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Research Article

The Effect of High Intensity Interval Training on the Intrinsic and Extrinsic Pathways of Myocardial Apoptosis in Diet-Induced Obese Rats

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Abstract

Background: Diet-induced obesity is associated with several cardiovascular diseases and myocardial apoptosis. This study aimed to investigate the effect of high intensity interval training (HIIT) on the myocardial apoptosis in the diet-induced obese rats.

Methods: Thirty-two male rats, after an obesity induction period, were randomly selected and assigned to four groups including non-obese control (NOC; n = 8), basal obese control (BOC; n = 8), obese control (OC; n = 8), and obese+HIIT (OT; n = 8). NOC and BOC groups were sacrificed before the training period. The OT group underwent a HIIT program performed 5 times/week over 12 weeks. Rat hearts were removed 48 hours after the last training session. The Bax, Bcl-2, cytochrome-c, Bid, caspase-8, as well as caspase-3 protein expressions were analyzed using western blotting. Data were analyzed using independent *t* test.

Results: After obesity induction period, the Bax, Bid, cytochrome-c, caspase-8, caspase-3 proteins, and Bax/Bcl-2 in BOC were found to be significantly higher than those in NOC ($P = 0.025$, $P = 0.0001$, $P = 0.013$, $P = 0.017$, $P = 0.01$, $P = 0.18$, respectively). However, Bcl-2 protein in BOC was detected to be significantly lower than that in NOC ($P = 0.025$). The results obtained after completing HIIT training showed that Bid, cytochrome-c, caspase-8, caspase-3, and Bax/Bcl-2 in OT were lower than those in OC ($P = 0.005$, $P = 0.039$, $P = 0.001$, $P = 0.04$, $P = 0.05$, respectively). However, Bcl-2 protein in OT was significantly higher than that in OC ($P = 0.004$). No significant difference was found between OT and OC regarding Bax protein ($P = 0.32$).

Conclusion: Diet-induced obesity may have exacerbated the myocardial apoptosis through both intrinsic and extrinsic pathways. However, it seemed that HIIT training significantly prevented the increase of myocardial apoptosis in obese rats.

Keywords: High intensity interval training, Myocardial, Apoptosis, Obesity

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Background

Obesity is a complex health problem worldwide caused by an imbalance between energy intake and expenditure. Despite the contribution of genetic predisposition to the incidence of obesity, the rapid development of the obesity epidemic in recent decades has reflected fundamental changes in environmental factors, especially the diet (1). In addition to hereditary obesity, high energy intake from the diet is associated with increased cardiovascular risk factors (2) as well as with dysfunction of the heart (3). Some evidence has emerged suggesting that apoptosis plays a significant role in the development of obesity-related heart diseases and, therefore, obesity may lead to the loss of cardiomyocytes and the acceleration of some heart diseases, such as heart failure, by increasing myocardial apoptosis (4). Whelan et al have suggested that level of apoptosis in the healthy heart is very small – about 0.001%-0.002% (5), but only an enhancement of 0.023% in apoptosis due to the increased caspase8 in rat myocardium causes dilated cardiomyopathy after two to six months (6). Programmed cell death occurs through two pathways that both of which lead to the activation of caspase 3 and breakdown of cellular components (7-9). There is no conclusive evidence to confirm that obesity-induced apoptosis is activated by any intrinsic or extrinsic pathways. In this regard, George has pointed out that dietary obesity increases myocardial apoptosis, which is associated with decreased phosphoinositide3-kinase (PI3K)/Protein kinase B (AKT) pathway activity as well as decreased mitochondrial function and integrity (10). However, the exact mechanisms of obesity-induced apoptosis have still remained complicated. The effect of various exercise training on the process of apoptosis has been the subject of several health studies in the last decade. Some of them have found that the occurrence of relatively heavy and intense stress during exercise may

increase the risk of apoptotic factors (11, 12). However, some other studies have suggested that exercise training, versus cardiac apoptosis, have protective function (13, 14). High intensity interval training (HIIT) has recently received research attention because it is a time-efficient mode of exercise and produces considerable benefits (15-18). However, there is no accurate information about the effect of HIIT training on the intrinsic and extrinsic pathways of myocardial apoptosis in diet-induced obese rats. A recent, limited study by Tolouei Azar et al investigating HIIT training and apoptosis in obese rats has found that 12 weeks of HIIT training reduces the number of apoptotic cells in testicular tissue of high-fat diet-induced obese rats (19). However, the effect of HIIT training on the apoptosis process in the cardiac tissue of diet-obese rats has still remained unclear.

