



# The Effect of Eight Weeks of Resistance Training and Testosterone Enanthate Consumption on TNF- $\alpha$ and IL-6 Genes' Expression in the Kidney Tissue of Female Wistar Rats

Khadijeh Molaei<sup>1</sup>, Sanaz Mirzayan Shanjani<sup>2, \*</sup>, Ali Gorzi<sup>3</sup>, Yaser Kazemzadeh<sup>4</sup> and Abdolali Banaeifar

<sup>5</sup>

<sup>1</sup>Ph.D. Student, Department of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

<sup>2</sup>Assistant Professor, Department of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

<sup>3</sup>Associate Professor, Department of Sport Sciences, University of Zanjan, Zanjan, Iran

<sup>4</sup>Assistant Professor, Graduate of Kharazmi University, Tehran, Iran

<sup>5</sup>Associate Professor, Department of Exercise Physiology, South Tehran Branch, Islamic Azad University, Tehran, Iran

\*Corresponding author: Assistant Professor, Department of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran. Email: san\_mir2000@yahoo.com

Received 2022 May 25; Revised 2022 August 31; Accepted 2022 September 05.

## Abstract

**Objectives:** This study aimed to evaluate the effect of testosterone enanthate anabolic steroid on the gene expression of inflammatory factors in the kidney tissue of female rats following eight weeks of resistance training.

**Methods:** Twenty-one female Wistar rats weighing  $200 \pm 20$  g, aged eight weeks, were prepared from the Iran Pasture Institute. Following a week for acclimatization, the rats were randomly divided into three groups: control ( $n = 7$ ), training + placebo ( $n = 6$ ), and training + testosterone enanthate ( $n = 8$ ). Resistance training included five days a week of climbing a 1-meter ladder with 26 stairs while bearing weights and two days of rest. In the first week, the weights were equal to 40% of the rats' body weight. In the following weeks, the weight was increased by 20% per week. The rats were weighed two times a week. In the testosterone group, the rats were injected with 20 mg of testosterone enanthate thrice a week. Forty-eight hours following the last training session and testosterone injection, kidney tissue samples were collected to determine the gene expression of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) using real-time PCR.

**Results:** The results showed that testosterone consumption significantly increased the gene expression of TNF- $\alpha$  and IL-6 in female rats undergoing testosterone and resistance training ( $P = 0.001$ ).

**Conclusions:** The findings of this study demonstrated that using testosterone increased inflammatory factors in the kidney tissue of female Wistar rats.

**Keywords:** Resistance Training, Testosterone Enanthate, Kidney Tissue, Inflammatory Factors

## 1. Background

Anabolic steroid hormones are often used to cure various diseases (1). Testosterone, known as the ancestor of most steroids, was first discovered by Adolf Butenandt et al., and since then, this hormone has been applied without any legal limitation to treat specific diseases and to strengthen general and particular body muscles (2). Moreover, athletes sometimes abuse this hormone to improve their performance (3). Statistics show that 67% of athletes in the USA had used anabolic-androgenic steroids in 2007 (4). Improper consumption or abuse of these medicines causes long-term and irrecoverable damage to some vital body organs like the heart, liver, brain, and

kidneys (5). Surprisingly, a survey in 2006 showed that only 38% of interviewed athletes were aware of the side effects of anabolic steroids (6). The medicinal type of anabolic steroids, known as testosterone enanthate, is a natural androgen hormone, and in recent years, its consumption has been widely increased among athlete women working in strength sports (7). This hormone affects the cell nucleus through complicated interactions, spreads in a cell due to its solvability in lipids, enters the cell nucleus in combination with proteins, and regulates the production of several proteins (8).

In the past, anabolic-androgenic steroids had been prescribed to cure anemia in patients suffering from chronic kidney disease (CKD). However, their dosage

to cure anemia was much less than their dosage in doping (9). Prescribing androgens to cure anemia in patients with CKD was stopped considering its numerous destructive effects on kidneys (10). Studies show that steroid abuse is closely related to kidney failure (11). Daher et al. demonstrated that the long-term consumption of high-dose anabolic-androgenic steroids led to renal damage, nephrotic inflammation, and tubular necrosis (12). In addition, Almkhatar et al. reported that the kidney tissues of four bodybuilders who used more than 400 mg of testosterone propionate in a week showed tubular necrosis and increased kidney inflammation (13). Apoptosis, an influential factor in developing kidney fibrosis, can be triggered by various inflammatory cytokines (14). Androgens also play an essential role in the induction of apoptosis. For example, androgens can induce kidney cell apoptosis by inducing a caspase-dependent apoptotic pathway (15). Also, testosterone can induce the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), leading to renal inflammation and CKD progression (16).

