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The Effect of Aerobic Training with Vitamin D on Extrinsic Pathway of Apoptosis and Anti- Apoptotic Indices of Heart Tissue of Rats Exposed to Oxidative Damage Induced by H_2O_2

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Abstract

Introduction: Apoptosis plays an important role in the development of cardiovascular diseases. The aim of this study was to investigate the effect of aerobic training (AT) with Vitamin D (VD) on extrinsic pathway of apoptosis and anti- apoptotic indices of heart tissue of rats exposed to oxidative damage induced by H_2O_2.

Methods: In this experimental study, 60 rats were selected and randomly placed into 10 groups of six, including 1) 1 mmol / kg H_2O_2, 2) 2 mmol / kg H_2O_2, 3) VD + 1 mmol / kg H_2O_2, 4) AT + 1 mmol / kg H_2O_2, 5) AT + VD + 1 mmol / kg H_2O_2, 6) VD + 2 mmol / kg H_2O_2, 7) AT + 2 mmol / kg H2O2, 8) AT + VD + 2 mmol / kg H_2O_2, 9) sham and 10) control. During eight weeks, the rats in groups 1-9 were subjected to intraperitoneal injection of H_2O_2 at specific doses three times a week; the rats in groups 4, 5, 7 and 8 performed aerobic trainings, and the rats in groups 3, 5, 6 and 8 received 0.5 µg / kg of vitamin D3 intraperitoneally daily. Protein concentrations of Caspase 8, Fas and FLIP were measured in rat heart tissue. For analysis of the research findings, Kolmogorov- Smirnov, one-way ANOVA, two-way ANOVA along with Bonferroni post hoc tests were used ($p \leq 0.05$).

Results: H_2O_2 has a significant effect on the increase of Caspase 8, Fas and FLIP in the heart tissue of rats ($p \leq 0.05$); eight weeks of aerobic training and Vitamin D intake has a significant effect on decreasing the concentration of Caspase 8, Fas and increase of FLIP in the heart tissue of rats exposed to oxidative damage induced by 1 and 2 m/mol/kg H_2O_2 ($p \leq 0.05$); aerobic training and Vitamin D intake has significant interactions in the reduction of Caspase 8, Fas in the heart tissue of rats exposed to oxidative damage induced by 1 and 2 m/mol/kg H_2O_2 ($p \leq 0.05$), however, it does not have interactive effect on the increased FLIP of the heart tissue of rats exposed to oxidative damage induced by 1 and 2 m/mol/kg H_2O_2 ($p \geq 0.05$).

Conclusion: It seems that performing aerobic training activities and simultaneous consumption of vitamin D supplement has interactive effects on reducing some of the extrinsic pathway indices of apoptosis in the heart tissue of H_2O_2-rats exposed to oxidative damage induced by H_2O_2.

Keywords: Training, Vitamin D, Apoptosis, H_2O_2

Introduction

Cardiovascular disease is one of the most common causes of mortality in the world (1), and an important part of this complication occurs following apoptosis of cardiac cells due to increased oxidative stress caused by the production of reactive oxygen species (ROS) and the reduction of antioxidants (2). Apoptosis is a highly organized and planned natural biological process that plays a vital role in monitoring various types of non-pathological cell events and is a process for the removal of damaged cells (3). Cells that suffer from apoptotic death show numerous

