

# The Effects of Administration of Vitamin D, Infliximab, and Leflunomide on Testosterone Concentrations in Rats under Atorvastatin Therapy

Duygun Altintas Aykan<sup>1</sup> , Muhammed Seyithanoglu<sup>2</sup> 



**Cite this article as:** Altintas Aykan D, Seyithanoglu M. The Effects of Administration of Vitamin D, Infliximab, and Leflunomide on Testosterone Concentrations in Rats under Atorvastatin Therapy. *Eurasian J Med* 2019; 51 (3): 224-7.

**ORCID IDs of the authors:**  
D.A.A. 0000-0001-8224-4006  
M.S. 0000-0002-8027-7549

<sup>1</sup>Department of Pharmacology, Kahramanmaraş Sütçü İmam University School of Medicine, Kahramanmaraş, Turkey

<sup>2</sup>Department of Biochemistry, Kahramanmaraş Sütçü İmam University School of Medicine, Kahramanmaraş, Turkey

Received: December 29, 2018  
Accepted: May 8, 2019

Correspondence to: Duygun Altintas Aykan  
E-mail: altintasduygun\_dr@yahoo.com

DOI 10.5152/eurasianjmed.2019.18481



Content of this journal is licensed under a Creative Commons Attribution 4.0 International License.

## ABSTRACT

**Objective:** Statins inhibit the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase enzyme and thus reduce plasma cholesterol levels. Although decreased cholesterol level is the main target of anti-lipidemic drugs, cholesterol has an important role in the synthesis of lipid-based hormones such as testosterone. In this study, the alterations in serum testosterone levels were examined in rats under atorvastatin therapy and their responses to vitamin D, infliximab, and leflunomide supplementation were evaluated.

**Materials and Methods:** Wistar rats were treated with atorvastatin (100 mg/kg) for 21 days to induce inhibition of the HMG-CoA reductase enzyme activity. Following statin therapy, rats received vitamin D (0.2 µg/kg/day) orally for 15 days, infliximab (7 mg/kg/day) intraperitoneally in two doses, or leflunomide (10 mg/kg/day) orally in two doses. Subsequently, the alterations in serum testosterone levels were measured by ELISA.

**Results:** Atorvastatin led to a decrease in the testosterone level compared to the vehicle group. Administration of vitamin D, infliximab, and leflunomide under HMG-CoA inhibition insignificantly increased the testosterone level compared to the atorvastatin control group. Furthermore, it appears that rats under statin administration respond better to treatment with leflunomide by achieving a greater induction in testosterone levels than with vitamin D or infliximab.

**Conclusion:** Our data provide evidence that administration of vitamin D, infliximab, and leflunomide in rats under atorvastatin treatment may ameliorate the serum testosterone levels.

**Keywords:** Vitamin D, infliximab, leflunomide, testosterone, atorvastatin

## Introduction

Statins reduce plasma cholesterol levels by inhibiting the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase enzyme. There are studies suggesting that statins impair testosterone production, because they reduce cholesterol biosynthesis [1, 2]. Testosterone is the main circulating androgen in men and has an important role in libido, bone density, fat distribution, muscle mass, strength, blood cell formation, and sperm production [3]. Androgen levels were reported to fluctuate in male patients under anti-lipidemic treatment [2].

Testosterone and vitamin D are involved in many medical conditions such as osteoporosis, cardiovascular diseases, and metabolic syndrome [4]. The protective role of vitamin D in several systemic diseases has been studied previously [5]. As the administration of androgenic hormone replacement therapy may lead to serious side effects such as prostate cancer, cardiovascular disorders, and polycythemia, vitamin D supplementation for patients with suboptimal testosterone levels may increase their androgen levels without leading to side effects of excess testosterone [6]. Some studies have shown a positive correlation between serum testosterone levels and 25(OH)vit D levels in men [7, 8]. However, there are also studies reporting the opposite [9, 10].

