International Pharmacy Acta. 2023;6(1):e5
Original Article
OPEN ACCESS
ISSN: 2645-3266

The effect of Atorvastatin and Endurance training and their Combination on Testosterone Levels, Leydig, Spermatogonia, Spermatocyte cells numbers, Diameter and Thickness of Seminiferous Tubules and testicular morphology in rats

Amin Abdi [a] *, Hadi Abdi[b], Nabi Shamsaei[c]

a. Faculty of Basic Sciences, Razi University, Kermanshah, Iran.
b. Department of Physical Education, Payame Noor University, Tehran, Iran.
c. Department of Sport Sciences, Ilam University, Ilam, Iran.

Article Info:
Received: December 2022
Accepted: February 2023
Published online:
April 2023

* **Corresponding Author:**
Amin Abdi
Email:
abdiamin1900@gmail.com

Abstract:

Many drugs and regular exercise may have effect on male fertility. Therefore in the present study we investigated the effect of Atorvastatin and Endurance training on serum levels of testosterone,Leydig ,spermatogonia, Spermatocyte cells numbers and testicular morphology and morphometric evaluation in rats. 30Wistar rats(230±10g)were randomly divided into 6 groups:control, endurance training,atorvastatin(5 mg/kg and 10 mg/kg),the endurance training + atorvastatin (5 mg/kg and 10 mg/kg),5 rats per group. Drug intervention groups received atorvastatin for 4 weeks using gavage.Training groups also run a rodent treadmill 5 days a week for 4 weeks(speed of 10 m/min for 10 min in first week and other week: 16 m/min for 50 min) Serum testosterone level was measured by ELISA Kit.The results showed in the endurance training+atorvastatin 10 mg/kg, morphological disruption of testicular was observed.The number of Leydig, spermatogonia and Spermatocyte as well as blood testosterone level and germinal epithelium thickness were significantly decreased in the endurance training+atorvastatin 10 mg/kg compared to the control group($p< 0.05$). In conclusion,the results of this study showed the negative effect of the combination of endurance training and high dose atorvastatin (10 mg/kg) on testicular tissue and male reproductive system due to decreased levels of testosterone in rats.

Keywords: Endurance Training; Atorvastatin; Testosterone; Leydig; Spermatogonia; Spermatocyte.

Please Cite this article as: Abdi A, Abdi Hadi, Shamsaei N. The effect of Atorvastatin and Endurance training and their Combination on Testosterone Levels, Leydig , Spermatogonia ,Spermatocyte cells numbers, Diameter and Thickness of Seminiferous Tubules and testicular morphology in rats. Int. Pharm. Acta. 2023;6(1):e5
DOI: https://doi.org/10.22037/ipa.v6i1.40193

1. Introduction

Today, due to the prevalence of cardiovascular disease and diabetes in developing countries, the use of statins is increasing. Studies have reported the beneficial effects of these drugs in preventing the progression of many cardiovascular diseases [1, 2]. Statins have special effects on various tissues of the body in pathological conditions [3, 4]. In addition to blood lipids lowering effects, these drugs have antioxidant, anti-inflammatory, anti-apoptotic, and protective effects, therefore, they are of special therapeutic importance [5]. Statins have side-effects on the male reproductive system [6].

The 3-hydroxy-3-methyl glutaryl coenzyme (HMG-CoA) reductase is the most important enzyme in cholesterol synthesis. This liver enzyme is responsible for the synthesis of mevalonate, which is a regulatory enzyme in cholesterol synthesis. Atorvastatin binds to this enzyme as a competitive inhibitor and replaces its substrate, and inhibits the cholesterol biosynthesis in the liver [7]. Ultimately, inhibition of intracellular cholesterol production leads to an increase in cholesterol

