Hormozgan Medical Journal

doi 10.34172/hmj.2023.8232

Hormozgan Med J. 2023; 27(3):146-152

Original Article

Vitamin D Supplementation Combined With Aerobic Training Increases the Gene Expression of Antioxidants in Kidney Tissue in Healthy Middle-Aged Rats

Amin Molaie[1], Abbas Ali Gaeini[2], Reza Nouri[2*], Mohammad Hemmatinafar[3]

[1]Department of Sport Sciences, Kish International Campus, University of Tehran, Kish, Iran
[2]Department of Exercise Physiology, Faculty of Sport Sciences, University of Tehran, Tehran, Iran
[3]Department of Sport Sciences, Faculty of Psychology and Educational Sciences, University of Shiraz, Shiraz, Iran

***Correspondence to**
Reza Nouri,
Email: nuri_r7@ut.ac.ir

Abstract

Background: Both exercise and vitamin D can affect oxidative stress indicators. However, their interaction at the level of kidney tissue has been less investigated. Hence, the main objective of the present study was to determine the effect of vitamin D supplementation and aerobic training on oxidative stress markers in the kidney tissue of middle-aged male rats.

Methods: To conduct this study, 20 Wistar male rats)14–12 months and 350–400 grams (were randomly divided into four groups: control (CON), aerobic training (EXE), vitamin D (VD), and aerobic training + vitamin D (EXE + VD). The exercise consisted of 8 weeks of aerobic training (5 days/week). The VD and EXE + VD groups received vitamin D (500 units/kg) daily. Olive oil was also used as a placebo, and 48 hours after the last intervention session, animal tissues were removed. The gene expression of oxidative stress biomarkers, including catalase (CAT), manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPx), and xanthine oxidase (XO) was measured by the real-time polymerase chain reaction (PCR) method. Data analysis was performed by one-way analysis of variance test at the significance level of $P<0.05$.

Results: The results showed that the EXE + VD increased the gene expression of Mn-SOD, CAT, and GPx ($P<0.05$) and decreased the expression of XO ($P<0.05$). Furthermore, VD alone had no effect on the expression of antioxidant enzymes or XO ($P>0.05$). Moreover, EXE increased CAT and Mn-SOD expression compared to the CON ($P<0.05$) and decreased XO expression ($P<0.05$). Additionally, CAT values in EXE were significantly higher than those in VD ($P<0.05$).

Conclusion: According to the results of the present study, it seems that the VD + EXE can increase antioxidant defense and reduce oxidative stress in kidney tissue.

Keywords: Aerobic exercise, Antioxidant, Oxidative stress, Kidney, Vitamin D

Received: October 27, 2022, **Accepted:** November 21, 2022, **Published Online:** May 8, 2023

Background

In mammals, the kidneys act as a major excretory organ to remove and refine the body's metabolic wastes (1). Most animal species die after one week of complete renal failure (2). Moreover, the balance of electrolytes, hormonal secretions such as renin, erythropoietin, and prostaglandin, along with the activation of vitamin D are important functions of the kidneys (1, 2). However, it has been demonstrated that with increasing age and aging, as in other tissues of the body, structural and functional changes occur in kidney tissue that can be associated with the incidence of kidney disease (1).

One of the underlying mechanisms of aging, which also increases in middle age, is damage caused by oxidative stress (3). Mammalian cells (e.g., kidney cells) often rely on aerobic metabolism (1). During aerobic metabolism, in addition to water and energy, some by-products called reactive oxygen species (ROS) are formed that are involved in inducing oxidative stress in kidney cells (1, 4). The synthesis of uric acid as a final product of purine metabolism can also increase oxidative stress directly by xanthine oxidoreductase activity (1). This enzyme is synthesized as xanthine dehydrogenase, which can be converted to xanthine oxidase (XO) by calcium-dependent proteolysis or the modification of cysteine residues (1). In doing so, the enzyme loses its capacity to bind nicotinamide adenine dinucleotide and instead transfers electrons to O2, producing the superoxide anion ($O2^-$), one of the most important ROS (1). Therefore, XO measurement is known as one of the important indicators in monitoring oxidative stress of kidney tissue. Under physiological conditions, the produced ROS are mainly inactivated through intracellular and extracellular defense mechanisms, namely, antioxidants

(1, 3, 5). However, the excessive ROS formation or the inability of antioxidants to remove them can lead to large-scale oxidative damage to cellular molecules, including membrane lipids, proteins, and DNA (1, 4, 6). These injuries lead to cell dysfunction due to the reduced function of various organs of the body and the occurrence of various diseases (1, 4). Therefore, strengthening antioxidant pathways is extremely crucial to prevent and combat kidney disorders, especially in middle age. The most important enzymes in the antioxidant defense system include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (1).