It is not known whether tumor necrosis factor-alpha (TNF-α) inhibitor, infliximab, and the pyrimidine inhibitor, leflunomide, were involved in the androgenic hormone disorders. A previous study examined the changes in sex hormones following initiation of anti-TNF-α therapy in adolescents with Crohn's disease. Improvements in disease activity and cytokine levels during the induction

therapy were associated with rapid and significant increases in sex hormones. The relationships linking sex hormone levels with systemic inflammatory factors were reported to implicate a role of inflammatory cytokines in this regulation [11]. Besides, the studies with leflunomide suggested its less efficient therapeutic effect, due to the counter action of estrogens on apoptosis, as also observed during the disease modifying anti-rheumatic drugs therapy [12]. To our knowledge, there is no study on the roles of infliximab or leflunomide in impairing the testosterone production in men under statin treatments.

The aim of this study is to investigate the relationship between the statin therapy and serum testosterone levels and to evaluate the effects of administration of vitamin D, infliximab, and leflunomide on serum testosterone alterations.

## Materials and Methods

### Animals

The study was carried out at our Pharmacology department. All experiments were conducted in strict accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. All protocols in this study were approved by the Laboratory Animal Care Committee (Ethics Committee File No: 2018/10/06, approval date: 10.10.2018). The rats were housed and maintained at 22°C, 60±5% humidity, and a 12:12 h light/dark cycle with free access to food and water *ad libitum*.

### Drugs

Atorvastatin (Ator, Sanovel, Turkey) 100 mg/kg/day, vitamin D (Monovit, Kocak Farma, Turkey) 0.2 µg/kg/day, and leflunomide (Reumil, Abdi Ibrahim, Turkey) 10 mg/kg/day were mixed with drinking water, and given orally to animals in 1 mL volume by oral gavage. Infliximab (Remicade, Merck Sharp Dohme, Singapore) 7 mg/kg/day was dissolved in 0.9% saline and administered intraperitoneally in 1 mL/kg volume. At the end of the experiment, rats were anesthetized with ketamine HCl (80 mg/kg) (Ketalar, Pfizer, Turkey) and xylazine (10 mg/kg) (Rompun, Bayer, Germany).

### Experimental Design

All groups, except Group 1, received 100 mg/kg/day of atorvastatin with oral gavage for 21 days in a 1 mL volume. The doses in the present study for atorvastatin, infliximab, leflunomide, and vitamin D are based on relevant studies and shown to be effective and safe from previous experimental studies [13-15].

The rats were randomly divided into five groups (n=6 per group) and treated as follows:

**Group 1 (vehicle):** received saline orally from 1 to 21 days, and then with an equal volume of vehicle (0.9% NaCl) intraperitoneally in two doses on the 21st day.

**Group 2 (atorvastatin):** received atorvastatin (100 mg/kg) orally from 1 to 21 days to induce HMG-CoA inhibition.

**Group 3 (atorvastatin+vitamin D):** received atorvastatin (100 mg/kg) orally from 1 to 21 days to induce HMG-CoA inhibition+received vitamin D (0.2 µg/kg) with oral gavage from 21 to 35 days.

**Group 4 (atorvastatin+infliximab):** received atorvastatin (100 mg/kg) orally from 1 to 21 days to induce HMG-CoA inhibition+received infliximab (7 mg/kg) intraperitoneally in two doses on the 21st day.

**Group 5 (atorvastatin+leflunomide):** received atorvastatin (100 mg/kg) orally from 1 to 21 days to induce HMG-CoA inhibition+received leflunomide (10 mg/kg) with oral gavage in two doses on the 21st day.

We implemented infliximab and leflunomide on a single day in two doses based on previous studies; regarding that agents showed anti-inflammatory and protective effects in rats [3, 16].

### Measurement of Serum Testosterone Levels

After drug treatments with vitamin D, infliximab, and leflunomide, blood sample was collected by cardiac puncture in rats under anesthetic condition at room temperature and centrifuged (4000 rpm, rt.) for 10 min, and then the supernatant was collected for biochemical analysis. Concentrations of testosterone in the plasma were measured by ELISA strictly in accordance with the manufacturer's instructions (201-11-5126; SunRed Biological Technology, Shanghai, China).

