International Journal of Research and Review
Vol. 10; Issue: 5; May 2023
Website: www.ijrrjournal.com
E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Vitamin E Increases Leydig Cells and Testosterone in Rats with Excessive Exercise

Naomi Hutabarat[1], Ida Sri Iswari[2], Ni Putu Sriwidyani[3]

[1]Master Program in Biomedical Science, Anti-Aging Medicine Concentration, [2]Department of Clinical Microbiology, [3]Department of Anatomical Pathology;
Faculty of Medicine, Udayana University, Bali, Indonesia

Corresponding Author: Naomi Hutabarat

DOI: https://doi.org/10.52403/ijrr.20230551

ABSTRACT

Background. Excessive exercise increases oxidative stress, which affects cell damage, including Leydig cells. Damage to Leydig cells leads to a drop in testosterone levels. Vitamin E is known to protect spermatogenesis by inhibiting Leydig cell apoptosis and lipid peroxidation. This study investigates vitamin E efficacy in increasing Leydig cells count and testosterone levels in male Wistar rats exposed to excessive physical exercise.

Methods. Thirty-six male Wistar rats were divided into the control and treatment groups in this posttest-only control group experimental study. The control group was a group that received excessive exercise and 1 ml/day of distilled water once daily. The treatment group was a group that received excessive exercise and vitamin E (20 mg/day) dissolved in distilled water up to 1 ml once daily. Excessive exercise in this study was swimming ≥ 60 minutes a day in an 80 cm x 50 cm pool with 60 cm deep. After 40 days; Leydig cells number and testosterone levels were examined and compared between groups.

Results. Leydig cells' mean value in the treatment group was 2.6 times higher than the control (10.23±0.73 vs. 3.83±0.67; p<0.001). For testosterone levels, the mean value was 2.3 times higher in the treatment group compared to control (11.42±0.27 ng/mL vs. 4.94±0.21 ng/mL; p<0.001).

Conclusions. Oral administration of vitamin E (20 mg/day) for 40 days increased Leydig cell number and testosterone levels in Wistar rats with excessive physical exercise compared to controls. Vitamin E might be considered a treatment for male fertility

Keywords: antioxidant; exercise; testosterone; vitamin E

INTRODUCTION

Switching to a healthier lifestyle is currently a trend among young adults. Exercise is one of the most popular lifestyles due to its comprehensive effects on health.[1,2] Nevertheless, excessive exercise has adverse effects, especially on male reproductive systems. Excessive physical exercise defined as a high-intensity physical activity without adequate rest, which leads to decreased physical performance and fatigue. According to the literature, appropriate exercise should be moderate and not exceed 6 hours per week. Proper exercise is based on frequency, intensity, time, and type (FITT). Frequency 3-5 times/week, moderate intensity (72-87% of maximum heart rate), 30-60 minutes/day; examples of exercise types include swimming or cycling. Physical training is excessive when the frequency, intensity, time, and type exceed the FITT principle.[6] Excessive exercise triggers free radicals that lead to oxidative stress, promoting rapid aging. During physical exercise, carbon dioxide production increases, lactic acid accumulates, and hemoglobin oxygen saturation decrease, increasing oxygen demand. Excessive exercise increases oxygen consumption 100- to 200-fold, which triggers oxidative stress that damages cells in the body if continued.[7] Excessive exercise leads to DNA damage that results in cell death.[8,9]

In men, excessive exercise can damage Leydig cells, resulting in a decrease in testosterone. In Leydig cells, cell death occurs through three pathways: DNA damage due to the formation of inflammatory factors, the formation of reactive oxygen species (ROS), and DNA damage mediated by NADPH oxidase.[8] Lower testosterone levels manifest in physical or psychological impairments such as decreased energy, muscle strength, sexual function, mood, and a decrease in overall quality of life.[10]