excretion by low-density lipoprotein (LDL) receptors and a decrease in plasma cholesterol [8].
Cholesterol is an essential substance for the synthesis of steroid hormones, especially testosterone. One of the most important side effects of atorvastatin is a decrease in testosterone levels. The effect of statins on sex hormone levels has been studied in several studies, but the results of these studies are different and equivocal. A study by Kant et al. (2009) found that taking atorvastatin in people with type 2 diabetes reduced sex steroids. Another study found that high doses of atorvastatin did not affect the production of sex hormones [9]. Atorvastatin may also inhibit the production of testosterone in the body [10]. Studies have also shown that atorvastatin significantly reduces sperm count and their survival [11].
Regular exercise is a key program in the prevention and treatment of cardiovascular disease and leading to a reduction of complications of this disease [12]. One of the most important physiological adaptations after physical activity is hormonal adaptation. Hormonal response to physical activity depends on the intensity, duration, type of exercise program, and level of physical fitness [13]. Researchers have shown that endurance training has an acute effect on hormonal response and chronic changes in basal hormonal concentration [14]. The results of studies show that endurance exercise lowers plasma cholesterol levels [15]. Endurance training has also been shown to reduce testosterone levels and lower testosterone to cortisol ratios [12, 16].
Various exercise training can have a significant impact on reproductive markers [17]. High-intensity exercise can adversely affect testicular function [18], moderate-intensity exercise improves diabetes-induced suppressed spermatogenesis and improves sperm parameters. [19].
In recent years, alongside pharmacological treatment, one of the recommendations of physicians for cardiovascular and hyperlipidemia patients is the use of exercise training, especially endurance exercise training. According to studies, concomitant use of statin drugs and endurance training may be more effective in treating and preventing cardiovascular disorders such as myocardial infarction. The combination of statin drugs and regular endurance training in cardiovascular patients has been reported to reduce arterial wall stiffness by reducing circulating basophils [20]. In this regard, Guazzi et al. (2007) reported that endurance training after myocardial infarction may double the beneficial effects of statin therapy [21]. On the other hand, due to the effect of atorvastatin on inhibiting cholesterol synthesis and the role of endurance training in reducing plasma cholesterol concentration and the need for cholesterol to synthesize sex hormones, it seems that the simultaneous use of endurance training and statin drugs can cause disorders in the production of sex hormones by lowering cholesterol and ultimately reduce reproductive function. Therefore, in the present study, the effect of atorvastatin and endurance training on serum testosterone levels, testicular morphology and morphometry in male rats has been investigated.

2. Materials & Methods

2.1. Animals

This study was a part of Ph.D dissertation of Hadi Abdi with number (2282058) and all of experimental procedures involving animals were approved by the ethics institute Committee of the Animal Care of Ilam University .30 adult male wistar rats (weighting 230 ± 10 g) were prepared and placed in standard cages and controlled environments (22-25^{o} C, 45-50% humidity, and 12-h light/dark cycle).

2.2. Study design

The rats were randomly divided into 6 groups: control (Group A), endurance training (Group B), atorvastatin (5 mg/kg(Group C) and 10 mg/kg(Group D)) , the endurance training + atorvastatin (5 mg/kg(Group E) and 10 mg/kg(Group F)) 5 rats per group.
Animals in pharmacological intervention groups (C,D,E,F) received atorvastatin dissolved in normal saline orally by gavage for 4 weeks daily and fed with high cholesterol diet (Normal diet mixed with 2% cholesterol + 0.5% cholic acid) The rats in the control group received Normal diet [22].
The rats in the endurance training intervention groups were trained to run on a treadmill (5-Lane rodents treadmill; Technic Azma Company, Iran) 5 days a week for 4 weeks. Initially, the rats were acclimatized to run for 5 min at 5 m/min, 5° slope for 5 days before the formal training sessions. Initially, electrical shock (0.5 mA) was used to force the animals to run forward. After acclimation sessions, the rats started formal training. The training program consisted of 5 sessions per week of progressive running on the treadmill at a speed of 10-16 meters per minute (m/min) for 10-50 minutes and 5° slope. The rats started the formal training program with a speed of 10 m/min for 10 min and 5° slope for 5 days per week in first week. The speed and duration of the training and slope of treadmill were increased progressively, so that animals were running at speed of: with 5° slope in the fourth week of the training [23, 24]. This exercise training program is a moderate intensity program with about 55% VO2max. Sedentary animals daily run on the treadmill at a speed of 5 m/min for 5min.

2.3. Specimen collections

Forty-eight hours after the last training session, all rats in different groups anesthetized using chloroform. Under

complete anesthesia, 5 cc of blood was collected directly from their hearts and poured into tubes containing EDTA anticoagulants. After the blood collection, the animals abdominal cavity were opened and their testicles were carefully removed and placed in 10% formalin fixative for histological examination and then were passaged for study with a light microscope.

2.4. Testosterone assay

30 minutes after collecting blood samples, by using a centrifuge apparatus, their serum was separated and stored at -20. C. A special ELISA kit (DiaPlus, made in the United States, measuring 0.30 nanograms per milliliter) was used to measure serum testosterone levels and expressed as ng/ml.