### Statistical Analysis

The data were defined as the arithmetic average and standard deviation. To apply parametric tests, the Kolmogorov Smirnov test was used to determine whether the samples had normal distribution and whether the variances were homogeneous. For multiple groups, analysis of variance test with *post-hoc* Tukey's test for significance difference was used for normally distributed data. Kruskal Wallis test with Mann Whitney U test under Bonferroni correction was used for the analysis of none normally distributed data. The p values less than 0.05 were considered significant. The data were evaluated at 95% confidence interval. The data were ana-

lyzed in The Statistical Package for the Social Sciences (SPSS) 17.0 program (SPSS Inc., IL, Chicago, USA).

## Results

### Effect of Atorvastatin on Serum Testosterone Levels

In rats receiving atorvastatin for 21 days, the activity of HMG-CoA decreases. As presented in Table 1 and Figure 1, the results of Group 2 showed that atorvastatin administration led to a decrease in the testosterone level compared to the vehicle group ( $p>0.05$ ). The mean testosterone content was 92.52 pg/mL in the vehicle group. The mean testosterone content was decreased to 83.26 pg/mL after administration of atorvastatin at the end of the 21st day. Thus, HMG-CoA inhibition by atorvastatin administration reduced the levels of testosterone in rats.

### Effect of Administration of Vitamin D on Serum Testosterone Levels under Atorvastatin Therapy

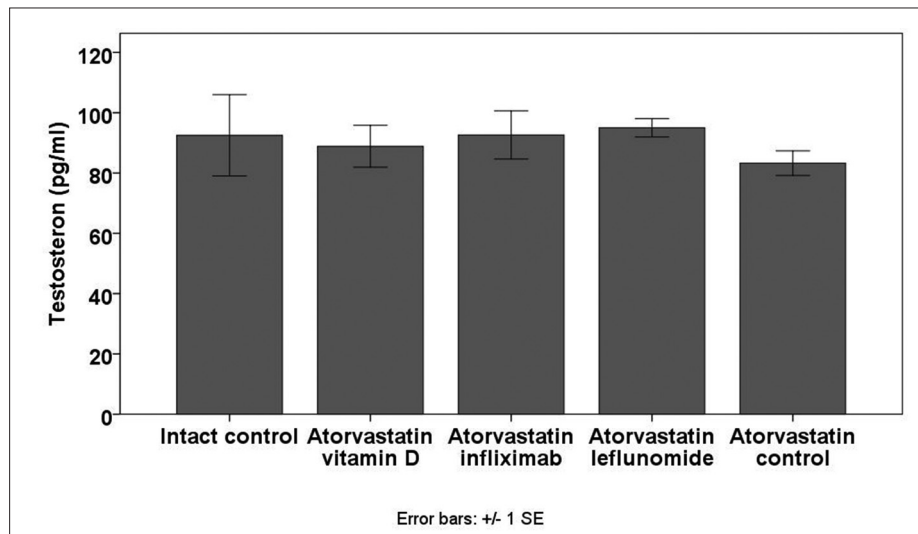
In Group 3 where rats received atorvastatin orally from 1 to 21 days to induce HMG-CoA inhibition, and subsequently received vitamin D with oral gavage from 21 to 35 days, the level of testosterone was found to be higher than the atorvastatin control group; but this increase was not significant. As shown in Table 1 and Figure 1, the mean testosterone content was 83.26 pg/mL in the atorvastatin control group. The mean testosterone content was increased to 88.87 pg/mL after subsequent administration of vitamin D. Thus, subsequent vitamin D administration in rats with HMG-CoA inhibition led to an increase in the testosterone level compared to the atorvastatin control group ( $p>0.05$ ).