morphological changes, including small and cellular aggregation, chromatin density, organized nucleic rupture, plasma membrane germination and cellular fragmentation, and the formation of apoptotic cells rapidly swallowed. In general, the pathways involved in the induction of apoptosis process are divided into two categories of intrinsic pathway (or mitochondrial pathway) and the extrinsic pathway (or route of death receptors) (4). In the extrinsic pathway of apoptosis, the binding of the receptor to the ligand initiates the action of protein-protein in the cell membrane, which activates the start-up caspases. Main receptors include Fas receptors (CD95 or Apo-1), Tumor necrosis factor receptor-1 (TNFR1), ligand receptors, TNF-induced apoptosis (TRAIL-R1), and TRAIL-R2 receptors (5). The Fas / FasL system is one of the systems that induces apoptosis by the death receptor. When connecting Fas/FasL, Fas together with the second death and pro-apoptosis 8, through endocytosis, form a Death-inducing signaling complex (Disc). Endosomal DISC releases from the receptor as cytosolic DISC directly or through a reinforcing loop including mitochondria and pro-apoptosis protein t-Bid leads to initial Caspase 8 activity. Caspase 8 is activated with the binding of TRADD to FADD and Procaspase 8, and subsequently activates Caspase 3, thereby inducing apoptosis (5). Although some researchers believe that exercise training reduces apoptosis by reducing the oxidative stress (6), concerning the effect of exercise on apoptotic indices, contradictory results have been reported. For example, some studies have reported reduced t-Bid, Bad, Bak, Bax, cytochrome- C cytosolic, activated caspase-9 and activated caspase-3 and increased expression of Bcl- 2 gene following endurance trainings in rats (3, 7- 9). Also, some studies reported significant increase in ¬sFas and ¬FasL levels following endurance training in rats (10), and lack of changes in Bcl- 2 levels and increased Bax following endurance training in the heart tissue of myocardial (11). Considering the contradictory results about the effect of exercise training on apoptosis, the use of antioxidant supplements has been considered by researchers. Vitamin D is a secreted steroid that is synthesized in the skin and subsequently metabolized in the liver and kidneys, respectively (13). Some studies have reported anti-oxidant and anti-inflammatory effects of vitamin D on Tumor necrosis factor-α (TNFα) and Interleukin 6 (IL-6) (14) in inactive men; also some studies reported increase in total antioxidant capacity and reduction in malondialdehyde (MDA) and catalase (15). It should be noted that simultaneous use of antioxidant supplements and exercise trainings has attracted many researchers in the field of sports. Despite studies conducted in this area, no study was observed to examine the interactive effect of endurance training and supplementation of vitamin D on apoptosis. Accordingly, finding a suitable way for people at risk for cardiovascular disease due to oxidative stress seems to be necessary. Thus, the present study aimed to investigate the effect of aerobic training with vitamin D on extrinsic pathway of apoptosis and anti- apoptotic indices of heart tissue of rats exposed to oxidative damage induced by H_2O_2.

Methods

In this experimental study, 60 adult Wistar rats weighting 200 ± 20 grams and 8 to 10 weeks old were purchased from the Animal Breeding Center of Shiraz University of Medical Sciences and transferred to the Animal House Physiology Research Center of Kerman University of Medical Sciences. Rats were kept for one week to adapt in a cage for animals under standard conditions, a temperature of 22 ± 2 ° C, a 12-hour light / dark cycle, and free access to water and rodent foods (purchased from the Pars Animal Food Company in Tehran, Iran). Rats, to get to know, ran on a treadmill for a week before grouping. Then they were randomly assigned

