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Exogenous Testosterone, Finasteride and Castration Effects on Testosterone, Insulin, Zinc and Chromium in Adult Male Rats

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ABSTRACT

Background: Although effects of trace elements on secretion of sex steroids and insulin have been studied, the effects of these hormones on serum level of trace elements have been rarely investigated. The aim of the present study was to evaluate the effect of testosterone and finasteride administration and castration on serum levels of testosterone, insulin, zinc and chromium. **Methods:** Male adult rats (n = 32) were divided into 4 groups (n = 8). Group 1, control; Group 2, castration, castration was done at the first day of the study; Group 3, finasteride (20 mg/kg/day, dissolved in drinking water) and Group 4, testosterone (5 mg/kg/day, i.p.). At the end of the period of the study (35 days), serum testosterone, insulin, zinc and chromium levels were determined in the blood samples collected directly from the right atrium of the heart of the animals. **Results:** The data indicated that the serum levels of testosterone, insulin and zinc were significantly increased ($P<0.01$) in testosterone-administrated and finasteride groups, but the level of chromium was decreased in both groups ($P<0.01$). Castrated group had the lowest serum levels of testosterone, insulin and zinc ($P<0.05$). Also, the levels of serum chromium in this group were increased. **Conclusion:** The study demonstrates that testosterone and finasteride increases insulin and zinc levels and decreases chromium levels in the serum of male adult rats. According to these data, it seems that testosterone may affect glucose cycle through effect on serum insulin levels and trace elements such as zinc and chromium. *Iran. Biomed. J. 17 (1): 49-53, 2013*

Keywords: Finasteride, Castration, Insulin, Zinc, Chromium

INTRODUCTION

Androgens are steroids, which are secreted primarily from the testes and adrenals [1]. The major gonadal androgen in males is testosterone. Testosterone can be converted in peripheral tissues by 5 alpha-reductase to the more potent androgen, dihydrotestosterone [2]. Finasteride is a synthetic anti-androgen that acts by inhibition of the 5 alpha-reductase enzyme and induces adverse effects on epidydims of testes and also erection of penis. Erection is a complex process which involves important signaling in the brain. It seems that finasteride affects on both central and peripheral neural pathways of erection [3]. Furthermore, some studies demonstrated that finasteride could be effective for the treatment of some central nervous system diseases such as epilepsy and Parkinson [4, 5].

Insulin is the major regulator of glucose homeostasis and studies have shown that testosterone and insulin interact in their actions at the level of target organs [6, 7]. The interaction between testosterone and insulin differs among individuals and depends on the obesity. In fact, testosterone supplements reduce body weight, plasma insulin and cholesterol levels and improve the oral glucose tolerance test only in obese Zucker rats besides the lean ones [8].

Zinc is the only metal that is found in almost all enzyme classes [9]. Presence of high concentration of zinc in testes and accessory sex glands shows that it has an important role in the function of male reproductive system [10]. Zinc also is necessary for the synthesis and secretion of luteinizing hormone and follicle-stimulating hormone, spermatogenesis, testicular steroidogenesis, androgen metabolism and interaction with steroid receptors [11]. The relationship between serum testosterone and plasma zinc concentrations has been reported [12].

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Moreover, it has been shown that zinc deficiency increased apoptotic degeneration in testes may cause irreversible changes in the germ cells associated with decreased epididymal sperm concentration, motility and fertility index, which contributes to the low efficiency of spermatogenesis thereby indicating a possible role of zinc in fertility [13]. Chromium is an essential nutrient that enhances the action of insulin [14], a hormone critical to the metabolism and storage of carbohydrate, fat and a protein in the body [15].

It has been shown the adverse effect of chromium on male reproduction in rats [16]. Furthermore, high dose of chromium is responsible for spermatogenic and steroidogenic impairments in rats [16]. According to literature, the effects of trace elements on secretion of androgens and insulin have been investigated and here in the present study was aimed to show the effects of testosterone, finasteride and castration on trace elements.

MATERIALS AND METHODS

Animals. Male Wistar rats (n = 32), weighing approximately 255.6 ± 5.797 g, were purchased from Pasteur Institute of Iran (Tehran) and maintained in our animal resource center under controlled conditions of lighting (12-h light, 12-h dark cycle) and temperature (22 ± 2°C). The animals were given free access to standard chow and water.