2.5. Histological and Morphometric Analysis

After preparing the paraffin blocks, coronal sections at 5µm thicknesses were cut (with a microtome) and placed on the slide. Testicular tissue sections were stained with H&E staining by standard method [25]. For the counting of Spermatogonia and Spermatocytes, seminephros tubes with relatively equal size in each testicle was selected then 8 sections per sample were examined by using 100_ objective lens. Also, the number of Leydig cells was counted in the interstitial tissue the cross-sections of the most circular seminiferous tubules were photographed and the diameters of the seminiferous tubule were measured across the minor and major axes, and the mean diameter was determined. The length and width of the seminiferous tubule are the minor and major axes. For each group, 110–130 tubules were measured. To estimate the epithelial thickness was subtracted the mean diameter of lumen from the mean diameter of the seminiferous tubule [26] Figure 1(C).

2.6. Statistical analysis

All data were analyzed using SPSS software (SPSS for Windows; SPSS Inc., Chicago, IL, USA; Version 21.00). The Colmogrov-Smirnov test was used to verify the normality of data distribution. One way analysis of variance (ANOVA) and Scheffe's post hoc tests were used to compare the differences between the groups. The significance level was set at $p<0.05$.

3. Results and Discussion

In the control group (A) the testis tissue structure was normal. Also the different categories of Spermatogonia cells and Leydig cells in interstitial areas were observed. The mean number of Spermatogonia, Spermatocyte, Leydig cells, Seminiferous tubules diameter and Epithelial thickness in Group B ,Group C, Group D and Group E were not significantly different ($p>0.05$), versus the control group. (Table 1) Also Normal histological structures of the testis were observed in them (Figure 1). The number of Spermatogonia ($25.46 \pm .944$), Spermatocyte ($23.61 \pm .622$),and Leydig cells($6.18 \pm .258$) in the Group (F) decreased significantly compared to the control group ($p<0.05$). We found that the thickness of the seminiferous tubule in the Group (F) significantly reduced ($p < 0.05$), whereas the diameters of the seminiferous tubule in that was non-significant. (Table 1).

Table 1. Comparison of the count of spermatogonia, spermatocyte, Leydig cells Seminiferous tubules diameter (µm) and Epithelial thickness (µm) in different groups of rats. Values are means ± SEM.

	Group A (control)	Group B (endurance training)	Group C (atorvastatin 5 mg/kg)	Group D (atorvastatin 10 mg/kg)	Group E (the endurance training + atorvastatin 5 mg/kg)	Group F (the endurance training + atorvastatin 10 mg/kg)
Spermatogonia	30.06 ±.814	27.14± .834	28.96 ±1.08	28.8 ± 1.11	28.38 ± .997	25.46 ± .944*
spermatocyte	31.38 ±.666	28.72 ± .744	30.98± .875	28.48 ± .465	28.18± .545	23.61 ± .622*
Leydig cells	8.9 ± .208	7.5 ± .287	7.84 ± .249	7.22 ± .309	7.58± .278	6.18 ± .258*
Seminiferous tubules diameter (µm)	206.62±4.95	194.51±2.15	202.60± 2.66	191.26±2.13	190.56 ± 2	184.72± 1.77
Epithelial thickness (µm)	114.26±1.36	110.85±1.30	109.2 ± 2.09	112.52± 2.25	112.89±2.79	99.68 ± 2.22*

* Significant difference compared to the control group ($p<0.05$)