Objectives

Therefore, identifying the effect of HIIT training on intrinsic and extrinsic pathways of myocardial apoptosis in order to reduce the diseases and related damages, especially in obese subjects, seemed an undeniable necessity. To this end, the present study aimed to investigate the effect of 12 weeks of high-intensity interval training on the intrinsic and extrinsic pathways of myocardial apoptosis among diet-induced obese rats.

Methods

Animals

Thirty-two male Wistar rats (aged 21 days; weighed 40 ±5 g) were purchased from the Pasteur Institute Animal Care Center (Karaj, Iran). To create acclimation, the rats were housed for one week in a well-controlled temperature of 20-24˚C, humidity of 50±10%, under a 12 h light/dark cycle. Animals were provided with standard chow and water *ad libitum*. The study was conducted in accordance with the instructions for treating animals in Medical Sciences of the Islamic Azad University of Tabriz (IR.IAU.TABRIZ.REC.1398.086).

Obesity Induction

After the acclimatization period was over, eight rats were randomly isolated from the others as a non-obese control group (NOC; n = 8) and 24 rats were fed with high-fat chocolate milk drink (960 kcal/L; 14% protein, 20% fat, 68% carbohydrate) made available over 6 weeks by using an extra beverage bottle (1). Consumption of chocolate milk, pellets, and weight changes were recorded every week. The obesity was confirmed by Lee index ([body weight^{-3} (grams)/height (centimeters)] × 1000). Following this, eight obese rats were randomly selected as the basal obese control group (BOC; n = 8). NOC and BOC groups were sacrificed at the beginning of the training period in order for evaluating the effect of diet-induced obesity on myocardial apoptosis.

High-Intensity Interval Training Protocol

Initially, all 16 obese rats were familiarized with a motor-driven treadmill (incline: 0%, speed:10-25 m/min, duration: 5-10 minutes) for one week. Then, all animals performed an incremental exhaustive exercise test to calculate the maximum running speed (equivalent to VO2peak). In this test, the rats performed an incremental test to exhaustion on a treadmill inclined to 15° (starting at 10 m/min with increments of 3 m/min every 2 minutes; the criterion was to go into shock three times or leave the treadmill bar). Then, animals were weight matched and randomly divided into two groups: obese control (OC; n = 8) and obese with HIIT training (OT; n = 8). The OC group was excluded from participating in any training intervention until the end of the course. The OT group was subjected to a high-intensity interval training program performed 5 sessions per week over 12 weeks (Table 1) (20). The treadmill speed was increased by five percent each week to meet the principle of overload.

Tissue Removal

All rats in OC and OT groups were anesthetized with intraperitoneal (IP) injection of ketamine (90 mg kg^{-1}) and xylazine (10 mg kg^{-1}) and sacrificed 48 h following the last exercise session; then the cardiac tissues were extracted and maintained at -80°C for later analyses.

Western Blotting

Table 1. Details of High Intensity Interval Training Program

Week of Training	1	2	3	4	5	6	7	8	9	10	11	12
High intensity interval (85-90% $VO2_{peak}$)												
Speed (m/min)	26	26	27	28	29	30	31	32	33	34	35	36
Time (min)	2	2	2	2	2	2	2	2	2	2	2	2
Low intensity interval (45-50% $VO2_{peak}$)												
Speed (m/min)	10	10	11	12	13	14	15	16	17	18	19	20
Time (min)	2	2	2	2	2	2	2	2	2	2	2	2
No. of repetitions	5	6	7	8	9	10	11	12	13	14	15	16
Treadmill grade (%)	15	15	15	15	15	15	15	15	15	15	15	15
Total workout time (min /day)	20	24	28	32	36	40	44	48	52	56	60	64