A study by Castaneda et al. clarified that moderate resistance training reduced the levels of inflammatory factors in the kidney tissue (17). Professional bodybuilders and powerlifters utilize androgenic-anabolic steroids to increase their performance and muscle mass and decrease body fat (18). Using androgenic-anabolic steroids has increasingly propagated among bodybuilders and non-competitive athletes who seek increasing muscle mass (19). Androgenic-anabolic steroids in females may lead to menstrual irregularity, clitoris hypertrophy, and uterus and breast atrophy (20). Moreover, despite the popularity of testosterone enanthate consumption among women, no study has yet evaluated changes in the inflammatory factors in the kidney tissue following testosterone enanthate consumption.

## 2. Objectives

Here we assessed if eight weeks of resistance exercise in combination with testosterone enanthate injection could influence inflammatory factors in the renal tissue of female Wistar rats.

## 3. Methods

### 3.1. Animals and Research Design

This experimental study was conducted on 21 female Wistar rats aged eight weeks on average and weighed 200  $\pm$  20 g. The rats were randomly divided into three groups:

control (n = 7), training + placebo (olive oil) (n = 6), and training + testosterone enanthate (n = 8). The rats were placed in polycarbonate cages (four animals per cage) and kept at a 12-hour dark/12-hour light cycle, 22  $\pm$  2°C, and 45%  $\pm$  5% humidity. The rats were provided with water and food ad libitum.

### 3.2. Ethical Considerations

In this study, all ethical principles published by the National Institute of Health (NIH) on working procedures with animals (e.g., food and water accessibility, suitable care conditions, imposing no obligation to do the resistance training, and being sure of animal anesthesia before autopsy) were closely watched. The exercise would be stopped during resistance training if the animal slipped while climbing the ladder with weights.

### 3.3. Research Protocol

The 8-week resistance training protocol included climbing a 1-meter ladder with 26 steps at a steep of 85° with weights being carried on the rats' tails. The protocol consisted of five days of training and two days of rest in a week (each session consisted of four sets of six repetitions with 60 to 90 seconds of rest). The weights were increased during the study. In the first week, the load weight was equal to 40% of the rats' weight and was increased by 20% per week till the fifth week. In the fifth week, the weight was reduced by 20%, but in the sixth week, it was increased by 40% of the rats' weight. In the final two weeks, the load weight was increased by 20% of the rats' weight per week (21). The rats were weighed two times a week (Table 1). The rats in the testosterone enanthate group were injected with 20 mg of testosterone enanthate three times a week (21).

### 3.4. Laboratory and Statistical Methods

At the end of the 8-week procedure, the animals were fasted of food and water for 12 to 14 hours. Tissue samples were collected 48 hours following the last training session and testosterone injection. Anesthesia was performed using a combination of ketamine (30 - 50 mg/kg) and xylazine (3 - 5 mg/kg) through intraperitoneal injection. After 40 to 50 seconds of administration, the animals were in a suitable anesthesia condition. According to a predefined timing, renal tissue sampling was performed in all three groups (i.e., control, training + placebo, and training + testosterone enanthate). After being washed with physiologic serum, the samples were immediately placed into special tubes. The tissues obtained were immediately frozen in liquid nitrogen (-196°C) and then kept at -80°C in the laboratory until further evaluation.

**Table 1.** Resistance Training Protocol

Groups	Time							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Intensity (%)	40	60	80	100	80	120	140	160
Repetition	6	4	5	6	4	4	5	6
Sets	1	2	2	2	2	4	4	4
Rest between repetitions (s)	15	15	30	30	15	30	30	30
Rest between sets (s)	0	60	90	90	60	100	120	120

### 3.5. Gene Expression Analysis

The gene expression of IL-6 and TNF- $\alpha$  in the kidney tissue was determined using real-time PCR. For this purpose, an RNA extraction kit (Cat. No., DQ383-40h), a cDNA synthesis kit (Cat. No. BR441-096, BioFACT TM, 5X RT Pre-Mix), and 2X Real-Time PCR Master Mix<sup>TM</sup> (BioFACT<sup>®</sup> h) at a final concentration of 2.5 mM for MgCl<sub>2</sub>, including SYBR Green I and w/o Rox reference dye, were used.