to 10 groups of six including: 1) 1 mmol / kg H_2O_2, 2) 2 mmol / kg H_2O_2, 3) VD + 1 mmol / kg H_2O_2, 4) AT + 1 mmol / kg H_2O_2, 5) AT + VD + 1 mmol / kg H_2O_2, 6) VD + 2 mmol / kg H_2O_2, 7) AT + 2 mmol / kg H_2O_2, 8) AT + VD + 2 mmol / kg H_2O_2, 9) sham and 10) control. During eight weeks, the rats in groups 1-9 were subjected to intraperitoneal injection of H_2O_2 at specific doses of three times a week; the rats in groups 4, 5, 7 and 8 performed aerobic trainings, and the rats in groups 3, 5, 6 and 8 received 0.5 µg / kg of Vitamin D3 intraperitoneally daily. In order to reach the appropriate dose of injection, normal saline was used for dilution and dimethyl sulfoxide (DMSO) was used to dissolve Vitamin D3 in saline. Due to the need to investigate the effect of the solvent, a group called the DMSO was defined, which received only solvents daily (18). Groups 4, 5, 7, and 8 performed daily aerobic trainings on a rodent treadmill for 8 weeks, the 10-degree gradient of the treadmill was steady, but the speed and duration of the training gradually increased from about 8 m / min for 30 minutes in the first week to 12 m / minute with the same time in the second week; 16 m / minute for 45 minutes in the third week, and 20 m / minute for 45 minutes in the fourth week. During the fifth to eighth weeks, the speed remained fixed at 20 m / min for 60 minutes (19). 24 hours after the last training session, the rats were anesthetized by inhalation of chloroform. The heart tissue of the rats was carefully isolated and immediately immersed and frozen in liquid nitrogen and stored for further experiments at -75 ° C. To measure the variables of the study, on the day of the experiment, the tissues were taken out of the freezer, a certain amount of tissue was weighed, and proportionate to it a homogeneous buffer (PBS) with pH = 7.2-7.7 was added. The test tubes containing the desired tissue and buffer were put inside a frozen container to avoid buffering and degradation of the proteins and using a sonicator were homogenized over 5 cycles for 10 seconds; then using the refrigerated centrifuge they were centrifuged for 15 minutes at 12,000 rpm, and the resulting topical solution was transferred to a new tube. To perform the test, the kits and specimens (supernatant) were removed from the refrigerator for at least 20 minutes and placed at the laboratory temperature. Then, standard solutions were made and diluted to the wells coated with antibodies and 40 Lambda was added to the wells for specimens from the sample and 10 Lambda was removed from the FasL antibody and added to the sample wells. At the end, about 50 Lambda from the HRP enzyme was added to all wells of standard and specimen, after 60 minutes incubation at 37 ° C, the plate was removed from the incubation, and after discharging the contents, it was washed 5 times using buffer; 50 Lambda of the chromogen A solutions followed by 50 Lambda of chromogen B were added to the wells and incubated again for 10 minutes at 37 ° C for the reaction. After removing the plate from the incubator, the stop solution was added and then the reaction was terminated. Within a time interval of less than 10 minutes, light absorption was observed and recorded for wells at 450 nm wavelength for research variables (FAS, Caspase 8 and FLIP). For analysis of the research findings, Kolmogorov-Smirnov, one-way ANOVA, two-way ANOVA and Bonferroni post hoc tests were used ($p \leq 0.05$). All data were analyzed by SPSS software version 21.

Results

The mean and standard deviation of the variables of the research are presented in Table 1. The results of one-way ANOVA test in Table 2 show that there is a significant difference in the caspase 8 levels ($F = 138.86$, $p = 0.001$), Fas ($F = 68.17$, $p = 0.001$) and FLIP ($F= 41.15$, $p=0.001$) in the control, sham, 1 mmol / kg H_2O_2 and 2 mmol / kg H_2O_2 groups. Tukey's post hoc test showed that 1 mmol / kg H_2O_2 has a significant effect on the increase of caspase 8 ($M = -0.12$, $p = 0.001$) and Fas ($M= -2.36$, $p = 0.001$) and also