Vitamin E is known as a non-enzymatic antioxidant that effectively counteracts the adverse effects (oxidation of lipids, membranes, and DNA) of oxidative stress. Thus, may reducing or preventing the damage of cell tissues, including Leydig cells, by oxidative reactions.[11,12] Vitamin E is essential in the biosynthesis of pituitary gonadotropins and testosterone. The receptors for vitamin E in the endocrine system are located in the pituitary gland. In mice deficient in vitamin E, the degeneration of Leydig cells is decreased, which may lead to a decrease in testosterone production.[13]

The efficacy of vitamin E in protecting spermatogenesis has been demonstrated in various animal studies with different exposure to oxidative stress.[4,14,15] However, the effect of vitamin E in excessive exercise as oxidative stress has rarely been studied. As knowledge of the health benefits of exercise increases, so does the need for information on the andrological consequences of exercise. Based on this description, this study evaluated the efficacy of vitamin E administration on Leydig cell number and testosterone levels in male Wistar rats subjected to excessive physical exercise.

MATERIALS & METHODS

Study design and experimental animals

The study is a true experimental design using randomized posttest-only control group. The study was conducted at the Integrated Biomedical Laboratory, Udayana University, Bali, Indonesia. All research procedures were approved by the Ethics Committee of Udayana University (B/231/UN14.2.9/ PT.01.04/2022).

The experimental animals were healthy male Wistar rats (*Rattus norvegicus*), 3-4 months old with a body weight ranging from 150 to 200 grams. The Wistar rats aged 3-4 months were adult rats that were sexually mature and suitable for adult reproductive system research. For this study, 32 male Wistar rats were needed. However, 10% was increased to 36 rats to prevent dropout.

Material and Procedure

The vitamin E (α-tocopherol) used in this study was 100 mg of vitamin E in capsule form manufactured by PT. Indofarma. The dose administered to the rats was 20 mg/day for 40 days.[11] The excessive exercise in this study consisted of swimming for 60 minutes daily in a pool size 80 cm x 50 cm with a water depth of 60 cm.

Wistar rats (n = 36 animals) were divided into two equal groups (n = 18 animals): the control and treatment groups. The control group, i.e., the group that received excessive exercise, was then administered 1 ml of distilled water once daily. The treatment group was the group that received excessive exercise and received oral vitamin E once daily at a dose of 20 mg/day dissolved in 1 ml of distilled water. The intervention lasted for 40 days. After 40 days, the testicular tissue of the Wistar rats was collected to count the number of Leydig cells, and venous blood was collected from the medial canthus orbitalis to determine testosterone levels.

Assessment

Leydig cell number was measured using

histological examination stained with hematoxylin-eosin (HE). The number of Leydig cells was observed and counted using a microscope (OptiLab, Miconos, Yogyakarta, Indonesia) by adding the total number of Leydig cells from the best five large fields of view (HPF) of the right and left testis using a zigzag method. These observations were observed under a 400× magnification microscope on the right and left testis and then averaged to determine the number of Leydig cells per HPF using ImageRaster version 4.0 software (Miconos, Yogyakarta, Indonesia).
The testosterone level was examined using the rat testosterone enzyme-linked immunosorbent assay (ELISA) kit (BT-LAB, Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd, Shanghai, China). The absorbance value of the color density related to the spectrum at a wavelength of 450 nm is determined using a spectrophotometer.

STATISTICAL ANALYSIS

Statistical analysis was performed with IBM SPSS statistics for Mac version 26 (IBM Corp, NY). The numerical variables were displayed using the central tendency parameters and standard deviation. The comparability test was assessed using an independent t-test to examine the difference between group.

RESULT

The distribution and homogeneity of Leydig cells number and testosterone level were normal and homogeneous in each group. The comparison test for the number of Leydig cells and testosterone level was performed to compare the mean values of each variable in the control and treatment groups. Significance analysis was performed using the independent t test. The results of the comparison test between the groups are shown in Table 1.