### 3.6. Statistical Analysis

To analyze the data, in addition to descriptive statistics (mean  $\pm$  SD) to present the variables, the Shapiro-Wilk test was used to assess the normality of data distribution, after which the Leven test at a  $P < 0.05$  significance level was applied to examine variance homogeneity. Then one-way ANOVA and the Bonferroni post-hoc test were utilized for intergroup and pair-wise comparisons, respectively. All statistical procedures were performed in SPSS software, version 20. Graphs were drawn using the GraphPad Prism (9.4.0.673) software.

## 4. Results

Table 2 represents the mean and standard deviation of TNF- $\alpha$  and IL-6 levels in female rats in the three study groups (i.e., control, training + placebo, and training + testosterone enanthate), along with the results of comparing groups using the ANOVA and Bonferroni tests.

**Table 2.** Mean  $\pm$  SD of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) and Interleukin-6 (IL-6) Levels<sup>a</sup>

Groups	N	TNF- $\alpha$ ( $\times 10^{-4}$ ) <sup>b</sup>	IL-6 ( $\times 10^{-3}$ ) <sup>b</sup>
Control	7	1.95 $\pm$ 0.16 <sup>c</sup>	2.16 $\pm$ 0.55 <sup>c</sup>
Training + placebo	6	1.40 $\pm$ 0.13 <sup>d</sup>	1.50 $\pm$ 0.26 <sup>d</sup>
Training + testosterone	8	4.14 $\pm$ 0.81 <sup>c, d</sup>	5.10 $\pm$ 0.41 <sup>c, d</sup>

<sup>a</sup> Values are expressed as mean  $\pm$  SD.

<sup>b</sup>  $P < 0.0001$ , significant difference between the three groups (one-way ANOVA).

<sup>c</sup>  $P < 0.0001$ , training + testosterone versus control (Bonferroni post-hoc test).

<sup>d</sup>  $P < 0.0001$ , training + testosterone versus training + placebo (Bonferroni post-hoc test).

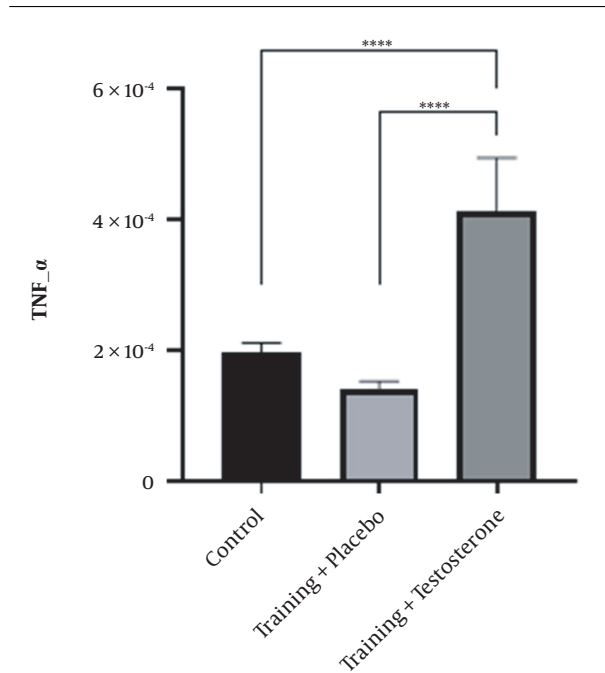
### 4.1. Effect of Testosterone Enanthate Plus Resistance Training on TNF- $\alpha$ and IL-6 Genes' Expression Levels in the Kidney Tissue of Female Wistar Rats