Experimental design. All animals received care as based on the Research Committee of the Razi University. The animals were allocated to 4 groups. Each group consisted of 8 animals. Group 1, control: the animals that received sesame oil (0.2 ml/day, i.p.); group 2, castration: castration was done at the first day of experiment; group 3, finasteride: the animals that received finasteride (20 mg/kg/day, dissolved in the drinking water) and group 4: testosterone: the animals received testosterone dissolved in 0.2 ml sesame oil and were used 5 mg/kg/day (i.p.). Castration was performed under general anesthesia induced by ether and ketamine (20 mg/kg) combination [17]. The scrotum was opened and spermatic cord was legated to remove the testes. Then, the scrotum was sutured. The period of the treatments was 35 days.

Sample collection. At the end of the study, blood samples from all experimental rats were collected directly from the right atrium of the heart [18]. Separation of serum was achieved by centrifugation. The samples were stored in tubes with plastic caps at -70 until analyses.

Biochemical analyses. The levels of zinc and chromium were measured by inductive coupled plasma-optical emission spectroscopy (Perkin Elmer, model 7300, USA). Results were expressed as µg/L. Serum hormones of testosterone and insulin were measured by radio immuno assay and human diagnostic test kit (Immunotech, catalogue no: IM 1119 and IM 1447) using a Gamma counter (Kontron, USA) [19]. Results were expressed as ng/ml for testosterone and µu/ml for insulin.

Statistical analysis. Data were presented as mean ± SE. Multiple comparisons were calculated by one-way ANOVA. For comparing the two groups, statistical student's *t*-test was used. The significance level chosen was $P<0.05$.

RESULTS

The mean serum level of testosterone, insulin, Zinc and chromium in experimental groups was compared statistically with control group (Fig. 1). Serum level of testosterone in testosterone and finasteride-administrated groups were increased ($P<0.01$), whereas in castration group, it decreased significantly ($P<0.05$) (Fig. 1A).

Serum zinc concentrations in testosterone and finasteride-administrated groups were increased ($P<0.01$ and $P<0.05$, respectively), while it was decreased significantly ($P<0.05$) in castrated rats (Fig. 1B). Chromium serum level in testosterone and finasteride-administrated groups was decreased significantly ($P<0.01$ and $P<0.05$, respectively), but in castrated group, it did not show any significant differences (Fig. 1C).

In testosterone-administrated group, there was a significant increase in the serum insulin levels ($P<0.01$), whereas testosterone deficiency due to castration significantly reduced serum levels of this hormone in castrated group ($P<0.05$). Also in finasteride administrated group, serum insulin levels were increased significantly ($P<0.05$) (Fig. 1D).

DISCUSSION

In the present study, serum testosterone level was significantly higher in testosterone and also in finasteride administrated groups. This finding is consistent with the finding of other researchers [20-22].

Increased serum testosterone in testosterone group shows that extra amounts of testosterone have high affinity to bind to albomin or sex hormone-binding globulin to turn over through the blood. The increased