reduction of FLIP (M = 1.60, p = 0.001) compared to control group; 2 mmol / kg H_2O_2 has a significant effect on the increase of caspase 8 (M = 0.22, p = 0.001) and Fas (M =-4.33, p = 0.001) and also reduction of FLIP (M = 2.00, p = 0.001) compared to the control group; also, in the 2 mmol / kg H_2O_2 group, the levels of Caspase 8 (M = -0.10, p = 0.001) and Fas (M = -2.27, p = 0.001) are significantly higher than those of 1 mmol / kg H_2O_2; however, there is no significant difference in FLIP levels (M = 0.40, p = 0.39) in groups of 1 mmol / kg H_2O_2 and 2 mmol / kg H_2O_2; In addition, there is no significant difference between the levels of Caspase 8 (M = 41.15, P = 0.001), Fas (M = 41.15, p = 0.001) and FLIP (M = 41.15, p = 0.001) in the control and sham groups. The results of two-way ANOVA test in Table 3 show that eight weeks of aerobic training (F = 65.83, p = 0.001, effect size of 0.60) and Vitamin D supplementation (F = 124.23, p = 0.001 and effect size of 0.74) have a significant effect on the decrease of caspase 8 levels in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2. Also, aerobic training and Vitamin D supplementation have interactive effects in reducing caspase-8 in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2 (F = 6.36, p = 0.01 and effect size 0.12). Eight weeks of aerobic training (F = 138.59, p = 0.001 and effect size of 0.76) and Vitamin D supplementation (F = 169.73, p = 0.001, and effect size of 0.79) have a significant effect on the reduction of Fas levels in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2. Also, eight weeks of aerobic training and Vitamin D supplementation have interactive effects on reducing Fas in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2 (F = 53.75, p = 0.001 and effect size of 0.55). Eight weeks of aerobic training (F = 108.82, p = 0.001, and effect size of 0.71) and Vitamin D supplementation (F = 87.57, p = 0.001, and effect size of 0.67) have a significant effect on the increase in FLIP levels in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2. However, aerobic training and Vitamin D supplementation have no interactive effects in increasing FLIP levels in the heart tissue of rats exposed to oxidative damage induced by 1 mmol / kg H_2O_2 (F = 0.12, p = 0.73, and effect size of 0.001). The results of two-way ANOVA test in Table 3 show that eight weeks of aerobic training (F = 172.19, p = 0.001, and effect size of 0.84) and Vitamin D supplementation (F=225.54, p = 0.001, and effect size of 0.87) have a significant effect on the reduction of caspase 8 levels in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2. Also, aerobic training and Vitamin D supplementation have interactive effects in reducing caspase 8 in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2 (F = 41.31, p = 0.001, and effect size of 0.57). Eight weeks of aerobic training (F = 209.99, p = 0.001, and effect size of 0.87) and Vitamin D supplementation (F = 187.18, p = 0.001, and effect size of 0.85) have a significant effect on the reduction of Fas levels in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2. Aerobic training and Vitamin D supplementation have interactive effects on reducing Fas in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2 (F= 118.85, p = 0.001, and effect size of 0.79). Eight weeks of aerobic training (F = 109.61, p = 0.001, and effect size of 0.78) and Vitamin D supplementation (F = 120.97, p = 0.001, and effect size of 0.79) have a significant effect on increasing levels of FLIP in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2. However, aerobic training and Vitamin D supplementation have no interactive effects on increasing FLIP levels in the heart tissue of rats exposed to oxidative damage induced by 2 mmol / kg H_2O_2 (F = 0.02, p = 0.87, and effect size of 0.001).

Table 1. *Mean and standard deviation of research variables in ten research groups*

Variable / Group	Caspase 8	Fas	FLIP
Control	0.24±.01	9.92±.52	5.16±.66
Sham	0.26±.01	10.31±.66	5.47±.52
1 mmol/ kg H_2O_2	0.36±.02	11.98±.67	3.55±.37
2 mmol/ kg H_2O_2	0.46±.03	14.26±.90	3.15±.51
VD+ 1 mmol/ kg H_2O_2	0.21±.02	8.22±.48	5.76±.52
VD+ 2 mmol/ kg H_2O_2	0.26±.02	8.22±.48	5.76±.52
AT+ 1 mmol/ kg H_2O_2	0.22±.02	8.21±.49	6.34±.49
AT+ 2 mmol/ kg H_2O_2	0.28±.03	8.86±.36	5.36±.73
AT+ VD+ 1 mmol/ kg H_2O_2	0.15±.02	7.39±.56	8.43±.70
AT+ VD+ 2 mmol/ kg H_2O_2	0.20±.03	8.19±.63	7.48±.48

Table 2. *Results of one way ANOVA to examine the levels of research variables in 1 mmol / kg H_2O_2, 2 mmol / kg H_2O_2, sham and control groups*