Table 1. Comparative Analysis of Leydig Cell and Testosterone

Variable	Groups		P
	Control	Treatment	
	Mean±SD	Mean±SD	
Leydig Cell Number (/HPF)	3.83±0.67	10.23±0.73	<0.001
Testosterone Level (ng/mL)	4.94±0.21	11.42±0.27	<0.001

Comparative analysis result conveyed that the average of Leydig cell number and testosterone level were higher in the treatment group than in the control ($p < 0.001$). The histological appearance of testicular tissue samples from both groups indicated that interstitial Leydig cells count was denser in the treatment group compared to control group (Figure 1).

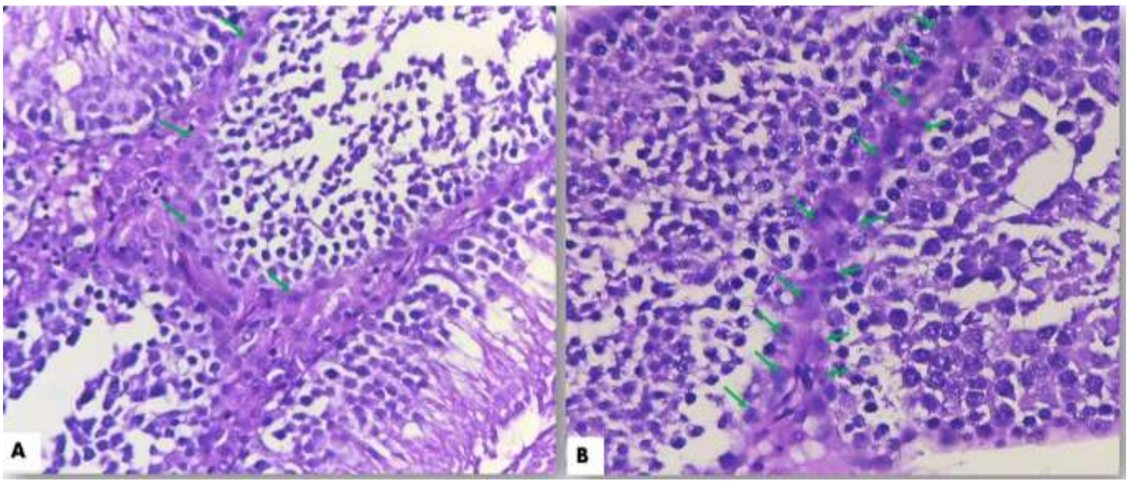

Figure 1. Comparison of Histological Features of Leydig Cells.

The picture includes control (A) and treatment (B). Interstitial Leydig cells are shown in green arrows in fig. The number of Leydig cells appeared denser in the treatment group than in the control at 400x magnification using HE staining.

DISCUSSION

The average number of Leydig cells in the control group (with excessive exercise) was 3.83±0.67 cells/HPF. Isdadiyanto and Tana (2020) revealed that the number of normal Leydig cells in male Wistar rats at 8-12 weeks of age was reported to be 14.70±2.65 cells.[16] The average testosterone level in a study conducted by Stephen et al. (2021) in male Wistar rats at three months of age was 10.03±1.28 ng/ml, while in this study, the serum testosterone level in control rats with excessive exercise was 4.94±0.21 ng/ml.**[17]** This shows that excessive exercise for 40 days reduces Leydig cell number and serum testosterone in male Wistar rats.