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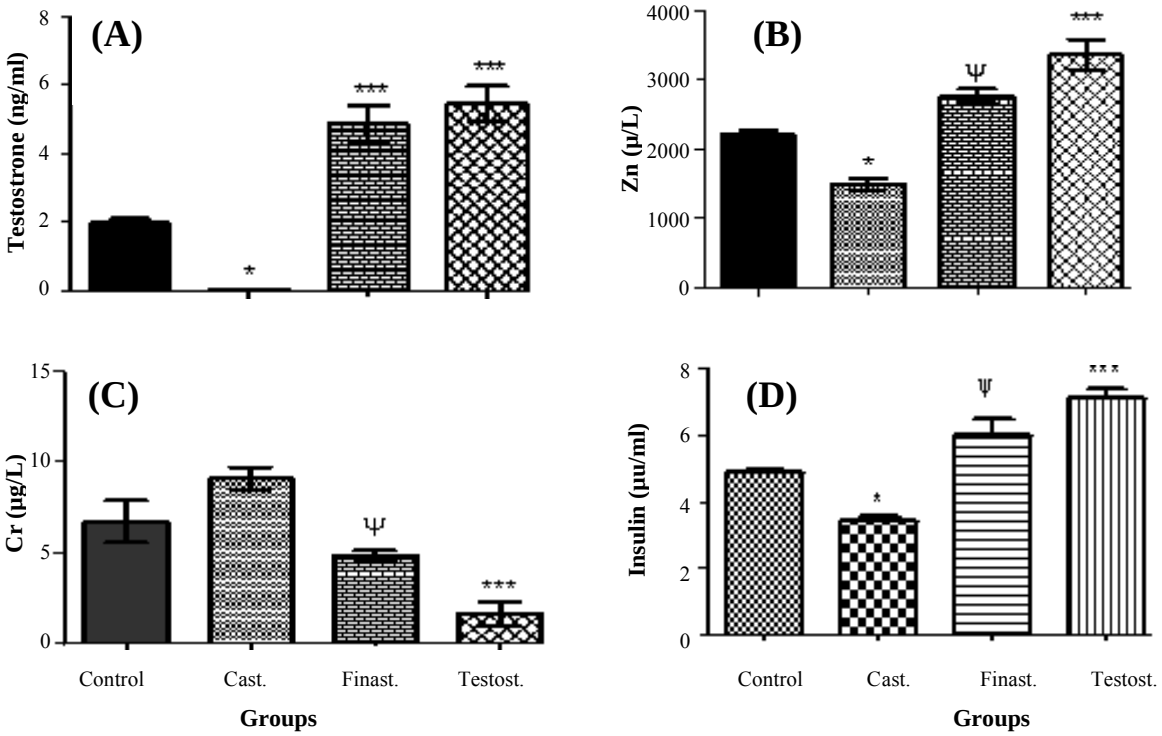

Fig. 1. Effect of testosterone (Testos.), finasteride (Finast.) administration and also castration (Cast.) on serum testosterone levels during 35 days of the study (n = 8). **(A)** The data show serum testosterone level significantly is decreased in castrated animals, whereas it increased in testosterone and finasteride administrated groups. **(B)** The data show serum zinc (Zn) level significantly is decreased in castrated animals, whereas it increased in testosterone and finasteride administrated groups. **(C)** The data show serum chromium (Cr) level does not show significant differences in castrated animals, whereas it decreased in testosterone and finasteride administrated groups. **(D)** The data show serum insulin level significantly is decreased in castrated animals, whereas it increased in testosterone and finasteride administrated groups. **P*<0.05, ****P*<0.001 and *t*-test (Ψ) = *P*<0.001

testosterone serum levels in finasteride-administrated group demonstrate that finasteride induces inhibition of conversion of testosterone to dihydrotestosterones in free testosterone as the same as the tissues such as prostate [22].

The present study indicates that serum insulin level following testosterone and finasteride administration was significantly increased. In contrast, castrated rats showed a significant decrease in serum insulin level.

More works investigating interactions between sex steroids and insulin action have been carried out in animals [7, 8, 18, 23]. It is clear that steroid hormones are important regulators of insulin. Studies have shown that serum insulin level following testosterone administration is significantly increased when compared to castrated rat, suggesting that normal circulating level of testosterone is essential for maintaining optimum insulin concen-tration in serum [23]. Morimoto *et al*. [18] have shown that testosterone modify serum insulin levels through direct effect on pancreatic islet function by favoring insulin gene expression and release.

The researchers have been focused on the effects of trace elements on secretion of androgens and insulin, and little information is available on the effects of androgens on serum levels of trace elements. In this study, we investigate the effects of testosterone, finasteride and castration on serum levels of trace elements (zinc and chromium) and insulin.

The data of the present study point to the positive relation between testosterone and zinc and negative correlation between testosterone and chromium. Baltaci *et al.* [12] reported a positive correlation between serum testosterone and serum zinc concentration that confirmed the two other studies [24, 25]. Om and Chung [26] established that zinc deficiency in male rats inhibited both testosterone and luteinizing hormone significantly. A similar finding was also reported by Martin *et al.* [27].

Chromium exposure at medium and high doses has been shown to reduce the activities of testicular steroidogenic enzymes [16]. Therefore, decreased serum testosterone level in medium and high dose of chromium in treated groups might be due to impaired activities of these enzymes. Degenerative changes in spermatogenic cycle were very much conspicuous in medium and high doses of chromium-treated group [14, 16].