SOD generally contains three isoenzymes: manganese superoxide dismutase (Mn-SOD), zinc superoxide dismutase (Zn-SOD), and copper superoxide dismutase (Cu-SOD) (1, 4). The main difference between these enzymes is their location at the cellular level, but functionally they all reduce the superoxide radical to a more stable radical called hydrogen peroxide (4) which is converted to water and molecular oxygen by CAT or GPx, using glutathione as a substrate (1, 4). Therefore, the coordinated action of SOD, GPx, and CAT is extremely important to maintain the oxidant-antioxidant balance of the body cells. For example, mitochondrial oxidative stress due to Mn-SOD dysfunction was found to be involved in the pathogenesis of several renal diseases, including acute kidney injury (AKI) and chronic kidney disease (CKD), as well as in the transmission of AKI to CKD (4, 7). Mn-SOD dysfunction is also associated with glomerular fibrosis and interstitial tubules, inflammation, excessive renal cell apoptosis, and tubular cell damage (4). Therefore, strategies that increase protein levels and enzyme activity of Mn-SOD CAT and renal GPx can play an important role in the prevention of kidney disease. It has been suggested that vitamin D is effective as one of the strategies to prevent diseases related to oxidative stress and inflammation in kidney tissue (8). This vitamin is involved in reducing oxidative stress by increasing NrF-2 and expressing genes encoding antioxidant enzymes (8). It has also been found in animal models that pretreatment with vitamin D3 prevents the decrease in the expression of SOD genes caused by AKI (9). Therefore, vitamin D supplementation may be useful for the upregulation of the expression of antioxidant genes such as SOD, CAT, and GPx.

Interestingly, metabolic stresses induced by exercise also stimulate the expression of antioxidant genes. In addition, exercise is one of the most important strategies proposed to improve kidney function or prevent kidney disease. However, the available evidence suggests inconsistent information regarding the effect of exercise on oxidative stress in renal tissue. For example, some studies have demonstrated an improvement in antioxidant profile (6, 10, 11) and a reduction in oxidative stress (12, 13) after exercise, but others have not exhibited significant changes in antioxidants (12, 14, 15). Given these inconsistent findings, further studies are needed. Further, since exercise and vitamin D supplementation both affect the regulation of oxidative stress and antioxidant defense, the question arises as to whether vitamin D supplementation combined with aerobic exercise can further stimulate the gene expression of antioxidants in the kidney. Thus, the present study aimed to clarify the effect of vitamin D supplementation and aerobic training on oxidative stress markers in the kidney tissue of middle-aged male rats.

Material and Methods

In the present study, 20 male rats (Wistar breed) with an age range of 14–12 months and a weight range of 350–400 g were purchased from Pasteur Animal Breeding Center. After two weeks of identification, maximum velocity test, and weight measurement, they were randomly divided into four groups: control (Con), aerobic training (EXE), vitamin D (VD), and aerobic training + vitamin D (EXE + VD) with five rats in each group.

Storage and Tissue Removal Methods

After marking polycarbonate cages for rats separately (3 rats in one cage), they were maintained at 25°C and an ambient humidity of 30-40% with a 12-hour dark and 12-hour light cycle and free access to water and food. The animals were weighed at the start of each week with a digital scale for animals. The diet of rats included the standard feeding pattern of rats prepared from the Behvarvar food industry, consisting of a total of 16.6 kJ/g: carbohydrate 66.40%, Fat 10.60%, and protein 23%. Then, 48 hours after the last intervention session, the animals were anesthetized (after 12 hours of fasting) with xylazine (10 mg/kg) and ketamine (75 mg/kg), and the kidney tissues were removed and kept at -80°C.

Exercise Protocol

To adapt the animals to environmental conditions such as changing the dark cycle to light, two weeks of familiarity with the laboratory environment and two weeks of familiarity with exercise on a treadmill were considered. Two weeks of familiarity with the exercise included a training program of 10 sessions for 10 minutes in each session with a zero-degree slope and a speed of 5-7 meters per minute in the first week and a speed of 8-10 meters per minute in the second week. The main training program consisted of 8 weeks and 5 days per week. The training period started with 20 minutes in the first session, and one minute was added to each session, so at the beginning of the seventh week, a maximum time of 50 minutes was reached, which was maintained until the eighth week. The highest intensity of exercise was at the beginning of the seventh week, which was maintained until the end of the eighth week. The maximum velocity was determined according to the Wisløff VO2max test

(16). Five minutes of warm-up time and 5 minutes of cooling- down time with 40-50% VO2max (10 meters per minute) were considered for each exercise session.