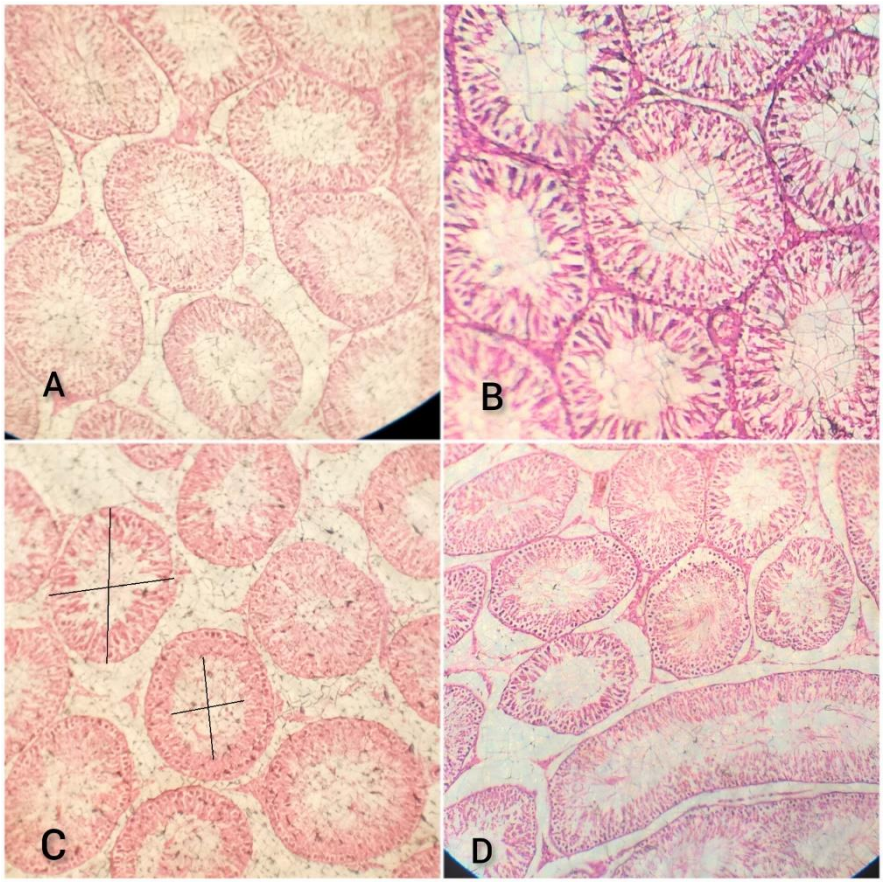

Figure 1. Tissue sections of testicular seminiferous tubules in different groups (hematoxylin-eosin staining, magnification 400X)(A) Normal histological structures of the testis in control group(A),(B) undisturbed pattern in the arrangement of seminiferous tubules in Group B(endurance training). (C) Normal germinal epithelium in Group C(atorvastatin 5 mg/kg). (D) Normal germinal epithelium arrangement in Group D(atorvastatin 10 mg/kg).

In the Group (F) severe testicular damages were found. These included: rupture of the primary layer of cells in the tubules , disruption and irregularity of the germinal epithelium , edema between the seminiferous tubule, congestion in the blood vessels. (Figure 2).

The results of serum testosterone levels (ng/ml) in different groups, Control group (4.01±0.37), Group B **(** 2.76 ±1.14), Group C,(3.21± 1.71), Group D (3.42±.68) Group E(2.48±1.67) Group F(1.95±0.26) showed that serum testosterone levels in the Group (F), were significantly lower than in the control group. (Figure 3).

The results showed that the combination of endurance exercise and atorvastatin (10 mg/kg) caused rupture of the primary layer of cells in the tubules and disruption and irregularity of the germinal epithelium in the testicular tissue of the male rats. Also in this group the thickness of the germinal epithelium and the density of the leydig cells have decreased significantly. The number of Leydig and spermatogonia cells, as well as .In the present work serum testosterone levels in the Group (F), decreased significantly.

The synthesis of steroid hormones depends on the supply of cholesterol, which may be obtained through the extraction of plasma lipoproteins or through external synthesis [27]. It has been shown that moderate to severe aerobic exercise reduced the levels of low-density lipoprotein (LDL), cholesterol, and triglycerides in adult men and women [28]. On the other hand, statin drugs inhibit the synthesis of the precursor of steroids by blocking the formation of mevalonate [29]. Kanat et al. (2009) reported that the hormonal effects of statins are related to their cholesterol-lowering effects [30].

Decreased levels of sex hormones have been reported in male athletes, therefore, possible impairment in male fertility is related to volume, intensity, and type of activity [31]. Intense exercise can lead to dysfunction of the male reproductive system. Some research has shown that long-term exercise lowers testosterone levels [32], which is consistent with the results of the present study. Evidence suggests that decreased gonadal function (gonads) is seen in men who are chronically or long-term involved in endurance training [33].

