and Valentine methods,\(^23\) and malonaldehyde (MDA) activity was measured via Mihara and Uchiyama method based on the manufacturer's protocol.\(^24\)

Statistical Analyses

For analyzing the findings, the Shapiro-Wilk procedure, one-way analysis of variance, and Tukey’s post hoc statistical test were employed (P≤0.05).

Results

GPx, MDA and SOD protein levels have been shown in Figures 1, 2, and 3, respectively. The results of the Shapiro-Wilk test showed that the data distribution of GPx, MDA, and SOD in the three groups of research was normal (P≤0.05). The results of one-way ANOVA showed that there was a significant difference in GPx levels between the research groups (Figure 1). However, there was no significant difference in MDA levels (Figure 2) as the P and F values confirmed these results (P=0.64). The SOD levels did not show a significant difference as P and F values (Figure 3, P=0.29). The results of Tukey’s post hoc test showed that GPx levels in the training + testosterone group was significantly lower compared to the control group (P=0.007).
Discussion

The results revealed that eight weeks of resistance training had no significant effect on the levels of SOD, MDA and GPx in kidney tissue. It has been reported that high intensity resistance activity can increase oxidative stress and impair cell redox status (oxidation-reduction). It has also been exhibited that oxidative stress during higher intensity activity is greater as compared to low-intensity resistance activity. Although the exact mechanism of ROS production and oxidative stress after resistance training is not known, possible mechanisms responsible for ROS formation due to resistance training could be xanthine oxidase pathway, respiratory burst of neutrophils, catecholamine autooxidation, localized muscle ischemia, conversion of weak superoxide to strong hydroxyl radical by lactic acid and altered calcium homeostasis. On the other hand, it has been shown that regular and continuous exercise declines lipid and protein peroxidation by enhancing antioxidant defense.

The results of the studies conducted by Venoojarvia et al. and Park & Kwak are similar to the results of the present study. Park and Kwak measured the effects of aerobic and anaerobic training on three groups of endurance athletes, resistance athletes, and non-trained individuals, in which no change in oxidative stress status and antioxidant capacity was observed, which could be attributed to redox/resuscitation balance. On the other hand, recent findings show that resistance training has different effects on oxidative stress. In contrast to the present findings, Ezabadi et al. showed that 4 weeks of resistance training leads to a significant increase in SOD and CAT levels as well as a significant decrease in the cerebral MDA of diazinon-poisoned rats, which is contradictory with the results of this study. The main reasons for these contradictory results are differences in the antioxidant capacity of the investigated tissue, differences in tissue sampling time, differences in the type of resistance training protocols and intensity and duration of exercise, as well as laboratory methods of measuring the desired concentrations.

The reason for the lack of change in the activity of antioxidant enzymes in this study can be explained by several possible factors. The intensity and duration of the exercise may not have been such that it increased the production of reactive oxygen species in the kidney tissue.

On the other hand, it is possible that normal levels of renal antioxidant enzymes are responsive to the radicals produced following this type of exercise, which may be a good justification for not altering the activity of these enzymes. Regarding interactive effects, the results of the present study showed that GPx levels in the training and testosterone group were significantly lower than the control group but there was no significant difference in MDA and SOD levels between the study groups.

Similar to the results of the present study, Riezzo et al. showed that the use of anabolic steroids along with high intensity training reduces the enzymatic activity of GR and the antioxidant GPx and thus reduces the ability of the kidneys to inhibit radicals. Also, the findings of this study are consistent with the findings of Kara et al. who showed that the use of anabolic steroids increases catalase, while the parameters of MDA, GSH, SOD did not change significantly. Research by de Lima et al. showed that nandrolone consumption increased oxidative stress in both proteins and lipids and subsequently decreased SOD.
activity. Oxidative stress is an important parameter that increases organ damage, and this metabolic disorder is characterized by either an increase in reactive oxygen species or a decrease in the antioxidant enzyme system. Decreased antioxidant enzymes, due to their high use to reduce ROS, may change the redox status to oxidative stress, and the decrease in GPx antioxidant activity in this study is probably due to the response to these conditions. Anabolic steroids may have a direct toxic effect on glomerular cells, leading to the accumulation of the mesangial matrix and the depletion of podocytes, which are independent of structural-functional adaptations.

The results of the previous studies have shown that testosterone can cause oxidative stress directly and also indirectly by activating the renin-angiotensin-aldosterone system and endothelin (by regulating NADPH oxidase). It can be mentioned that the measuring of the variables by western blotting and real-time polymerase chain reaction (PCR) methods, and also studying the effects of resistance training and testosterone consumption on liver and heart tissues can be interesting issues in the future research.

Conclusion
In our study, there were no significant differences in MDA and SOD levels between the research groups while GPx levels revealed a significant difference in the training + testosterone group in comparison to the control group. In addition, it appears that testosterone consumption can be effective in increasing anti-oxidative stress factors in the kidney tissue of resistance trained rats.

Ethical Approval
The study was approved by the Ethical Committee of Tehran Islamic Azad University of Medical Sciences (IR. IAU.TMU.REC.1399.347).

Conflict of Interest Disclosure
The authors have no conflict of interest to declare.

Authors’ Contributions
All authors had equal contribution to the writing and revision of this paper.

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