Variable	Factor	Sum of Squares	df	Mean Square	F	Sig
Caspase 8	Between Groups	0.29	3	0.09	*138.86	0.001
	Within Groups	0.02	30	0.001		
	Total	0.31	33			
Fas	Between Groups	106.78	3	11.47	*68.17	0.001
	Within Groups	15.66	30	0.27		
	Total	122.44	33			
FLIP	Between Groups	34.41	3	11.47	*41.15	0.001
	Within Groups	8.36	30	0.27		
	Total	42.77	33			

* The significance level is 0.05

Discussion

The results showed that administration of 1 mmol / kg H_2O_2 and 2 mmol / kg H_2O_2 has a significant effect on increase of Caspase 8, Fas and decrease of FLIP in rat heart tissue. Recent studies have clearly shown that activation of apoptosis in cardiac cells following an increase in oxidative stress, which itself results in changes in the expression of genes and proteins, occurs inside the cell (5), thereby weakening the anti-oxidative and immune system of body (20-22); therefore, the balance or imbalance between antioxidant defense and oxidative stress plays a decisive role in the occurrence of many cardiovascular diseases and the aging process (23, 24). The two processes of inflammation and apoptosis play an essential role in the pathogenesis of heart failure and are the main key in regulating the cardiovascular system (25). One of the main systems for regulating apoptosis is the Fas (CD95) FasL system. The activation of apoptosis in this way is linked to the progression of heart failure and death of heart cells. Increase in caspase 8 following oxidative stress, suppresses the FLIP complex, which inhibits the binding of FasL ligands (FasL or CD95L). FasL or CD95L is a glycosylated peptide in the outer surface of the cell membrane belonging to the family of tumor necrosis factors (TNFs) (26, 27).

Table 3. *Results of two-way ANOVA test to investigate the effect of aerobic training and Vitamin D on the concentration of Caspase-8, Fas and FLIP proteins in the heart tissue of rats exposed to oxidative damage caused by H_2O_2*

H_2O_2- Induced Oxidative Damage	Variable	Factor	Sum of Squares	df	F	p	Partial Eta Squared
1 mmol/ kg H_2O_2	Caspase-8	Training	0.08	1	65.83	0.001	0.60
		Vitamin D	0.15	1	124.23	0.001	0.74
		Interaction of Training and Vitamin D	0.008	1	6.36	0.01	0.12
	Fas	Training	48.83	1	138.59	0.001	0.76
		Vitamin D	59.80	1	169.73	0.001	0.79
		Interaction of Training and Vitamin D	18.94	1	53.75	0.001	0.55
	FLIP	Training	67.40	1	108.82	0.001	0.71
		Vitamin D	54.24	1	87.57	0.001	0.67
		Interaction of Training and Vitamin D	.075	1	0.12	0.73	0.001
2 mmol/ kg H_2O_2	Caspase-8	Training	0.128	1	172.19	0.001	0.84
		Vitamin D	0.168	1	225.54	0.001	0.87
		Interaction of Training and Vitamin D	0.031	1	41.31	0.001	0.57
	Fas	Training	83.839	1	209.99	0.001	0.87
		Vitamin D	74.732	1	187.18	0.001	0.85
		Interaction of Training and Vitamin D	47.450	1	118.85	0.001	0.79
	FLIP	Training	38.285	1	109.61	0.001	0.78
		Vitamin D	42.254	1	120.97	0.001	0.79
		Interaction of Training and Vitamin D	0.009	1	0.02	0.87	0.001

Fas exists on the surface of lymphocytes B and T, types of cancer cells and a number of human cells. Increasing the Fas protein level at the cell surface stimulates interferon gamma and TNF-α and activates lymphocytes. Fas binding to Fas-L or anti-Fas antibody leads to trimerization and then peptide binding, which forms the DISC inducer set and starts the apoptotic process (28). In confirmation of the research findings, the researchers noted injection of 0.5 µg / ml H2O2 reduced FLIP and increased caspase 8 in rats (29). The results of this study showed that eight weeks of aerobic training has a significant effect on decreasing caspase 8, and Fas levels and increasing FLIP in heart tissue of rats exposed to oxidative damage induced by H_2O_2. Cell death plays an important role in controlling the