Physical exercise increases carbon dioxide production, causing lactic acid to accumulate, and decreases hemoglobin oxygen saturation, increasing oxygen demand. During excessive exercise, oxygen consumption increases 100- to 200-fold. This condition can trigger oxidative stress, and if it continues continuously, cells in the body will eventually be damaged.**[7]** Excessive physical training has been shown to induce DNA damage that leads to cell death.**[1,4]** Conditions of continuous overtraining or over-exercising alter the redox balance by accumulating excessive oxidative stress, resulting in damage to cells and tissues, including Leydig cells.**[18]**

The results showed that the average number of Leydig cells and testosterone levels were significantly higher in the treatment group ($p > 0.05$) than in the control group. These results suggest that administering 20 mg/day of vitamin E to Wistar rats in conjunction with excessive exercise for 40 days could significantly increase Leydig cells and testosterone levels compared to the control. In line with prior studies that showed the efficacy of oral administration of alpha-tocopherol to increase testosterone levels in Wistar rats compared to the control group. However, this study did not examine excessive exercise.**[11]**

Antioxidants are needed to protect ROS from excessive exertion. Vitamin E is a potent fat-soluble antioxidant because it can scavenge free radicals by acting as an electron donor that stabilizes lipid peroxidase so that free radicals are not formed.**[13,19]** Vitamin E is also one of the natural antioxidants in sperm, protecting sperm from free radicals, preventing lipid peroxide formation and damage by ROS, and stored in mitochondrial and microsomal membranes.**[11,20,21]** Vitamin E contributes to the inhibition of oxidative stress in testicular tissues, plays a distinguish role in spermiogenesis, also prevents apoptosis induction, particularly in germinal epithelium. Vitamin E inhibiting the spermatogenic cells, Sertoli cells, and Leydig cells oxidative damage.**[22]** Deficiency of vitamin E in testicular tissues generates oxidative stress, hence reducing the testosterone synthesis.

This study acknowledges limitations. Even though we refer to a dose of 20 mg/day for 40 days, studies with various doses to determine the optimal dose need to be carried out to prove the most appropriate effect of vitamin E and the side effects that may arise. Further human studies are needed to evaluate whether vitamin E provides similar benefits.

CONCLUSION

The influence of the social environment on aspects of self-image encourages the trend of exercising as a lifestyle considered positive. As a result, it is common for someone to exercise excessively as a quick method to achieve the desired goal. However, excessive exercise has a negative impact due to increased oxidative stress, which ultimately affects cell damage, one of which is Leydig cells. Damage to Leydig cells can impact decreasing testosterone levels in men. In this study, the mean

Leydig cells number and testosterone levels of male Wistar rats given oral vitamin E (20 mg/day for 40 days) and excessive physical exercise were higher than the controls. Vitamin E can block the adverse effects of excessive physical exercise.