Vitamin D Supplementation Method

Vitamin D supplementation was performed after the second week of familiarity. At the beginning of each week after weighing the animals, the amount of vitamin D at the rate of 500 units (12.5 micrograms) per kilogram per day was calculated and divided into three servings with 0.3 mL of olive oil. The supplement was administered to the animals by oral gavage every other day. It should be noted that the animals of the EXE and Con groups were fed olive oil as much as the VD group by gavage.

Measurement of Biochemical Variables

Real-time polymerase chain reaction (PCR) was used to measure antioxidant genes. For this purpose, tissue homogenization and RNA extraction process were performed first. The resulting RNA was then stored in a -80°C freezer, and cDNA synthesis was performed according to the instructions in the Easy cDNA synthesis kit (made by Pars Toos, Iran). The prepared cDNA was then stored at -20°C. To evaluate the expression of genes using real-time PCR, all primers were designed by Allele IDv7.8 software, and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the internal control (Table 1). Then, for each gene, a mixture of different PCR components including SYBER Green Master Mix was prepared, and after mixing and spinning, it was distributed inside the microtubes for the device, and 1 µL of cDNA sample was added to each vial. Afterward, the program of the real-time PCR device was adjusted, and after completing the activity of the device and observing the graphs and the amount of fluorescence propagation, the amount of change in the expression of the desired gene compared to GADPH was measured by calculating ΔΔCt. Then, the relative expression of the gene was calculated using the formula $2^{-\Delta\Delta Ct}$. The mean and standard deviation of the measured variables are reported in Table 2.

Table 1. List of Primers Used in This Study

Genes	Primer Sequences
Mn-SOD	Forward: 5′- CGGTCCAGCGGATGAAGAGAGG-3′
	Reverse: 5′- GGCAATCCCAATCACACCACAAGC-3′
CAT	Forward: 5′-CAGAGCCTGAAGTCACCA-3′
	Reverse: 5′-CAGAACGTGAGGCAAGAGGG-3′
GPx	Forward: 5′-CTG AGG GGA TTT TTC TGG-3′
	Reverse: 5′-GGT TTT TCC ATG ACG GTG T-3′
XO	Forward: 5′- CATCCCCATTGAGTTCAGAGTATCC-3′
	Reverse: 5′-TTGTTTTGCGTTATCTCCGTGCT -3′
GAPDH	Forward: 5′- AAG TTC AAC GGC ACA GTC AAG G-3′
	Reverse: 5′-CAT ACT CAG CAC CAG CAT CAC C -3′

Note. Mn-SOD: Manganese superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; XO: Xanthine oxidase; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

Table 2. Mean and Standard Deviation of the Measured Variables

Variable	Groups	Mean ± SD
XO (Normalized gene expression)	EXE + VD (n = 5)	0.017 ± 0.004
	VD (n = 5)	0.51 ± 0.25
	EXE (n = 5)	0.25 ± 0.08
	Con (n = 5)	0.55 ± 0.15
CAT (Normalized gene expression)	EXE + VD (n = 5)	1.45 ± 0.29
	VD (n = 5)	0.24 ± 0.056
	EXE (n = 5)	0.61 ± 0.17
	Con (n = 5)	0.16 ± 0.02
Mn-SOD (Normalized gene expression)	EXE + VD (n = 5)	0.12 ± 0.037
	VD (n = 5)	0.069 ± 0.020
	EXE (n = 5)	0.075 ± 0.030
	Con (n = 5)	0.024 ± 0.010
GPx (Normalized gene expression)	EXE + VD (n = 5)	2.24 ± 1.04
	VD (n = 5)	1.13 ± 0.52
	EXE (n = 5)	0.62 ± 0.12
	Con (n = 5)	0.33 ± 0.09

Note. SD: Standard deviation; XO: Xanthine oxidase; CAT: Catalase; Mn-SOD: Manganese superoxide dismutase; GPx: Glutathione peroxidase; Con: Control; EXE: Aerobic training; VD: Vitamin D.

Data Analysis

All statistical analyses in the present study were performed using SPSS software version 22. The distribution and homogeneity of variance were checked by Shapiro-Wilk and Leven tests, respectively. After confirming the assumptions (i.e., normal distribution and homogeneity of variances), a one-way analysis of variance was used to analyze the data. In addition, the Tukey post hoc test was used to compare the pairs, and the significance level was considered $P < 0.05$.