natural physiology of the body and many pathological conditions of the heart. The onset and implementation of apoptosis is a multistage process dependent on a program (30). The involved molecules in this program are present in various intracellular portions, including the plasma membrane, Golgia complex, mitochondria, and nucleus. Interactions between these sectors and the exchange of specific signal molecules are vital for the systematic development of apoptosis. Exercise seemingly improves apoptosis in the heart of rats through the mitochondrial pathway, and physical activity seems to be beneficial in reducing cardiac apoptosis by decreasing ROS and preventing subsequent mitochondrial cytochrome C (30). In addition, aerobic exercise by inhibition of nitric oxide (NO) in inverse physiological concentrations inhibits cytochrome oxidase (Complex IV from the electron transfer chains), which results in hyperpolarization of the mitochondrial membrane and therefore prevents apoptosis. Types of direct and indirect anti-apoptotic molecules are incrementally regulated by NO (31). Various intracellular anti-apoptotic proteins, such as iNOS, myeloid leukemia or bone marrow (Mcl-1), Glucose 8-regulated protein (Grp 78) and Interleukin-8 (IL-8) increase during moderate intensity training, and after detraining, they remain in high levels. The levels of Mcl-1 as an anti-apoptotic mediator of the NO signal fall due to the discharging of the downstream NO molecule, i.e. circular guanosine monophosphate (cGMP). As soon as the NO-cGMP signal is activated, neutrophils preserve the increased expression of Mcl-1 and slow down the process of apoptosis. Therefore, aerobic exercise activity seems to slow down the process of apoptosis by increasing the iNOS-NO-cGMP-Mcl-1 pathway (31). In line with the current study, the researchers pointed out that one bout resistance training with 80% of one maximal repeat can be effective in regulating and preventing apoptosis (32); levels of t-Bid, Bad, Bak, Bax, cytochrome-C cytosolic, Caspase activated -9 and Caspase activated -3 had significant reductions in the ovariectomized and trained groups of rats (33). Among the reasons for the coherence of these studies with the present study, one can note the same statistical population and similarity in the method of measuring variables; it has been reported that 12 weeks of endurance training with a gradient of 15% increases the expression of Bcl- 2 gene and decreases Bad, Bak, Bax in rats (3)

It was also stated that the levels of the Bad, Bax, and Bax to Bcl- 2 proteins in the trained rats were lower than the detrained group (8). Among the reasons for the coherence of these studies with the present study, the long term of the training period and the same measurement method can be mentioned. In this regard, aerobic training was reported for three months with an intensity of 75- 80% of maximal oxygen uptake (VO_{2max}) increased the expression of AIF and decreased the expression of Caspase 9 in rat soleus muscle (34). Concerning the reasons for matching the above study with the present study, one can mention the similarity of the statistical population and similarity in measuring the levels of measurement of these variables in the tissue. On the other hand, dissimilar to the present study, the researchers stated that training with a weight attached to the tail and 3 hours of forced swimming increased the Bax ratio to Bcl- 2 levels, indicating an increase in apoptosis signaling; and acute exercise led to impairment of the mitochondrial matrix system (35). Among the reasons for inconsistency of the above study with the present study, we can point out the intensity and duration of trainings in the present study, with eight weeks duration, and the moderate intensity of trainings as compared to those of Ola *et al.* with one session high-intensity resistance training. Also, one bout high intense exercise activity led to the formation of oxygen radicals beyond the antioxidant capacity of the body, which was indicated by a