Declaration by Authors

Ethical Approval: Approved
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Jóźków P, Rossato M. The Impact of Intense Exercise on Semen Quality. Am J Mens Health 2017;11:654. https://doi.org/10.1177/1557988316669045.
2. Paley CA, Johnson MI. Abdominal obesity and metabolic syndrome: exercise as medicine? BMC Sports Sci Med Rehabil 2018;10:7. https://doi.org/10.1186/s13102-018-0097-1.
3. Yi X, Tang D, Cao S, Li T, Gao H, Ma T, et al. Effect of Different Exercise Loads on Testicular Oxidative Stress and Reproductive Function in Obese Male Mice. Oxid Med Cell Longev 2020;2020: e3071658. https://doi.org/10.1155/2020/3071658.
4. Ismalia KR, Pangkahila W, Sriwidyani NP. Oral administration of Bali Robusta coffee (Coffea canephora) extract prevented the reduction of Leydig cells and testosterone levels in male Wistar rats (Rattus norvegicus) with excessive physical training. Neurol Spinale Medico Chir 2021;4:37–41. https://doi.org/10.36444/nsmc.v4i1.151.
5. Joseph A-M, Nguyen LM-D, Welter AE, Dominguez JM, Behnke BJ, Adhihetty PJ. Mitochondrial adaptations evoked with exercise are associated with a reduction in age-induced testicular atrophy in Fischer-344 rats. Biogerontology 2014;15:517–34. https://doi.org/10.1007/s10522-014-9526-z.
6. Adhikari SP, Tretriluxana J, Dev R, Eglitis E, Shrestha N, Kerfeld C. Fitt-correct: updated dynamic and evidence-based principle of exercise prescription. J Nov Physiother Rehabil 2021;5:005–9.
7. Pangkahila W. Tetap Muda, Sehat dan Berkualitas: Konsep Anti-Aging Medicine. Jkt Kompas Media Nusant 2017.
8. Dong J, Chen P, Wang R, Yu D, Zhang Y, Xiao W. NADPH oxidase: a target for the modulation of the excessive oxidase damage induced by overtraining in rat neutrophils. Int J Biol Sci 2011;7:881.
9. Giri MKW. Immunological side in overtraining exercise. Int J Health Med Sci 2019;2:1–6.
10. Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biol Reprod 2018;99:101–11.
11. Zubair M. Effects of dietary vitamin E on male reproductive system. Asian Pac J Reprod 2017;6:145.
12. Kataoka T, Hotta Y, Kimura K. A Review of foods and food supplements increasing testosterone levels 2021.
13. Hidayatik N, Purnomo A, Fikri F, Purnama MTE. Amelioration on oxidative stress, testosterone, and cortisol levels after administration of Vitamins C and E in albino rats with chronic variable stress. Vet World 2021;14:137.
14. Akindele DOO. Effects of vitamin E and melatonin on serum testosterone level in sleep deprived Wistar rats. Afr J Med Med Sci 2014;43:295–304.
15. Acharya UR, Mishra M, Patro J, Panda MK. Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. Reprod Toxicol 2008;25:84–8.
16. Isdadiyanto S, Tana S. Jumlah Sel Leydig dan Mikroanatomi Testis Tikus Putih (Rattus norvegicus) Setelah Pemberian Teh Kombucha Konsentrasi 50% Waktu Fermentasi 6, 9 dan 12 Hari. Bul Anat Dan Fisiol 2020;5:67–74. https://doi.org/10.14710/baf.5.1.2020.67-74.
17. Stephen AO, Rotimi OA, Adegoke AT, Samson OO. Reprotoxic activities of vildagliptin administration in male Wistar rats. Braz J Pharm Sci 2021;57.
18. Wang J-Y, Lee Y-J, Chou M-C, Chang R, Chiu C-H, Liang Y-J, et al. Astaxanthin protects steroidogenesis from hydrogen peroxide-induced oxidative stress in mouse Leydig cells. Mar Drugs 2015;13:1375–88.
19. Abdelazim AM, Saadeldin IM, Swelum AA-A, Afifi MM, Alkaladi A. Oxidative stress in the muscles of the fish Nile tilapia caused by zinc oxide nanoparticles and its

modulation by vitamins C and E. Oxid Med Cell Longev 2018;2018.
20. Chatziralli IP, Theodossiadis G, Dimitriadis P, Charalambidis M, Agorastos A, Migkos Z, et al. The Effect of Vitamin E on Oxidative Stress Indicated by Serum Malondialdehyde in Insulin-dependent Type 2 Diabetes Mellitus Patients with Retinopathy. Open Ophthalmol J 2017;11:51–8. https://doi.org/10.2174/1874364101711010051.
21. Medina J, Gupta V. Vitamin E. StatPearls, Treasure Island (FL): StatPearls Publishing; 2022.
22. Malmir M, Mehranjani MS, Faraji T, Noreini SN. Antioxidant effect of Vitamin E on the male rat reproductive system by a high oral dose of Bisphenol-A. Toxicol Res Appl 2021;5:23978473211005560.

How to cite this article: Naomi Hutabarat, Ida Sri Iswari, Ni Putu Sriwidyani. Vitamin E increases Leydig cells and testosterone in rats with excessive exercise. *International Journal of Research and Review*. 2023; 10(5): 449-454. DOI: *https://doi.org/10.52403/ijrr.20230551*

******