### Effect of Administration of Infliximab on Serum Testosterone Levels under Atorvastatin Therapy

In Group 4 where rats received atorvastatin orally from 1 to 21 days to induce HMG-CoA inhibition, and subsequently received infliximab intraperitoneally in two doses on the 21st day, the level of testosterone was found to be higher than that of the atorvastatin control group,

**Table 1. Serum testosterone concentrations of groups**

	Testosterone, pg/mL
Vehicle	92.52±33.05
Atorvastatin control	83.26±10.05
Atorvastatin+Vitamin D	88.87±17.06
Atorvastatin+Infliximab	92.62±19.60
Atorvastatin+Leflunomide	95.00±07.50



**Figure 1.** Alterations in serum testosterone concentrations of groups. "Intact control" refers to the rats in the vehicle group. An improved but insignificant induction in testosterone levels after administration of vitamin D, infliximab, or leflunomide was seen in rats under atorvastatin therapy.

but this increase was not significant. As shown in Table I and Figure 1, the mean testosterone content was 83.26 pg/mL in the atorvastatin control group. The mean testosterone content was increased to 92.62 pg/mL after subsequent administration of infliximab. Thus, subsequent infliximab administration in rats with HMG-CoA inhibition led to an increase in the testosterone level compared to the atorvastatin control group ( $p>0.05$ ).

#### Effect of Administration of Leflunomide on Serum Testosterone Levels under Atorvastatin Therapy

In Group 5 where rats received atorvastatin orally from 1 to 21 days to induce HMG-CoA inhibition, and subsequently received leflunomide with oral gavage in two doses on the 21st day, the level of testosterone was found to be higher than that of the atorvastatin control group, but this increase was not significant. As shown in Table I and Figure 1, the mean testosterone content was 83.26 pg/mL in the atorvastatin control group. The mean testosterone content was increased to 95.00 pg/mL after subsequent administration of leflunomide. Thus, subsequent leflunomide administration in rats with HMG-CoA inhibition led to an increase in the testosterone level compared to the atorvastatin control group ( $p>0.05$ ).

#### Discussion

In the current analysis, we investigated the effect of vitamin D, infliximab, and leflunomide supplementation on serum testosterone concentrations in rats under atorvastatin therapy. We found that HMG-CoA inhibition by atorvastatin reduced the levels of testosterone in rats. On the other hand, subsequent administrations

of vitamin D, infliximab, or leflunomide under statin therapy led to an insignificant increase in the testosterone levels.

Statins improve endothelial function by lowering low-density lipoprotein (LDL) levels and through their pleiotropic effects [17]. Lipophilic properties also play an important role in the effects of statins [18]. In this study, we used atorvastatin, which is potent, has long lasting effect, and is the most used statin [19]. Reduced cholesterol levels may suppress the production of testosterone and dihydrotestosterone, which are the main androgens, since cholesterol is a necessary substrate in the synthesis of androgens in testis and adrenal glands. Previous studies have reported that testosterone was synthesized by plasma lipoproteins or endogenous synthesis [1]. Some studies have investigated the circulating androgen levels in men using statins due to dyslipidemia, and have proven that statins had affected the level of androgens [20]. The possibility of the relationship between decrease in cholesterol and the decrease in testosterone has been uncovered with confounding results from previous studies [21, 22]. In our study, we found that atorvastatin administration led to an insignificant decrease in the testosterone level compared to the vehicle group. Although statins decrease testosterone levels, they are known to be beneficial in erectile dysfunction. It was reported that anti-oxidant mechanisms of statins, pleiotropic effects, increased availability of nitric oxide, and decreased LDL may lead to their improvement in erectile dysfunction [17].

Vitamin D is an important agent in calcium cycle, bone metabolism, and muscle activity [23]. Vitamin D deficiency may cause various health

problems, as determined in the recent epidemiological studies such as autoimmune diseases, cardiovascular diseases, cancer, depression, and diabetes mellitus [6, 24]. Vitamin D receptors (VDR) were found in human testis, ejaculatory duct, and mature spermatozoa, suggesting that vitamin D may have a role in male fertilization [25]. This has been supported by studies of hypogonathropic hypogonadism, in which decreased testicular weight, abnormal testicular histology, and decreased number of functional sperms in VDR knockout mice were shown [26]. However, studies on the relationship between vitamin D and testosterone, or the effects of vitamin D supplementation on testosterone levels revealed heterogeneous results [27, 28]. In the present study, co-administration of vitamin D improved testosterone levels in rats compared to the atorvastatin control group, which shows that vitamin D might have some regulatory mechanism in controlling the testosterone production.