The ANOVA test clarified that treatment with testosterone enanthate led to significant changes in TNF- $\alpha$  ( $F(2, 18) = 57.093$ ,  $P < 0.0001$ ) and IL-6 ( $F(2, 18) = 29.240$ ,  $P < 0.0001$ ) levels in the kidney tissue of female rats. Paired comparison using the Bonferroni post-hoc test revealed that the mean differences in TNF- $\alpha$  (mean difference<sub>(j-i)</sub> = -0.56E-4,  $P = 0.205$ ) and IL-6 (mean difference<sub>(j-i)</sub> = -0.65E-3,  $P = 0.693$ ) levels were small and insignificant between the control and training + placebo groups. However, the mean difference in renal TNF- $\alpha$  level was considerable and statistically significant between the training + testosterone and control groups (mean difference<sub>(j-i)</sub> = 2.18E-4,  $P < 0.001$ ), as well as between the training + testosterone and training + placebo groups (mean difference<sub>(j-i)</sub> = 2.74E-4,  $P < 0.001$ ) (Figure 1). Likewise, the mean differences in renal IL-6 levels between the training + testosterone and control groups (mean difference<sub>(j-i)</sub> = 2.91E-3,  $P < 0.001$ ), as well as between the training + testosterone and training + placebo groups (mean difference<sub>(j-i)</sub> = 3.56E-3,  $P < 0.001$ ) were remarkable and statistically significant (Figure 2).

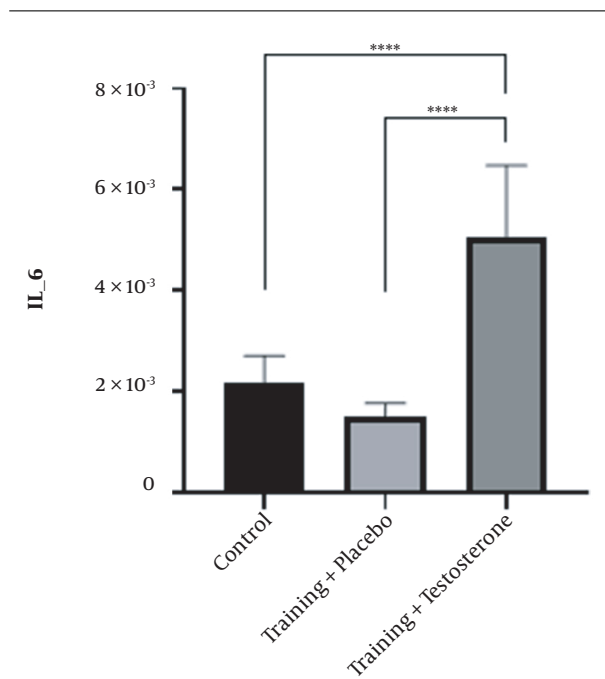
In summary, it can be concluded that the supplementation of testosterone led to a significant increase in the levels of inflammatory factors in rats compared to control and placebo groups. Additionally, performing resistance exercises by the placebo group did not have a significant impact on the levels of inflammatory factors compared to the control group.

## 5. Discussion

After eight weeks of resistance training and testosterone enanthate injection, IL-6 and TNF- $\alpha$  were measured in rats' kidney tissues. The results demonstrated that resistance training alone led to an insignificant reduction in the inflammatory cytokines assessed while consuming testosterone, either alone or



**Figure 1.** Tumor necrosis factor-alpha (TNF- $\alpha$ ) levels in different studied groups



**Figure 2.** Interleukin-6 (IL-6) levels in different studied groups

in combination with resistance training, significantly increased the levels of IL-6 and TNF- $\alpha$  inflammatory factors. Castaneda et al. showed that 12 weeks of moderate resistance training reduced IL-6 levels in patients suffering from CKD (17). As an influential factor, resistance training can direct inflammatory factors toward the kidney tissue (15). We also observed that although resistance training could reduce renal IL-6 and TNF- $\alpha$ , the decline was not significant, which can be attributed to the differences among the subjects. The level of primary inflammation differs between healthy and functionally impaired kidneys due to chronic diseases, justifying the reduction observed in the levels of inflammatory factors (22).