In consideration of the above data, we can claim that serum chromium is negatively correlated with serum testosterone levels.

Moreover, it has been established that hormones affect the metabolism of trace elements at the different levels including the excretion and transfer [28].

Possibly, testosterone through influenced the absorption and transmission of zinc and boron effects on serum levels of these elements. However, further studies are needed to elucidate the mechanism of the effect of testosterone on the zinc and chromium concentrations in serum.

In the present study, testosterone-administrated group in which we obtained the highest insulin levels also had the highest zinc and lowest chromium levels, whereas in castrated group in which we obtained the lowest insulin levels had the lowest zinc and highest chromium levels.

Zinc plays an important role in the synthesis, storage and secretion of insulin [29]. Zinc deficiency has been also described in diabetic patients [30] and zinc deficiency was shown to predispose to glucose intolerance, diabetes mellitus and insulin resistance [31]. Marques *et al.* [32] have reported that fastening insulin level is increased significantly after zinc supplementation.

Morris *et al.* [30] have shown a significant inverse relationship between plasma insulin and plasma chromium levels under conditions where plasma glucose is unchanged. This issue strengthens the association between chromium and insulin action and suggests that further investigation of the role of chromium in insulin action is merited.

In conclusion, the study demonstrates that testosterone and finasteride increases insulin and zinc levels and decreases chromium levels in the serum of male adult rats. These data also suggest that testosterone affects glucose cycle through effect on serum levels of insulin and trace elements such as zinc and chromium.

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REFERENCES

1. Riggs BL, Khosla S, Melton LJ. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev.2002 Jun;23(3):279-302*.
2. Vanderschueren D, Vandenput L, Boonen S, Lindbrg MK, Bouilon R, Ohlsson C. Androgens and bone. *Endocr Rev.2004 Jun;25(3):389-425*.
3. Zhang MG, Wu W, Zhang CM, Wang XJ, Gao PJ, Lu YL, Shen ZJ. Effects of oral finasteride on erectile function in a rat model. *J Sex Med.2012; 9(5):1328-36*.
4. Samba Reddy D, Ramanathan G. Finasteride inhibits the disease-modifying activity of progesterone in the hippocampus kindling model of epileptogenesis. *Epilepsy Behav.2012 Sep;25(1):92-7*.
5. Bortolato M, Cannas A, Solla P, Bini V, Puligheddu M, Marrosu F. Finasteride attenuates pathological gambling in patients with Parkinson disease. *J Clin Psychopharmacol.2012 Jun;32(3):424-5*.
6. Sheffield-Moore M, Urban RJ. An overview of the endocrinology of skeletal muscle. *Trends Endocrinol Metab.2004 Apr;15(3):110-5*.
7. Livingstone C, Collison M. Sex steroid and insulin resistance. *Clin Sci(Lond).2002 Feb;102(2):151-66.*
8. Davis DD, Ruiz AL, Yanes LL, Iliescu R, Yuan K, Moulana M, Racusen LC, Reckelhoff JF. Testosterone supplementation in male obese Zucker rats reduces body weight and improves insulin sensitivity but increases blood pressure. *Hypertension.2012 Mar;59(3):726-31*.
9. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev.1993 Jan;73(1):79-118*.
10. Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM. Copius-Peereboom JH et al. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reprod Toxicol.2001 Mar;15(2):131-6*.
11. Bedwal RS. and Bahoguna A. Zinc, copper, selenium in reproduction. *Experientia.1994 Jul;50(7):626-40*.
12. Baltaci A.K., Mogulkoc R., Ozturk A. Testosterone and zinc supplementation in castrated rat: Effects on plasma leptin levels and relation with LH, FSH and testosterone. *Life Sci.2006 Jan;78(7):746-52*.
13. Kumari D, Nair N, Bedwal RS. Testicular apoptosis after dietary zinc deficiency: ultrastructural and TUNEL studies. *Syst Biol Reprod Med.2011;57(5):233-43*.
14. Jain SK, Kahlon G, Morehead L, Dhawan R, Lieblong B, Stapleton T et al. Effect of chromium dinicocysteinate supplementation on circulating levels of insulin, TNF-α, oxidative stress, and insulin resistance in type 2 diabetic subjects: randomized, double-blind, placebo-controlled study. *Mol Nutr Food Res.2012 Aug;56(8):1333-41*.
15. Porte JrD, Sherwin RS, Baron A editor. Ellengerg & Rifkin's Diabetes Mellitus. New York: McGraw-Hil; 2003.
16. Chowdhury AR. Spermatogenic and steroidogenic impairment after chromium treatment in rats. *Indian J Exp Biol.1995 Jul;33(7):480-4*.
17. Udayakumar TS, Tyagi A, Rajalakshmi M, Das SN, Hashim S, Bajaj JS. Changes in structure and functions of prostate by long-term administration of an androgen testosterone enanthate in rhesus monkey (Macaca mulatta). *Anat Rec.1998 Dec;252(4):637-45*.
18. Morimoto S, Fernandez-Mejia C, Romero-Navarro G, Morales-Peza N, Díaz-Sánchez V. Testosterone effect on insulin content, messenger ribonucleic acid levels, promoter activity, and secretion in the rat. *Endocrinology.2001 Apr;142(4):1442-7*.
19. Capkova J, Ivanyi P, Rehakova Z. Sexual dimorphism,