TNF- $\alpha$  plays a role in various processes associated with inflammation, such as the release of chemotactic cytokines, upregulation of endothelial adhesion molecules, and migration of leukocytes. Infliximab is an anti-TNF- $\alpha$  monoclonal antibody that binds to soluble TNF- $\alpha$  and membrane-attached TNF- $\alpha$  [29]. Since the effect of TNF- $\alpha$  blockade can differ depending on the time of its administration, we used infliximab either immediately after or 6 h after inducing HMG-CoA inhibition in two doses in a single day. We found in our study that infliximab seems to be effective in increasing testosterone levels in rats under HMG-CoA inhibition.

Leflunomide, used in rheumatic diseases, is an inhibitor of pyrimidine synthesis. Anti-inflammatory and immunomodulatory effects of leflunomide are associated with its ability to suppress proinflammatory cytokines and to inhibit dendritic cell maturation and IL-17 production [30]. Leflunomide also presents anti-oxidative activity by suppressing the release of reactive oxygen species from white blood cells [31]. Inhibition of both pyrimidine synthesis and tyrosine-kinase activity leads to restriction of immunologic responses. Consequently, the mechanism results in the direct inhibition of T and B lymphocytes, decreased serum allo-specific immunoglobulin molecule production, and a decrease in the expression of adhesion molecules [32]. We administered leflunomide in two doses in a single day, as the benefit of short-term therapy with leflunomide has been proved; however, the benefit of long-term administration (more than 20 days) still remains unclear regarding adverse effects [33]. We found in our study that short-

term therapy of leflunomide seems to be effective in increasing testosterone levels in rats under HMG-CoA inhibition. Furthermore, it appears that rats under statin administration respond better to treatment with leflunomide by achieving a greater induction in testosterone levels than with vitamin D or infliximab.

We have some limitations in this study. First, we neglected the concentrations of the binding proteins in the serum. As vitamin D binding protein and testosterone binding globulin concentrations are influenced by the same factors, excessive vitamin D supplementation may affect testosterone binding globulin concentrations, causing alterations in total testosterone levels. Second, we used a single measurement to obtain the outcomes of our study. We may strengthen our data with measurements of LDL, luteinizing hormone, follicle-stimulating hormone, serum sex hormone binding globulin, TNF- $\alpha$ , and histopathological analysis. Third, the treatment with infliximab or leflunomide may generate an extensive list of important and serious side effects. These side effects are mainly associated with the dosage of drugs, formulations, and in combinations with other agents. However, we implemented the doses in the present study based on the relevant experimental studies which were shown to be effective and safe [13-15].

Although this experimental study has limited power, our data provide evidence that vitamin D, infliximab, and leflunomide administrations to rats under atorvastatin treatment resulted in an amelioration in serum testosterone levels. Our small-scale study requires further large-scaled studies to investigate the underlying mechanisms of exposure, before application of the drugs in the subsequent clinical trials.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Kahramanmaraş Sütçü İmam University laboratory animals experimental ethics committee (Ethics Committee File No: 2018/10/06, approval date: 10.10.2018)

**Informed Consent:** N/A.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – D.A.A.; Design – D.A.A.; Supervision - D.A.A., M.S.; Resources – D.A.A.; Materials – D.A.A., M.S.; Data Collection and/or Processing – D.A.A., M.S.; Analysis and/or Interpretation – D.A.A., M.S.; Literature Search – D.A.A., M.S.; Writing Manuscript – D.A.A.; Critical Review – D.A.A.