Results

One-way analysis of variance showed that the expression of the GPx gene is significantly different between the studied groups [F(3,16) = 10.08, $P = 0.001$]. Post hoc test revealed that GPx expression in the EXE + VD group was significantly higher than that in the VD (MD = 1.1, $P = 0.04$), EXE (MD = 1.61, $P = 0.003$), and Con (MD = 1.90, $P = 0.001$) groups. However, no significant difference was observed between the other groups ($P > 0.05$), as depicted in Figure 1.

CAT gene expression [F(3,16) = 57.61, $P = 0.000$] was significantly different between the groups. Post hoc test showed that CAT expression in the EXE + VD group was significantly higher than that in VD (MD = 1.21, $P = 0.000$), EXE (MD = 0.84, $P = 0.000$), and Con (MD = 1.30, $P = 0.000$) groups. CAT expression was also

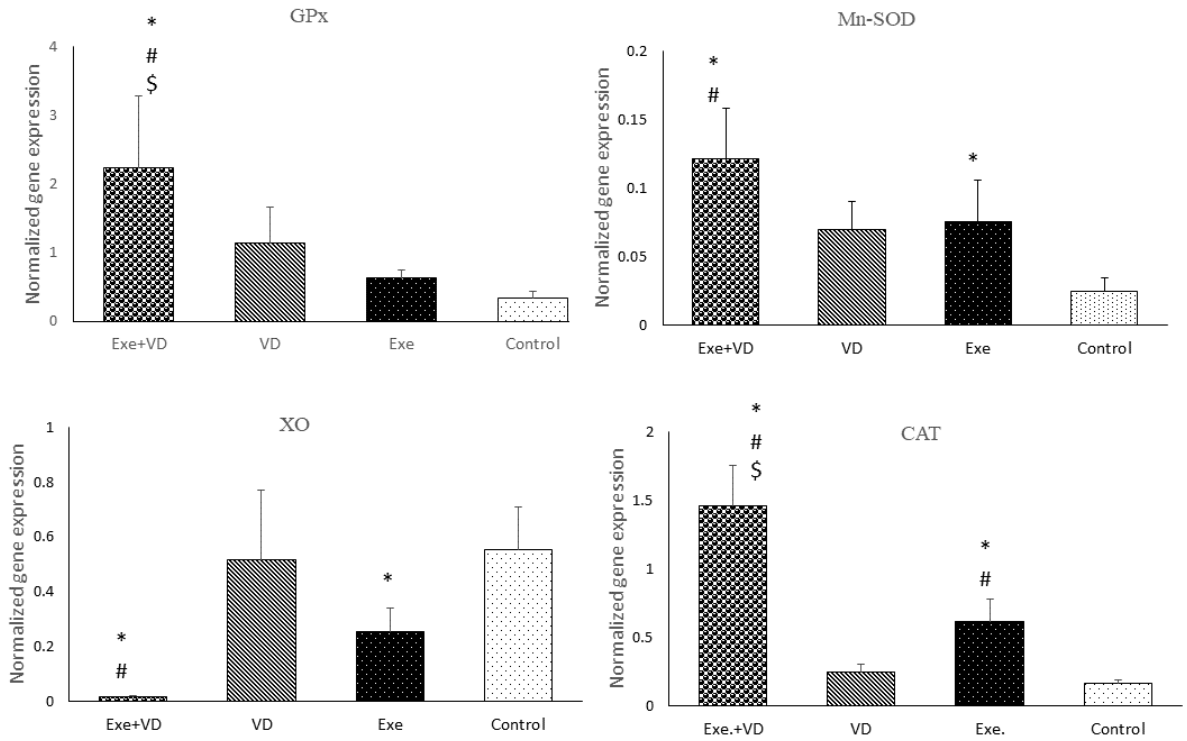

Figure 1. Changes in the expression of antioxidant genes in the study groups. GPx: glutathione peroxidase, CAT: catalase, Mn-SOD: manganese superoxide dismutase, XO: xanthine oxidase. * Significant difference with control group, #: Significant difference with VD group, $ Significant difference with EXE group
Figure 1

higher in the EXE group than that in the VD (MD = 0.36, $P = .021$) and Con (MD = 0.44, $P = 0.004$) groups. However, no significant difference was observed between VD and Con ($P > 0.05$) groups, as illustrated in Figure 1.