decrease in the activity of superoxide dismutase and catalase. These results also contradicted the results of this study. The difference between the findings of this study and the findings of those studies can be attributed to the type and intensity of training used (36-38). The results of this study showed that eight weeks of Vitamin D supplements has a significant effect on decreasing caspase 8 and Fas levels and increasing FLIP in heart tissue of rats exposed to oxidative damage induced by H_2O_2. In recent studies, the role of antioxidant Vitamin D in animal models and culture media has been reported. Studies have also shown that lack of Vitamin D leads to mild oxidative stress and increased proteolysis in the muscle (15). Although studies on the mechanism of Vitamin D in the signaling pathway of apoptosis are limited, some researchers believe that various types of Vitamin D isoforms inhibit NF-κB activity by decreasing the expression of TNF-α and decreasing the activity of IKBα (39). Vitamin D supplements also appear to inhibit caspase 8 and increase Bcl- 2, and subsequently control apoptosis from the intrinsic and extrinsic signaling pathways (40). Regarding the effects of Vitamin D on apoptosis, studies have been conducted. For example, in line with the present study, Vitamin D treatment in the osteoblasts of the calvaria bone reduced Fas, Caspase 8 and increased expression of Bcl- 2 (40, 41). Treatment of human stomach cells with 1.25 (OH) 2D3 in the culture medium reduced Bax levels, decreased ERK and phosphorylation of AKT (42, 43); the use of 50,000 units of Vitamin D once-biweekly decreased TNF-α in healthy young men (14) receiving 1,000 units of Vitamin D supplements increased total antioxidant capacity (TAC) and reduced MDA and did not significantly alter the glutathione (GSH) and catalase (CAT) levels in children aged 6-13 years (15). Differences in statistical population, dosage and levels of measurement of variables can be reasons for the inconsistency of the results of studies. The findings of this study showed that aerobic training and Vitamin D supplementation have interactive effects on the reduction of Caspase 8, Fas in rat heart tissue of rats to oxidative damage induced by H_2O_2, however, they have no interactive effects in increasing levels of FLIP. It seems that aerobic training with Vitamin D simultaneously result in a significant reduction of Caspase 8, Fas levels in the heart tissue of rats exposed to oxidative damage caused by H_2O_2. Also, physical activity seems to inhibit cytochrome oxidase (Complex IV) from the electron transfer chains by reducing ROS and preventing increased mitochondrial cytochrome C and inhibiting nitric oxide (NO) in physiologically inverse concentrations, and hence is beneficial in reducing apoptosis of heart cell (15, 32). Nontheless, Vitamin D by reducing the expression of TNF-α and decreasing the activity of IKBα, inhibits the activity of NF-κB, controls caspase 8 and increases Bcl- 2, and subsequently controls apoptosis from the intrinsic and extrinsic signaling pathways (39, 40). Therefore, aerobic training and Vitamin D are effective in reducing caspase 8, Fas, despite the interactive effect of two different signal paths. It seems that lack of interactive effect between aerobic exercise and vitamin D in increasing the levels of the FLIP protein are due to the different anti-apoptotic signaling pathways of these two agents. Concerning the simultaneous use of Vitamin D supplements and sports exercises, researchers reported that TNF-α concentrations decreased significantly after once-biweekly use of 50,000 units of vitamin D and eight weeks of resistance training, but this interactive effect was not significant on IL -6 (14); 12 weeks; three sessions per week resistance training with 70-80% of one maximal repetition (1RM); and simultaneous use of 4000 units of Vitamin D had no interacting effect on the reduction of serum levels of TNF-α in overweight men and women; However, this interactive effect was not significant in reducing IL-6 and CRP (44). Despite numerous studies, no study has been found to investigate the simultaneous effect of Vitamin D supplements and training on the

extrinsic pathway of apoptosis. Therefore, the findings of this study cannot be compared with other studies. The lack of control of biological behaviors affecting inflammation, which leads to an increase in apoptosis in cardiac cells, is one of the limitations of the present study. Most studies have shown that the antioxidant effects of Vitamin D depend on the dosage of it. Considering the non-significance of interaction between aerobic exercise and Vitamin D, one of the limitations of the present study is the lack of consideration of other doses along with this amount to compare the results. Therefore, it is suggested that in future studies, Vitamin D supplementation with different doses should be used.

Conclusion

According to the findings of this study, it can be concluded that performing aerobic exercise activities and the simultaneous consumption of Vitamin D supplement has interactive effects on reducing some of the extrinsic pathway indices of apoptosis in the heart tissue of H_2O_2 rats exposed to oxidative damage induced by H_2O_2.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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