In the present study, a significant increase in IL-6 and TNF- $\alpha$  was observed in the rats simultaneously receiving testosterone supplementation and resistance training. Our results in this study were consistent with that of Liborio et al. and Daher et al. (12, 22). As an explanation, it can be said that testosterone may have a role in inducing the production of inflammatory cytokines such as IL-6, TNF- $\alpha$ , and IL-1 (16, 17). The overexpression of TNF- $\alpha$  leads to its binding to the receptor type 1, causing an inflammatory response and cell proliferation. In this process, NF- $\kappa$ b is activated and induces the transcription of the genes involved in cellular survival and proliferation. In order for NF- $\kappa$ b to be activated, after the binding of TNF- $\alpha$  to the receptor type 1, TNF-receptor-associated factor 2 (TRAF-2), which is associated with the TNF- $\alpha$  receptor, binds to TRADD, followed by the recruitment of receptor-interacting protein (RIP) to the complex. Finally, both RIP and TRAF-2 contribute to the activation of the inhibitor of nuclear factor- $\kappa$ B (I $\kappa$ B) kinases. These kinases phosphorylate I $\kappa$ B and release NF- $\kappa$ b, and NF- $\kappa$ b triggers the transcription of the genes involved in inflammation, such as endothelial adhesion molecules, IL-6, and other inflammatory mediators. So, TNF- $\alpha$  invigorates its expression by activating NF- $\kappa$ b, and these cytokines lead to renal inflammation and the progression of CKDs (23). In addition, inflammatory cytokines like TNF- $\alpha$  and IL-6 can augment the activity of androgen receptors (12). Metcalfe et al. demonstrated that in male rats, TNF- $\alpha$  increased proptosis, profibrotic signaling, tubular interstitial fibrosis, and kidney dysfunction, accompanied by endogenous testosterone production in female rats, which could be alleviated by the administration of exogenous testosterone (11).

Our results were inconsistent those of Mohamad et al. (24), which may be due to differences in testosterone dosage and subjects' characteristics. Patil et al. conducted a study on male rats and reported that a lower dose of testosterone protected kidneys against ischemia-reperfusion injury; however, a higher dosage

induced the overexpression of TNF- $\alpha$  in the kidney, negating a renal protective effect (25). Therefore, TNF- $\alpha$  is a critical cytokine involved in ASS-related renal injury (25). Loh et al. declared that the increased expression of aquaporins 1 and 7 in the complex proximal tubule and aquaporins 2, 4, and 6 in collecting ducts caused a testosterone-dependent elevation of the arterial pressure in rats (26). These findings indicated that ASS could elevate water reabsorption and, subsequently, blood pressure in patients with CKD (24).

Another critical issue that should be considered in relation to the inflammatory responses associated with testosterone is the difference between males and females in their reactions to hormonal mediators (24). Zhao and Schooling reported that testosterone consumption caused more kidney injuries and related diseases in women than in men, while consuming a suitable dose of testosterone may even avoid CKDs in men (27). The exact mechanism of this phenomenon is unknown, but it may be attributed to the dominant role of testosterone in men's reproduction and its compatibility with male physiology.

### 5.1. Conclusions

According to our results and former studies, enanthate steroids can be an essential factor in inducing renal inflammation, evidenced by kidney damage observed in animal models and even bodybuilders consuming steroids. Animal cells can be suitable replacements for expressing metabolic characteristics in humans. Therefore, experiments in female rats can provide valuable information with regard to human beings. As a result, women athletes must be aware of the harmful and sometimes unrecoverable effects of testosterone enanthate, which is consumed to increase performance, gain weight, and build muscles.

### Acknowledgments

This article was a result of a research project approved under the ethics code of IR.IAU.TMU.REC.1399.347. We appreciate all those who helped us in this study.

### Footnotes

**Authors' Contribution:** Ali Gorzi and Khadijeh Molaei designed the study and drafted the manuscript. Sanaz Mirzayan Shanjani participated in the study design, conducted parts of statistical analysis, and helped draft the manuscript. Khadijeh Molaei collected, re-evaluated, and interpreted the clinical data, revised the manuscript, and performed the statistical analysis. Yaser Kazemzadeh

and Abdolali Banaeifar re-analyzed the clinical and revised the manuscript. All authors read and approved the final manuscript.