Likewise, Mn-SOD gene expression was significantly different between the groups [$F(3,16) = 11.15$, $P = 0.000$]. In addition, Mn-SOD in EXE + VD was significantly higher than that in the VD (MD = 0.52, $P = 0.32$) and Con (MD = 0.1, $P = 0.000$) groups. Further, Mn-SOD expression was higher in the EXE group than in the Con group (MD = 0.51, $P = 0.036$). However, no significant difference was observed between the other groups ($P > 0.05$), as depicted n Figure 1.

Similarly, XO expression was significantly different between the groups [$F(3,16) = 13.06$, $P = 0.000$]. Post hoc test showed that XO expression in the EXE + VD group was significantly lower than in VD (MD = 0.49, $P = 0.001$) and Con (MD = 0.53, $P = 0.000$) groups. XO expression was also lower in the EXE group than in the CON group (MD = 0.29, $P = 0.035$). However, no significant difference was observed between the other groups ($P > 0.05$), as presented in Figure 1.

Discussion

The findings of the present study indicated that the combination of vitamin D supplementation and aerobic training increased the expression of Mn-SOD, CAT, and GPx antioxidant genes and decreased XO expression. Further, according to the findings, vitamin D supplementation alone did not seem to have an effect on antioxidant defense or XO. However, aerobic training can increase CAT and Mn-SOD expression and decrease XO expression compared to the control group. In summary, the combination of vitamin D supplementation and aerobic exercise can increase antioxidant defense and reduce the oxidative stress of kidney tissue compared to any other interventions.

Although the combined effects of vitamin D supplementation and aerobic training on antioxidant defense or oxidative stress of kidney tissue have not been studied, some studies have exhibited the separate effects of these interventions (aerobic exercise and vitamin D supplementation) on oxidative stress. Consistent with the findings of the present study, Ishikawa et al found that in rats with diabetic nephropathy, 8 weeks of low to moderate-intensity aerobic exercise reduce the progression of diabetic nephropathy by reducing oxidative stress (6). Another study also revealed that exercise in diabetic nephropathy rats reduces oxidative stress and markers of cell apoptosis in kidney tissue by increasing SOD (10). In addition, Tucker et al in a study on animal models with early-stage CKD indicated that 8 weeks of high-intensity interval exercise (85% VO2max) increased mRNA-SOD and mRNA-CAT compared to inactive rats (11). Likewise, de Souza et al demonstrated that 8 weeks of aerobic training increase the activity of SOD and GPx in renal tissue in rats with CKD (17). However, some studies have reported that exercise

does not affect the expression or activity of antioxidant enzymes in kidney tissue (12). For example, Coelho et al found that 8 weeks of aerobic training do not significantly change the SOD and CAT levels of healthy male Wistar rats with CKD (12). However, levels of oxidative damage markers to lipids or carbonyl proteins decreased after exercise training (12). These findings suggest that exercise through mechanisms independent of the enhancement of antioxidant enzymes can also reduce oxidative damage in kidney tissue. Due to the decrease in XO activity in the present study, it seems that the decrease in the activity of ROS production sources in kidney tissue is one of these mechanisms. In addition, it was shown that increased uremic toxins such as uric acid can also be involved in inducing oxidative stress in kidney tissue (1). However, regular exercise can reduce the oxidative stress of kidney tissue by reducing the production of uric acid in the body (18-20). Nevertheless, in the present study, blood uric acid levels were not measured, so it is suggested that the role of this possible mechanism be further investigated in future studies. In addition, antioxidant enzymes in kidney tissue have been found to decrease with age (21, 22), and exercise can reverse this process (6, 10-12, 15, 17, 18). Given that the samples were middle-aged male rats, it is also possible that the observed changes were due to a decreasing trend of antioxidant enzymes and an up-regulation of XO due to aging (1).