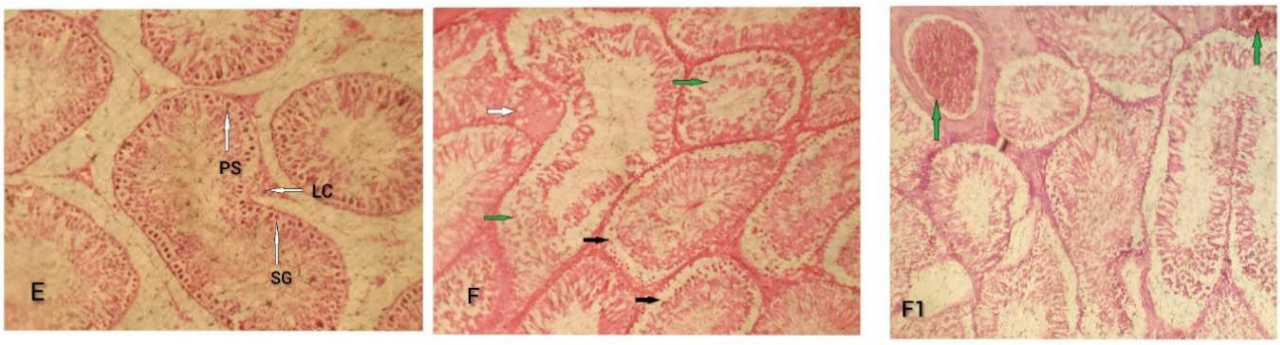

Figure 2. (E) Group E(the endurance training + atorvastatin5 mg/kg) (E),SG :Spermatogonia cell PS:Spermatocyte cell. LC: Leydig cells.(F) In Group F(the endurance training + atorvastatin10 mg/kg). (F) seminiferous tubules and interstitial tissue showed pathological changes. These included: disruption of the germinal epithelium(green arrows), rupture of the primary layer (black arrows), edema (white arrow). (F1)Group F congestion in the blood vessels (green arrows).

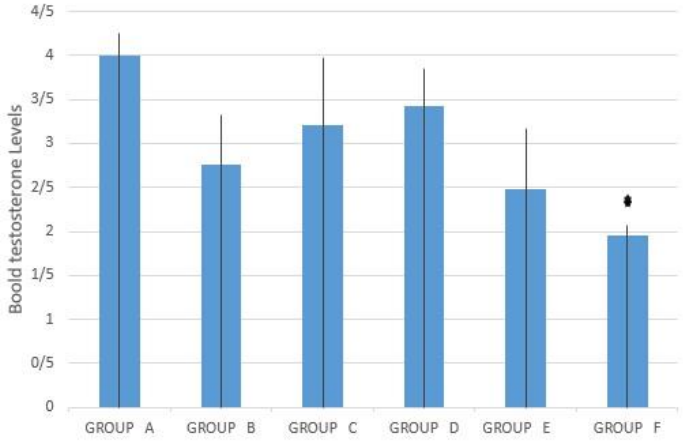

Figure 3. Blood testosterone (ng/ml) levels in different groups.

* Significant difference compared to the control group ($p < 0.05$)

Decreased serum testosterone levels have been reported during and after long-term activities and up to a few hours after intense short-term activities. Several mechanisms, including reducing gonadotropins, increasing cortisol, increasing catecholamine or prolactin levels, and even the accumulation of metabolic wastes, can affect testosterone synthesis [34].

Studies on the relationship between statins and testosterone production show conflicting results [27, 35]. Haipa et al. (2003) reported that 12 weeks of taking simvastatin reduced serum testosterone levels in men with high blood cholesterol [35]. Stanworth et al. (2009) also reported that total testosterone levels in men taking statin drugs decreased significantly compared with the control group [36]. The results of the above research are consistent with the results of the present study. However, Jay et al. (1991) reported that 12 weeks of pravastatin use did not alter serum testosterone levels [27]. The contradiction between the results of Jay study and the present study is probably related to the type of statin drug used. Atorvastatin has been used in the present study, while pravastatin has been used in the study of Jay et al. It has been shown that statins such as pravastatin have hydrophilic properties and their function is focused on the liver and are eliminated by other tissues, but lipophilic statins, such as atorvastatin, affect both the liver and areas outside the liver. It has also been shown that lowering cholesterol and blood lipids is better done by atorvastatin than other statins [37]. Atorvastatin is also a very potent statin that further lowers total cholesterol and thus reduces total testosterone levels. Studies show that atorvastatin has significant biological effects on testosterone [36].

Under normal physiological conditions, luteinizing hormone (LH) increases cholesterol taking by the testicls testosterone production stimulation. Decreased testosterone is perceived by the hypothalamic-pituitary axis and leads to further release of luteinizing hormone,