**Conflict of Interest:** The authors declared no conflicts of interest.

**Financial Disclosure:** The authors declared that this study has received no financial support.

## References

- Corona G, Boddi V, Balercia G, et al. The effect of statin therapy on testosterone levels in subjects consulting for erectile dysfunction. *J Sex Med* 2010; 7: 1547-56. [\[CrossRef\]](#)
- Hsieh CJ, Huang B. Rosuvastatin decreases testosterone levels but not sexual function in men with type 2 diabetes. *Diabetes Res Clin Pract* 2016; 120: 81-8. [\[CrossRef\]](#)
- Shalet SM. Normal testicular function and spermatogenesis. *Pediatr Blood Cancer* 2009; 53: 285-8. [\[CrossRef\]](#)
- Yeap BB. Testosterone and ill-health in aging men. *Nat Clin Pract Endocrinol Metab* 2009; 5: 113-21. [\[CrossRef\]](#)
- Morioka TY, Lee AJ, Bertisch S, Buettner C. Vitamin D status modifies the association between statin use and musculoskeletal pain: a population based study. *Atherosclerosis* 2015; 238: 77-82. [\[CrossRef\]](#)
- Chin KY, Ima-Nirwana S, Wan Ngah WZ. Vitamin D is significantly associated with total testosterone and sex hormone-binding globulin in Malaysian men. *Aging Male* 2015; 18: 175-9. [\[CrossRef\]](#)
- Lee DM, Tajar A, Pye SR, et al. Association of hypogonadism with vitamin D status: the European Male Ageing Study. *Eur J Endocrinol* 2012; 166: 77-85. [\[CrossRef\]](#)
- Nimptsch K, Platz EA, Willett WC, Giovannucci E. Association between plasma 25-OH vitamin D and testosterone levels in men. *Clin Endocrinol (Oxf)* 2012; 77: 106-12. [\[CrossRef\]](#)
- Lerchbaum E, Pilz S, Trummer C, et al. Serum vitamin D levels and hypogonadism in men. *Andrology* 2014; 2: 748-54. [\[CrossRef\]](#)
- Heijboer AC, Oosterwerff M, Schroten NF, et al. Vitamin D supplementation and testosterone concentrations in male human subjects. *Clin Endocrinol (Oxf)* 2015; 83: 105-10. [\[CrossRef\]](#)
- DeBoer MD, Thayu M, Griffin LM, et al. Increases in Sex Hormones during Anti-Tumor Necrosis Factor  $\alpha$  Therapy in Adolescents with Crohn's Disease. *J Pediatr* 2016; 171: 146-52.e1-2. [\[CrossRef\]](#)
- Montagna P, Brizzolara R, Soldano S, Pizzorni C, Sulli A, Cutolo M. Sex hormones and leflunomide treatment of human macrophage cultures: effects on apoptosis. *Int J Clin Exp Med* 2009; 2: 221-32.
- El-Ganainy SO, El-Mallah A, Abdallah D, Khatib MM, Mohy El-Din MM, El-Khatib AS. Elucidation of the mechanism of atorvastatin-induced myopathy in a rat model. *Toxicology* 2016; 359-360: 29-38. [\[CrossRef\]](#)
- Zhang Q, Ji Y, Lv W, He T, Wang J. Protective effects of leflunomide on renal lesions in a rat model of diabetic nephropathy. *Ren Fail* 2016; 38: 124-30. [\[CrossRef\]](#)
- Ozer EK, Goktas MT, Kilinc I, et al. Infliximab alleviates the mortality, mesenteric hypoperfusion, aortic dysfunction, and multiple organ damage in septic rats. *Can J Physiol Pharmacol* 2017; 95: 866-72. [\[CrossRef\]](#)
- Ozturk E, Surucu M, Karaman A, Samdanci E, Fadilloglu E. Protective effect of leflunomide against oxidative intestinal injury in a rodent model of sepsis. *J Surg Res* 2014; 187: 610-5. [\[CrossRef\]](#)