In general, according to previous studies and the results of the present study, the increase in antioxidant enzymes in the Exe + VD can be considered mainly due to the mechanisms resulting from exercise training. In particular, the expression of antioxidant enzymes in the VD group did not change significantly compared to the control group. Nevertheless, some previous studies have reported that vitamin D supplementation can reduce oxidative damage to kidney tissue after induction of kidney damage (9, 23, 24) and reverse the decreasing trend of antioxidant enzymes (9). It should be noted that differences in the type of samples and methodology of studies can partly explain this discrepancy in the findings. Xu et al indicated the antioxidant effects of vitamin D on kidney tissue in animal samples after the induction of AKI induced by lipopolysaccharides (9). BaSalamah et al also showed the antioxidant effects of vitamin D on the kidney after the induction of lead toxicity (24). The samples of the present study were healthy middle-aged male rats. Further, the length of the supplementation period in previous studies (9, 23, 24) is different from the present study. Despite this increase in GPx and CAT expression in the VD group compared to the Exe group, the presence of vitamin D may increase the antioxidant adaptations induced by exercise. This can be explained by the interaction of vitamin D receptor signaling pathways with exercise. In fact, it has been suggested that vitamin D or exercise may alter the morphology of mitochondria (the site of ROS production) in renal tissue (25). Moderate-intensity exercise prevented the reduction of age-related mitochondrial enzyme activity in the kidney, leading to a decrease in oxidative stress markers (25). However, some studies have reported that vitamin D receptor (VDR) signaling is involved in reducing the formation of mitochondrial ROS (26). These effects are in part due to the inhibition of nicotinamide adenine dinucleotide phosphate oxidase expression and increased SOD (5, 27). It has also been reported that after treatment with paricalcitol (VDR activators) in hemodialysis patients, levels of oxidative stress markers including malondialdehyde, nitric oxide, and carbonyl proteins in serum decreased, and levels of antioxidant defense, including glutathione, CAT activity, and SOD increased (28). One of the possible mechanisms for protecting the kidneys against oxidative stress by vitamin D is the Nrf2-Keap1 pathway (5). Nrf2 controls the expression of antioxidants and ROS detoxifiers through the antioxidant response element (5). Moreover, vitamin D3 can increase the expression of Nrf2 and also reduce the expression of Keap1 (an Nrf2 suppressor) (6). Interestingly, exercise can also help increase antioxidant defense by stimulating Nrf2 (29, 30). Therefore, the significant increase of CAT and Mn-SOD in the Exe + VD compared to other groups can be due to the synergistic effects of signaling caused by exercise and vitamin D on Nrf2 activation. Additionally, it has been reported that peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) can act as a VDR coactivator (31). On the other hand, the up-regulation of renal PGC-1α has been confirmed in response to exercise (13). Hence, another possible mechanism can be attributed to the molecular interaction between PGC1 and VDR. However, the role of these mechanisms in the regulation of oxidative stress in renal tissue, especially after the combination of vitamin D supplementation and exercise, requires further study.

It should be noted that the present study had some limitations. For example, in this study, the measurement of oxidative stress indices was evaluated only at the level of gene expression, while their protein levels and enzyme activity were not measured; therefore, it is suggested to be considered in future studies. Further, plasma/serum vitamin D levels and their relationship with oxidative stress indices were not evaluated. Therefore, the different absorption of vitamin D in the subjects may have influenced the results. Finally, in this study, only the effect of one dose of exercise training and vitamin D supplementation was investigated, so it is suggested to investigate the interactive role of vitamin D supplementation and aerobic exercise with different doses in future studies.

Conclusion

Overall, the findings of the present study revealed that 8

weeks of aerobic exercise leads to the increased expression of antioxidant enzymes in the tissues of middle-aged male rats. However, although vitamin D supplementation alone did not have a significant effect on the expression of antioxidant enzymes in kidney tissue, it seemed that it can enhance the adaptations of aerobic exercise in the expression of antioxidant = CAT, Mn-SOD, and GPx enzymes. The combination of aerobic training and vitamin D supplementation also reduced XO expression as one of the main sources of free radical formation in kidney tissue. These findings suggest that exercise training alone or in combination with VD supplementation can be effective in preventing age-related kidney disease and its associated oxidative stress.

Acknowledgments

This article is an extract from the approved doctoral thesis of the Department of Sports Physiology, University of Tehran. Therefore, we appreciate the efforts of the officials and professors of the Faculty of Physical Education and Sports Sciences of Tehran University, who provided the necessary facilities for the implementation of this research.

Authors' Contribution

Conceptualization: Abbas Ali Gaeini, Reza Nouri.
Data curation: Amin Molaei, Mohammad Hemmatinafar.
Formal analysis: Mohammad Hemmatinafar, Reza Nouri.
Funding acquisition: Abbas Ali Gaeini, Amin Molaei.
Investigation: Abbas Ali Gaeini, Reza Nouri.
Methodology: Amin Molaei, Mohammad Hemmatinafar.
Project administration: Abbas Ali Gaeini, Reza Nouri.
Resources: Abbas Ali Gaeini, Mohammad Hemmatinafar.
Software: Mohammad Hemmatinafar.
Supervision: Abbas Ali Gaeini, Reza Nouri, Mohammad hemmatinafar.
Validation: Amin Molaei, Abbas Ali Gaeini.
Visualization: Abbas Ali Gaeini, Reza Nouri, Amin Molaei.
Writing–original draft: Amin Molaei, Mohammad Hemmatinafar.
Writing–review & editing: Abbas Ali Gaeini, Reza Nouri, Mohammad Hemmatinafar, Amin Molaei.