which maintains testosterone levels through a negative feedback loop. Factors such as TNF-α and interleukin-6 have been reported to inhibit the release of the luteinizing hormone from the pituitary gland, leading to decreased blood testosterone levels [36]. Safarinejad et al. (2009) reported that high-intensity exercise reduced free testosterone, LH, and FSH levels. They suggested that the hypothalamic-pituitary-testicular axis suppression, probably as a result of reducing the production of the gonadotropin-releasing hormone (GnRH), may reduced LH and FSH response to exercise [38].Testosterone is a cholesterol-derived hormone that has a steroid chemical structure. This hormone is produced by biochemical pathways in Leydig cells. About 95% of the testosterone in the bloodstream is produced in the testicles and the rest in the adrenal glands. Most circulating testosterone (about 98%) is bound to carrier proteins. The other two percent are freely circulating in the bloodstream, indicating the active biological form of the hormone [39]. Our findings show that the number of Spermatogonia and Spermatocyte decreased significantly in the Group (F). Testosterone plays an important role in development and proliferation germminal cells [40]. A further decrease in testosterone levels in the Group (F), as mentioned earlier, disrupts the spermatogenesis process, resulting in a greater decrease in the number of spermatogonia and Spermatocyte cells than in the other groups. These results were in agreement with a study done by Akdeniz et al that reported atorvastatin reduced the number of Sertoli, spermatogonia and Spermatocyte cells [41]. The study showed that the thickness of the seminiferous tubule in the Group (F) significantly reduced. The effect was probably the result of the decrease in the number of spermatogonia and Spermatocyte cells. Various testicular damages in the Group (F) were observed during this histological study, These damages included: rupture of the primary layer of cells in the tubules,disruption and irregularity of the germinal epithelium, edema between the seminiferous tubule, congestion in the blood vessels.

Leite et al. (2014) found that statin consumption was associated with histopathological changes in seminiferous tubule and the death of germ cells in the testicles of rats [42]. Das and Indira (2015) also reported that taking atorvastatin caused histopathological changes (interstitial cells edema) in rats testicles. They attributed these histopathological changes to the reducing effects of androgen (testosterone) of atorvastatin [43]. These results are consistent with the results of the present study. Ouf et al. (2015) also showed in a study that the use of atorvastatin in rats causes congestion and vasodilatation in the blood vessels of the testes [44]. Moreover the study showed that the number of Leydig cells decreased significantly in the Group (F). An increase in oxygen consumption and subsequent increase in the production of free radicals and reactive oxygen species as a result of aerobic exercise, as well as an increase in oxidative damage due to atorvastatin use that Pal et al. (2015) reported in the investigation [45], may causes histopathological changes in the present study and as a result reducing the number of Leydig cells and disruption of the spermatogenesis process, followed by a decrease in the number of spermatogonia and Spermatocyte cells in the endurance exersice + atorvastatin(10 mg/kg) group(Group (F)).

It seems that the possible double effects of endurance exercise and atorvastatin (10 mg/kg) leads to a further decrease in testosterone levels and testosterone has the the basic role on the function Sertoli cells. In the seminiferous tubules only Sertoli cells express receptors for testosterone thus these cells are the main targets signals that regulate spermatogenesis [46]. In addition Sertoli cells form tight junctions with other cell in result to the organization of testicullar structure [47]. Thus This is a possibility that decrease in testosterone levels has negative effects on the function Sertoli cells that, leads to a reduce the number of spermatogonia and Spermatocyte cells, as well as morphological disruption of testicular tissue in the Group (F).

4. Conclusion

The results of the present study showed that the combination of endurance training and atorvastatin (10 mg/kg) causes degeneration of testicular morphology, followed by a decrease in the number of Leydig, spermatogonia, Spermatocyte cells and serum testosterone levels in male rats. These changes can lead to reduced reproductive activity in males. Therefore, in cases where there is a fear of infertility and reduced reproductive function, in order to reduce lipid profile and reduce the risk of cardiovascular disease, it is recommended that avoid simultaneous use of high dose statin drugs and endurance training.

Acknowledgements

The authors are very grateful to all those who provided the financial and administrative facilities for the present study.

Conflict of interest

The authors (Amin Abdi, Hadi Abdi and Nabi Shamsaei) declare that they have no conflict of interest.

References

1. Kishi T, Hirooka Y, Konno S, Sunagawa K. Atorvastatin improves the impaired baroreflex sensitivity via anti-oxidant effect in the rostral ventrolateral medulla of SHRSP. Clin Exp Hypertens. 2009;31(8):698-704.