**Conflict of Interests:** The authors declare no conflict of interests.

**Ethical Approval:** IR.IAU.TMU.REC.1399.347.

**Funding/Support:** No grant was received for this study.

### References

- Lundholm L, Kall K, Wallin S, Thiblin I. Use of anabolic androgenic steroids in substance abusers arrested for crime. *Drug Alcohol Depend.* 2010;111(3):222-6. [PubMed ID: 20627426]. <https://doi.org/10.1016/j.drugalcdep.2010.04.020>.
- Nieschlag E, Nieschlag S. The medical and cultural history of testosterone and the testes. In: Nieschlag E, Behre HM, editors. *Testosterone*. Cambridge, England: Cambridge University Press; 2012. p. 1-14. <https://doi.org/10.1017/cbo9781139003353.002>.
- Borjesson A, Ekebergh M, Dahl ML, Ekstrom L, Lehtihet M, Vicente V. Women's Experiences of Using Anabolic Androgenic Steroids. *Front Sports Act Living.* 2021;3:656413. [PubMed ID: 34859201]. [PubMed Central ID: PMC8632252]. <https://doi.org/10.3389/fspor.2021.656413>.
- Fortunato RS, Rosenthal D, Carvalho DP. [Abuse of anabolic steroids and its impact on thyroid function]. *Arq Bras Endocrinol Metabol.* 2007;51(9):1417-24. Portuguese. [PubMed ID: 18209883]. <https://doi.org/10.1590/s0004-27302007000900003>.
- Herbert ME, Roy RR, Edgerton VR. Influence of one-week hindlimb suspension and intermittent high load exercise on rat muscles. *Exp Neurol.* 1988;102(2):190-8. [PubMed ID: 3181357]. [https://doi.org/10.1016/0014-4886\(88\)90093-3](https://doi.org/10.1016/0014-4886(88)90093-3).
- Sjoqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. *Lancet.* 2008;371(9627):1872-82. [PubMed ID: 18514731]. [https://doi.org/10.1016/S0140-6736\(08\)60801-6](https://doi.org/10.1016/S0140-6736(08)60801-6).
- Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2010;95(6):2536-59. [PubMed ID: 20525905]. <https://doi.org/10.1210/jc.2009-2354>.
- Khajevand N. [Anabolic steroids and nutritional supplements in sports]. Tehran, Iran: Bamdad Ketab Publication; 2011. Persian.
- Schaefer F, Mehls O, Ritz E. New insights into endocrine disturbances of chronic renal failure. *Miner Electrolyte Metab.* 1992;18(2-5):169-73. [PubMed ID: 1465053].
- Dousdampanis P, Trigka K, Fourtounas C, Bargman JM. Role of testosterone in the pathogenesis, progression, prognosis and comorbidity of men with chronic kidney disease. *Ther Apher Dial.* 2014;18(3):220-30. [PubMed ID: 24119223]. <https://doi.org/10.1111/1744-9987.12101>.
- Metcalfe PD, Leslie JA, Campbell MT, Meldrum DR, Hile KL, Meldrum KK. Testosterone exacerbates obstructive renal injury by stimulating TNF-alpha production and increasing proapoptotic and profibrotic signaling. *Am J Physiol Endocrinol Metab.* 2008;294(2):E435-43. [PubMed ID: 18073317]. <https://doi.org/10.1152/ajpendo.00704.2006>.
- Daher EF, Silva Junior GB, Queiroz AL, Ramos LM, Santos SQ, Barreto DM, et al. Acute kidney injury due to anabolic steroid and vitamin supplement abuse: report of two cases and a literature review. *Int Urol Nephrol.* 2009;41(3):717-23. [PubMed ID: 19387860]. <https://doi.org/10.1007/s11255-009-9571-8>.
- Almukhtar SE, Abbas AA, Muhealdeen DN, Hughson MD. Acute kidney injury associated with androgenic steroids and nutritional supplements in bodybuilders(dagger). *Clin Kidney J.* 2015;8(4):415-9.