Competing Interests

The authors express that they have no conflict of interest.

Ethical Approval

This research has been approved by the Research Ethics Committee of the Institute of Physical Education and Sports Sciences of the Islamic Republic of Iran (Code: IR.SSRC.REC.1399.104).

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology (Carlton). 2012;17(4):311-21. doi: 10.1111/j.1440-1797.2012.01572.x.
2. Finco DR. Kidney function. In: Kaneko JJ, Harvey JW, Bruss ML, eds. Clinical Biochemistry of Domestic Animals. 5th ed. San Diego: Academic Press; 1997. p. 441-84. doi: 10.1016/b978-012396305-5/50018-x.
3. Kumar A, Yegla B, Foster TC. Redox signaling in neurotransmission and cognition during aging. Antioxid Redox Signal. 2018;28(18):1724-45. doi: 10.1089/ars.2017.7111.
4. Kitada M, Xu J, Ogura Y, Monno I, Koya D. Manganese superoxide dismutase dysfunction and the pathogenesis of kidney disease. Front Physiol. 2020;11:755. doi: 10.3389/fphys.2020.00755.
5. Mokhtari Z, Hekmatdoost A, Nourian M. Antioxidant efficacy of vitamin D. J Parathyr Dis. 2017;5(1):11-6.
6. Ishikawa Y, Gohda T, Tanimoto M, Omote K, Furukawa M, Yamaguchi S, et al. Effect of exercise on kidney function, oxidative stress, and inflammation in type 2 diabetic KK-A(y) mice. Exp Diabetes Res. 2012;2012:702948. doi: 10.1155/2012/702948.
7. Parajuli N, MacMillan-Crow LA. Role of reduced manganese superoxide dismutase in ischemia-reperfusion injury: a possible trigger for autophagy and mitochondrial biogenesis? Am J Physiol Renal Physiol. 2013;304(3):F257-67. doi: 10.1152/ajprenal.00435.2012.
8. Norris KC, Olabisi O, Barnett ME, Meng YX, Martins D, Obialo C, et al. The role of vitamin D and oxidative stress in chronic kidney disease. Int J Environ Res Public Health. 2018;15(12):2701. doi: 10.3390/ijerph15122701.
9. Xu S, Chen YH, Tan ZX, Xie DD, Zhang C, Xia MZ, et al. Vitamin D3 pretreatment alleviates renal oxidative stress in lipopolysaccharide-induced acute kidney injury. J Steroid Biochem Mol Biol. 2015;152:133-41. doi: 10.1016/j.jsbmb.2015.05.009.
10. Ghosh S, Khazaei M, Moien-Afshari F, Ang LS, Granville DJ, Verchere CB, et al. Moderate exercise attenuates caspase-3 activity, oxidative stress, and inhibits progression of diabetic renal disease in db/db mice. Am J Physiol Renal Physiol. 2009;296(4):F700-8. doi: 10.1152/ajprenal.90548.2008.
11. Tucker PS, Briskey DR, Scanlan AT, Coombes JS, Dalbo VJ. High intensity interval training favourably affects antioxidant and inflammation mRNA expression in early-stage chronic kidney disease. Free Radic Biol Med. 2015;89:466-72. doi: 10.1016/j.freeradbiomed.2015.07.162.
12. Coelho BL, Rocha LG, Scarabelot KS, Scheffer DL, Ronsani MM, Silveira PC, et al. Physical exercise prevents the exacerbation of oxidative stress parameters in chronic kidney disease. J Ren Nutr. 2010;20(3):169-75. doi: 10.1053/j.jrn.2009.10.007.
13. Wu F, Li Z, Cai M, Xi Y, Xu Z, Zhang Z, et al. Aerobic exercise alleviates oxidative stress-induced apoptosis in kidneys of myocardial infarction mice by inhibiting ALCAT1 and activating FNDC5/Irisin signaling pathway. Free Radic Biol Med. 2020;158:171-80. doi: 10.1016/j.freeradbiomed.2020.06.038.
14. Kutlu M, Naziroğlu M, Simşek H, Yilmaz T, Sahap Kükner A. Moderate exercise combined with dietary vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic-rat. Int J Vitam Nutr Res. 2005;75(1):71-80. doi: 10.1024/0300-9831.75.1.71.
15. Small DM, Beetham KS, Howden EJ, Briskey DR, Johnson DW, Isbel NM, et al. Effects of exercise and lifestyle intervention on oxidative stress in chronic kidney disease. Redox Rep. 2017;22(3):127-36. doi: 10.1080/13510002.2016.1276314.
16. Wisløff U, Helgerud J, Kemi OJ, Ellingsen O. Intensity-controlled treadmill running in rats: VO(2 max) and cardiac hypertrophy. Am J Physiol Heart Circ Physiol. 2001;280(3):H1301-10. doi: 10.1152/ajpheart.2001.280.3.H1301.
17. de Souza PS, da Rocha LG, Tromm CB, Scheffer DL, Victor EG, da Silveira PC, et al. Therapeutic action of physical exercise on markers of oxidative stress induced by chronic kidney disease. Life Sci. 2012;91(3-4):132-6. doi: 10.1016/j.lfs.2012.06.028.