2. Rosenson RS, Farkouh ME, Mefford M, Bittner V, Brown TM, Taylor B, Monda KL, Zhao H, Dai Y, Muntner P. Trends in Use of High-Intensity Statin Therapy After Myocardial Infarction, 2011 to 2014. J Am Coll Cardiol 2017; 69(22): 2696-2706.
3. Grip O, Janciauskiene S, Bredberg A. Use of atorvastatin as an anti-inflammatory treatment in Crohn's disease. Br J Pharmacol. 2008;155(7):1085-92.
4. Li J, Sun YM, Wang LF, Li ZQ, Pan W, Cao HY. Comparison of effects of simvastatin versus atorvastatin on oxidative stress in patients with coronary heart disease. Clin Cardiol. 2010;33(4):222-7.
5. Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol. 2005;45:89-118.
6. Banihani SA. Effect of statin on semen quality characteristics. Andrologia 2020; 52(6): e13592.
7. Graveline D. Cholesterol-lowering injectables:more harm than good? Journal of AmericanPhysicians and Surgeons 2015; 20(4):119–22.
8. Zhao SP, Wu ZH, Hong SC, Ye HJ, Wu J. Effect of atorvastatin on SR-BI expression and HDL-induced cholesterolefflux in adipocytes of hypercholesterolemic rabbits. Clin Chim Acta. 2006;365(1-2):119-24.
9. Kanat M, Serin E, Tunckale A, Yildiz O, Sahin S, Bolayirli M, Arinc H, Dirican A, Karagoz Y, Altuntas Y, Celebi H, Oguz A. A multi-center, open label, crossover designed prospective study evaluating the effects of lipid lowering treatment on steroid synthesis in patients with Type 2 diabetes (MODEST Study). J Endocrinol Invest. 2009;32(10):852-6 .
10. Ramezani Moghadam M, Heydarieh N, Khoshsokhan M. The Effect of Atorvastatin on Depression by Forced Swimming Stress model in Gonadectomized Male Mice, Journal of Shahid Sadoughi University of Medical Sciences. 2016;24(5):429-40.
11. Kawa F, Dizaye KF, Ahmed SR. Combination of atorvastatin and fenofibrate altered androgenic activities of male rats. Zanco J Med Sci, 2019;23(2):273-82.
12. Wilund KR, Feeney LA, Tomayko EJ, Weiss EP, Hagberg JM. Effects of endurance exercise training on markers of cholesterol absorption and synthesis. Physiol Res. 2009;58(4):545-52 .
13. Kraemer WJ, Ratamess N. Hormonal responses and adaptations to resistance exercise and training.Sports Med. 2005;35:(4)339-61.
14. Chwalbinska-Moneta J, Kruk B, Nazar K, Krzeminski K, Kaciuba-Uscilko H, Ziemba A. Early effects of short-term endurance training on hormonal responses to graded exercise. J Physiol Pharmacol. 2005;56(1):87-99 .
15. Swain D, Barry A, Franklin M. Comparison of cardioprotective benefits of vigorous versus moderate intensity aerobic exercise. Am J Cardiol. 2006;97:141-7.
16. Mirghani SJ, Agha alinejad H, Arshadi S, Ayaz A, Gerayli Korpi J, Fakourian A. Effect eight weeks strength, endurance and concurrent training on blood serum cortisol/testosterone ratio and muscular fitness in soldiers wrestler, J Army Univ Med Sci. 2013;11(3):211-18.
17. Hajizadeh Maleki B, Tartibian B, Chehrazi M. The effects of three different exercise modalities on markers of male reproduction in healthy subjects: a randomized controlled trial. Reproduction. 2017;153(2):157-74.
18. Matos B, Howl J, Ferreira R, Fardilha M. Exploring the effect of exercise training on testicular function. Eur J Appl Physiol. 2019;119(1):1-8.
19. Samadian Z, Tofighi A, Razi M, Tolouei Azar J, Ghaderi Pakdel F. Moderate intensity exercise training ameliorates the diabetes suppressed spermatogenesis and improves sperm parameters: Insole and simultaneous with insulin. Andrologia, 2019;51(11):e13457.
20. Toyama K, Sugiyama S, Oka H, Iwasaki Y, Sumida H, Tanaka T, et al. Combination treatment of rosuvastatin or atorvastatin, with regular exercise improves arterial wall stiffness in patients with coronary artery disease. PLoS One. 2012;7:e41369.
21. Guazzi M, Tumminello G, Reina G, Vicenzi M, Guazzi M. Atorvastatin therapy improves exercise oxygen uptake kinetics in post-myocardial infarction patients. Eur J Clin Invest. 2007;37:454-62.
22. Sharma, R. D, Raghuram, T. C., Dayasagar RaoV, 1991. Hypolipidaemic effect of fenugreek seeds. A clinical study. Phytother,3, PP:145-147.
23. Kregel K, Allen D, Booth F, Fleshner M, Henriksen E, O' Leary D, et al. Resource book for the design of animal exercise protocols. American Physiological Society. 2006:23-57.
24. Aboutaleb N, Shamsaei N, Khaksari M, Erfani S, Rajabi H, Nikbakht F. Pre-ischemic exercise reduces apoptosis in hippocampal CA3 cells after cerebral ischemia by modulation of the Bax/Bcl-2 proteins ratio and prevention of caspase-3 activation. J Physiol Sci. 2015;65(5):435-43.
25. Pearse AE. Histochemistry: Theoritical and applied. Analytical technology. 4th ed. Edinburgh: Churchill-Livingstone. 1985:441-555
26. Costa D.S., Paula T.A., Matta S.L. The intertubular compartment morphometry in capybaras (Hydrochoerus hydrochaeris) testis. Anim Reprod Sci.2006;91(1- 2):173-9..
27. Jay RH, Sturley RH, Stirling C, McGarrigle HH, Katz M, Reckless JP, Betteridge DJ. Effects of pravastatin and cholestyramine on gonadal and adrenal steroid production in familial hypercholesterolaemia. Br J Clin Pharmacol. 1991;32(4):417-22 .
28. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc. 2001;33(6 Suppl):S502-15 .
29. La Vignera S, Condorelli RA, Vicari E, Calogero AE. Statins and erectile dysfunction: a critical summary of current evidence. J Androl. 2012;33(4):552-8 .
30. Kanat M, Serin E, Tunckale A, Yildiz O, Sahin S, Bolayırlı M, et al. A multi-center, open label, crossover designed prospective study evaluating the effects of lipid lowering treatment on steroid synthesis in patients with Type 2 diabetes (MODEST Study). J endocrinological invest. 2009;32(10):852-6.
31. Manna I, Jana K, Samanta, PK. Effect of different intensities of swimming exercise on testicular oxidative stress and reproductive dysfunction in mature male albino Wistar rats. Indian J Expe Bio. 2004;42:816-22.
32. du Plessis S, Kashou A, Vaamonde D, Agarwal A. Is there a link between exercise and male factor infertility? Open Reproductive Sci J. 2011;3:105-13.
33. Hackney AC. Effects of endurance exercise on the reproductive system of men: the exercise-hypogonadal male condition. J Endocrinol Invest. 2008;31(10):932-8 .
34. Cumming DC, Wheeler GD, McColl EM. The effects of exercise on reproductive function in men. Sports Med. 1989;7(1):1-17 .
35. Hyyppa 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 .
36. 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(4):541-6 .
37. Shuhaili MFRMA, Samsudin IN, Stanslas J, Hasan S, Thambiah SC. Effects of different types of statins on lipid profile: a perspective on Asians. Int J Endocrinol Metab. 2017;15(2): e43319.
38. Safarinejad MR, Azma K, Kolahi AA. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus–pituitary– testis axis, and semen quality: a randomized controlled study. J Endocrinology. 2009;200(3):259-71.
39. Hackney AC. The male reproductive system and endurance exercise. Med Sci Sports Exerc. 1996;28(2):180-9 .
40. McLachlan RI, O'Donnell L, Meachem SJ, Stantontification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Horm Res. 2002;57:149-79
41. Ekrem Akdeniz, Mehmet Emin Onger, Mustafa Suat Bolat, Fatih Firat, Metin Gur, Onder Cinar, Mustafa Bakirtas5, Abdullah