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but not testosterone itself, is responsible for ankylosing enthesitis of the ankle in B10.BR (H-2k) male mice. *Ann Rheum Dis.2006 Jan;65(1):130-2*.

20. Huynh H, Seyam RA, Brock GB. Reduction of ventral prostate weight by finasteride is associated with suppression of insulin-like growth factor I (IGF-I) and IGF-I receptor genes and with an increase in IGF binding protein 3. *Cancer Res.1998 Jan;58(2):215-8*.
21. Roehrborn CG, Lee M, Meehan A, Waldstreicher J; PLESS Study Group. Effects of finasteride on serum testosterone and body mass index in men with benign prostatic hyperplasia. *Urology.2003 Nov;62(5):894-9*.
22. Ma Z, Hung Nguyen T, Hoa Huynh T, Tien Do P, Huynh H. Reduction of rat prostate weight by combined quercetin-finasteride treatment is associated with cell cycle deregulation. *J Endocrinol.2004 Jun;181(3):493-507*.
23. Muthusamy T, Dhevika S, Murugesan P, Balasubramanian K. Testosterone deficiency impairs glucose oxidation through defective insulin and its receptor gene expression in target tissues of adult male rats. *Life Sci.2007 Jul;81(7):534-42*.
24. Fuse H, Kazama T, Ohta S, Fujiuchi Y. Relationship between zinc concentrations in seminal plasma and various sperm parameters. *Int Urol Nephrol.1999; 31(3):401-8*.
25. Prasad AS, Mantzoros CS, Beck FW, Hess JW, Brewer GJ. Zinc status and serum testosterone levels of healthy adults. *Nutrition.1996 May;12:344-8*.
26. Om AS, Chung KW. Dietary zinc deficiency alters 5-α reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. *J Nutr. 1996 Apr;126(4):842-8*.
27. Martin GB, White CL, Markey CM, Blackberry MA. Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. *J Reprod Fertil.1994 May;101(1):87-96*.
28. Henkin RI. Trace metals in endocrinology. *Med Clin North Am.1976 Jul;60(4):779-97*.
29. Chausmer AB. Zinc, insulin and diabetes. *J Am Coll Nutr.1998 Apr;17(2):109-15*.
30. Morris BW, MacNeil S, Stanley K, Gray TA, Fraser R. The inter-relationship between insulin and chromium in hyperinsulinaemic euglycaemic clamps in healthy volunteers. *J Endocrinol.1993 Nov;139(2):339-45*.
31. Partda-Hernandez G, Arreola F, Fenton B, Cabeza M, Roman-Ramos R, Revilla-Monsalve MC. Effect of zinc replacement on lipids and lipoproteins in type 2-diabetic patients. *Biomed Pharmacother.2006 May; 60(4):161-8*.
32. Marques LF, Donangelo CM, Franco JG, Pires L, Luna AS, Casimiro-Lopes G et al. Plasma zinc, copper and serum thyroid hormones and insulin levels after zinc supplementation followed by placebo in competitive athletes. *Biol Trace Elem Res.2010 Sep;142(3):415-23*.