- Ishimitsu T, Ohno E, Ueno Y, et al. Effects of atorvastatin and ezetimibe on endothelial function in dyslipidemic patients with chronic kidney disease. *Clin Exp Nephrol* 2014; 18: 704-10. [\[CrossRef\]](#)
- Kostis JB, Dobrzynski JM. The effect of statins on erectile dysfunction: a meta-analysis of randomized trials. *J Sex Med* 2014; 11: 1626-35. [\[CrossRef\]](#)
- Hall SA, Page ST, Travison TG, Montgomery RB, Link CL, McKinlay JB. Do statins affect androgen levels in men? Results from the Boston area community health survey. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1587-94. [\[CrossRef\]](#)
- Hyppä MT, Kronholm E, Virtanen A, Leino A, Jula A. Does simvastatin affect mood and steroid hormone levels in hypercholesterolemic men? A randomized double-blind trial. *Psychoneuroendocrinology* 2003; 28: 181-94. [\[CrossRef\]](#)
- Stanworth RD, Kapoor D, Channer KS, Jones TH. Statin therapy is associated with lower total but not bioavailable or free testosterone in men with type 2 diabetes. *Diabetes Care* 2009; 32: 541-6. [\[CrossRef\]](#)
- Kocum TH, Ozcan TI, Gen R, et al. Does atorvastatin affect androgen levels in men in the era of very-low LDL targeting therapy? *Exp Clin Endocrinol Diabetes* 2009; 117: 60-3. [\[CrossRef\]](#)
- Heijboer AC, Oosterwerff M, Schroten NF, et al. Vitamin D supplementation and testosterone concentrations in male human subjects. *Clin Endocrinol (Oxf)* 2015; 83: 105-10. [\[CrossRef\]](#)
- Nandan P, Nayanatara AK, Poojary R, Bhagyalakshmi K, Nirupama M, Kini RD. Protective Role of Co-administration of Vitamin D in Monosodium Glutamate Induced Obesity in Female Rats. *J Natl Med Assoc* 2018; 110: 98-102. [\[CrossRef\]](#)
- Blomberg Jensen M, Nielsen JE, Jørgensen A, et al. Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Hum Reprod* 2010; 25: 1303-11. [\[CrossRef\]](#)
- Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 2000; 141: 1317-24. [\[CrossRef\]](#)
- Jorde R, Grimnes G, Hutchinson MS, Kjærgaard M, Kamycheva E, Svartberg J. Supplementation with vitamin D does not increase serum testosterone levels in healthy males. *Horm Metab Res* 2013; 45: 675-81. [\[CrossRef\]](#)
- Pilz S, Frisch S, Koertke H, et al. Effect of vitamin D supplementation on testosterone levels in men. *Horm Metab Res* 2011; 43: 223-5. [\[CrossRef\]](#)
- Arango-Dávila CA, Vera A, Londoño AC, et al. Soluble or soluble/membrane TNF- $\alpha$  inhibitors protect the brain from focal ischemic injury in rats. *Int J Neurosci* 2015; 125: 936-40. [\[CrossRef\]](#)
- Diamantopoulos AP, Hetland H, Myklebust G. Leflunomide as a corticosteroid-sparing agent in giant cell arteritis and polymyalgia rheumatica: a case series. *Biomed Res Int* 2013; doi: 10.1155/2013/120638. [\[CrossRef\]](#)
- Karaman A, Turkmen E, Gursul C, Tas E, Fadilloglu E. Prevention of renal ischemia/reperfusion-induced injury in rats by leflunomide. *Int J Urol* 2006; 13: 1434-41. [\[CrossRef\]](#)
- Bloudíková S, Rajnoch J, Lodererová A, Honsová E, Viklický O. Leflunomide derivate FK 778 in accelerated renal injury in transgenic rat. *Folia Biol (Praha)* 2010; 56: 72-7.
- Deuse T, Schrepfer S, Reichenspurner H. Immunosuppression with FK778 and mycophenolate mofetil in a rat cardiac transplantation model. *Transplantation* 2003; 76: 1627-9. [\[CrossRef\]](#)