Acıkgoz, Fikret Erdemir. Tropical Journal of Pharmaceutical Research December 2020; 19 (12): 2609-2614
42. Leite GAA, de Lima Rosa J, Sanabria M, Cavariani MM, Franci JAA, Pinheiro PFF, et al. Delayed reproductive development in pubertal male rats exposed to the hypolipemiant agent rosuvastatin since prepuberty. Reproductive toxicology. 2014;44:93-103.
43. Das SS, Indira M. Combined effects of atorvastatin and nicotine on testicular expression of steroidogenic enzymes in experimental rats. Int J Pharm Sci Research. 2015;6(6):2511.
44. Ouf A, Mohammed A, Hanafi S. Effect of atrovastatin on the testes od adult male aAlbino rats and the possible protective effect of vitamin E. Al-Azhar Assiut Med J. 2015;13(4):100-18.
45. Pal, S.; Ghosh, M.; Ghosh, S.; Bhattacharyya, S. and Sil, P. Atorvastatin induced hepatic oxidative stress and apoptotic damage via MAPKs, mitochondria, calpain and caspase12 dependent pathways.Food and Chemical Toxicology.; (2015): 83 : 36-47.
46. William H Walker and Jing Cheng ,FSH and testosterone signaling in Sertoli cells, Reproduction (2005) 130 15–28
47. Holstein AF, Schulze W and Davidoff M: Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol 2003, 1: 107.