- [PubMed ID: 26251708]. [PubMed Central ID: PMC4515889]. <https://doi.org/10.1093/ckj/sfv032>.
14. Verzola D, Villaggio B, Procopio V, Gandolfo MT, Gianiorio F, Fama A, et al. Androgen-mediated apoptosis of kidney tubule cells: role of c-jun amino terminal kinase. *Biochem Biophys Res Commun*. 2009;**387**(3):531-6. [PubMed ID: 19615976]. <https://doi.org/10.1016/j.bbrc.2009.07.056>.
  15. D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, et al. Sex hormones modulate inflammatory mediators produced by macrophages. *Ann NY Acad Sci*. 1999;**876**:426-9. [PubMed ID: 10415638]. <https://doi.org/10.1111/j.1749-6632.1999.tb07667.x>.
  16. Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. *Clin Exp Rheumatol*. 1993;**11**(2):157-62. [PubMed ID: 8508557].
  17. Castaneda C, Gordon PL, Parker RC, Uhlin KL, Roubenoff R, Levey AS. Resistance training to reduce the malnutrition-inflammation complex syndrome of chronic kidney disease. *Am J Kidney Dis*. 2004;**43**(4):607-16. [PubMed ID: 15042537]. <https://doi.org/10.1053/j.ajkd.2003.12.025>.
  18. Tidmas V, Brazier J, Hawkins J, Forbes SC, Bottoms L, Farrington K. Nutritional and Non-Nutritional Strategies in Bodybuilding: Impact on Kidney Function. *Int J Environ Res Public Health*. 2022;**19**(7). [PubMed ID: 35409969]. [PubMed Central ID: PMC8998289]. <https://doi.org/10.3390/ijerph19074288>.
  19. Hartgens F, Kuipers H. Effects of androgenic-anabolic steroids in athletes. *Sports Med*. 2004;**34**(8):513-54. [PubMed ID: 15248788]. <https://doi.org/10.2165/00007256-200434080-00003>.
  20. Turillazzi E, Perilli G, Di Paolo M, Neri M, Riezzo I, Fineschi V. Side effects of AAS abuse: an overview. *Mini Rev Med Chem*. 2011;**11**(5):374-89. [PubMed ID: 21443513]. <https://doi.org/10.2174/138955711795445925>.
  21. Gorzi A, Rajabi H, Qarakhanlu R, Dehkhoda M, Hedayati M. [The effect of eight weeks of resistance training on total acetylcholinesterase activity and type A12 in the horseshoe muscles of rats]. *Research in Sports Medicine and Technology*. 2018;**(13)**:7. Persian.
  22. Liborio AB, Nasseralla JC, Gondim AS, Daher EF. The case: renal failure in a bodybuilder athlete. Diagnosis: Nephrocalcinosis secondary to exogenous vitamin D intoxication. *Kidney Int*. 2014;**85**(5):1247-8. [PubMed ID: 24786891]. <https://doi.org/10.1038/ki.2013.230>.
  23. van Horssen R, Ten Hagen TL, Eggermont AM. TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. *Oncologist*. 2006;**11**(4):397-408. [PubMed ID: 16614236]. <https://doi.org/10.1634/theoncologist.11-4-397>.
  24. Mohamad NV, Wong SK, Wan Hasan WN, Jolly JJ, Nur-Farhana MF, Ima-Nirwana S, et al. The relationship between circulating testosterone and inflammatory cytokines in men. *Aging Male*. 2019;**22**(2):129-40. [PubMed ID: 29925283]. <https://doi.org/10.1080/13685538.2018.1482487>.
  25. Patil CN, Wallace K, LaMarca BD, Moulana M, Lopez-Ruiz A, Soljancic A, et al. Low-dose testosterone protects against renal ischemia-reperfusion injury by increasing renal IL-10-to-TNF-alpha ratio and attenuating T-cell infiltration. *Am J Physiol Renal Physiol*. 2016;**311**(2):F395-403. [PubMed ID: 27252490]. [PubMed Central ID: PMC5008676]. <https://doi.org/10.1152/ajprenal.00454.2015>.
  26. Loh SY, Giribabu N, Salleh N. Effects of gonadectomy and testosterone treatment on aquaporin expression in the kidney of normotensive and hypertensive rats. *Exp Biol Med (Maywood)*. 2017;**242**(13):1376-86. [PubMed ID: 28399644]. [PubMed Central ID: PMC5529000]. <https://doi.org/10.1177/1535370217703360>.
  27. Zhao J, Schooling CM. The role of testosterone in chronic kidney disease and kidney function in men and women: a bi-directional Mendelian randomization study in the UK Biobank. *BMC Med*. 2020;**18**(1):122. [PubMed ID: 32493397]. [PubMed Central ID: PMC7271464]. <https://doi.org/10.1186/s12916-020-01594-x>.