18. Asad A, Burton JO, March DS. Exercise as a therapeutic option for acute kidney injury: mechanisms and considerations for the design of future clinical studies. BMC Nephrol. 2020;21(1):446. doi: 10.1186/s12882-020-02098-9.
19. Hou Y, Ma R, Gao S, Kaudimba KK, Yan H, Liu T, et al. The effect of low and moderate exercise on hyperuricemia: protocol for a randomized controlled study. Front Endocrinol (Lausanne). 2021;12:716802. doi: 10.3389/fendo.2021.716802.
20. Francis K, Hamrick ME. Exercise and uric Acid: implication in cardiovascular disease. J Orthop Sports Phys Ther. 1984;6(1):34-9. doi: 10.2519/jospt.1984.6.1.34.
21. Gomes P, Simão S, Silva E, Pinto V, Amaral JS, Afonso J, et al. Aging increases oxidative stress and renal expression of oxidant and antioxidant enzymes that are associated with an increased trend in systolic blood pressure. Oxid Med Cell Longev. 2009;2(3):138-45. doi: 10.4161/oxim.2.3.8819.
22. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012;2012:465897. doi: 10.1155/2012/465897.
23. Ke CY, Yang FL, Wu WT, Chung CH, Lee RP, Yang WT, et al. Vitamin D3 reduces tissue damage and oxidative stress caused by exhaustive exercise. Int J Med Sci. 2016;13(2):147-53. doi: 10.7150/ijms.13746.
24. BaSalamah MA, Abdelghany AH, El-Boshy M, Ahmad J, Idris S, Refaat B. Vitamin D alleviates lead induced renal and testicular injuries by immunomodulatory and antioxidant mechanisms in rats. Sci Rep. 2018;8(1):4853. doi: 10.1038/s41598-018-23258-w.
25. Little JP, Safdar A, Benton CR, Wright DC. Skeletal muscle and beyond: the role of exercise as a mediator of systemic mitochondrial biogenesis. Appl Physiol Nutr Metab. 2011;36(5):598-607. doi: 10.1139/h11-076.
26. Yang S, Li A, Wang J, Liu J, Han Y, Zhang W, et al. Vitamin D receptor: a novel therapeutic target for kidney diseases. Curr Med Chem. 2018;25(27):3256-71. doi: 10.2174/0929867325666180214122352.
27. Finch JL, Suarez EB, Husain K, Ferder L, Cardema MC, Glenn DJ, et al. Effect of combining an ACE inhibitor and a VDR activator on glomerulosclerosis, proteinuria, and renal oxidative stress in uremic rats. Am J Physiol Renal Physiol. 2012;302(1):F141-9. doi: 10.1152/ajprenal.00293.2011.
28. Izquierdo MJ, Cavia M, Muñiz P, de Francisco AL, Arias M, Santos J, et al. Paricalcitol reduces oxidative stress and inflammation in hemodialysis patients. BMC Nephrol. 2012;13:159. doi: 10.1186/1471-2369-13-159.
29. Merry TL, Ristow M. Nuclear factor erythroid-derived 2-like 2 (NFE2L2, Nrf2) mediates exercise-induced mitochondrial biogenesis and the anti-oxidant response in mice. J Physiol. 2016;594(18):5195-207. doi: 10.1113/jp271957.
30. Esgalhado M, Stenvinkel P, Mafra D. Nonpharmacologic strategies to modulate nuclear factor erythroid 2-related factor 2 pathway in chronic kidney disease. J Ren Nutr. 2017;27(4):282-91. doi: 10.1053/j.jrn.2017.01.022.
31. Savkur RS, Bramlett KS, Stayrook KR, Nagpal S, Burris TP. Coactivation of the human vitamin D receptor by the peroxisome proliferator-activated receptor gamma coactivator-1 alpha. Mol Pharmacol. 2005;68(2):511-7. doi: 10.1124/mol.105.012708.