This technique was applied in order to evaluate the expression of Bax, Bcl-2, Bid, cytochrome-c, caspase-8, and caspase3 proteins in cardiac tissue. Protein lysates were isolated using lysis buffer (20mM Tris-HCL (pH7.5), 150mM NaCl, 1mM Na2EDTA, 1mM EGTA, 1%NP-40) supplemented with complete protease inhibitor cocktail, and centrifuged at 12000×g for 15 minutes at 4ºC. The protein concentration of the supernatant was determined by the Bradford method. Proteins (20 µg proteins) were separated using SDS–polyacrylamide gel electrophoresis using 8%–12% denatured, ready gel and transferred onto a polyvinylidene difluoride (PVDF) membrane (Sigma). The membrane was blocked for 1 hour in 5% BSA in tris-buffered saline and 0.1% Tween 20 (TBST) to block nonspecific bindings. Subsequently, blots were incubated overnight at 4ºC with primary antibodies: β-actin (sc-47778), Bax (sc-7480), Bcl-2 (sc-492), Bid (ab10640), cytochrome-c (sc-13156), caspase-8 (ab138485), caspase-3 (sc-7272) (purchased from Santa Cruz (1:500)), and Acetyl-p53 (Lys382; purchased from cell Signaling Technology (1:500)). The membrane was then washed three times and incubated with the appropriate secondary antibody for 1 hour at room temperature in 5% milk in TBST. Protein bands were visualized using an enhanced chemiluminescence reagent, and radiographic film (Fujifilm, Tokyo, Japan) was quantified performing densitometry analysis by ImageJ software (National Institute of Health, Bethesda, Maryland, USA). Then, the density of the target protein bands was normalized against beta-actin control loading. Finally, the results were presented as relative density (compared to the NOC group).

Statistical Analysis

The data are presented as Means ± SD. Shapiro Wilk test was applied to assess the distribution of the data and it was confirmed to be normal. Thus, independent *t* test was used to determine the difference between NOC and BOC groups during obesity period and OC and OT groups during HIIT training period. Statistical analysis was defined to be significant if $P<0.05$. Statistical analyses were performed using SPSS 19.

Results

Table 2 shows some characteristics of the studied rats (means ± SD). According to the results presented in Table 2, body weight and Lee index were significantly higher in the BOC group than the NOC group after the obesity induction period ($P=0.0001$, $P=0.001$, respectively). At the end of this period, the heart/body weight ratio in obese rats was significantly lower than that in non-obese rats ($P=0.02$), although heart weight in obese rats was non-significantly higher than that in non-obese rats ($P=0.34$). In other words, 6 weeks of fattening diet significantly increased the body weight and Lee index, but significantly reduced heart/body weight ratio in the rats. Furthermore, body weight and Lee index were significantly lower in the OT group than the OC group after the HIIT training ($P=0.002$, $P=0.01$, respectively). However, the ratio of heart to body weight in OT group was significantly higher than that in OC group at the end of this period ($P=0.034$). In other words, 12 weeks of HIIT training significantly reduced body weight and Lee index, but significantly increased the heart-to-body weight ratio in obese rats.

The results demonstrated that the Bax, Bid, cytochrome-c, caspase-8, caspase-3 proteins, and Bax/Bcl-2 in BOC group were significantly higher than those in the NOC group after obesity induction period ($P=0.025$, $P=0.0001$, $P=0.013$, $P=0.017$, $P=0.01$, $P=0.18$, respectively). However, the Bcl-2 protein in the BOC group was significantly lower than that in the NOC group ($P=0.025$). In other words, six weeks of fattening diet significantly increased the pro-apoptotic proteins, but significantly reduced the anti-apoptotic protein in the myocardium of rats (Figure 1).

After completing HIIT training, the results revealed that the Bid, cytochrome-c, caspase8, caspase3, and Bax/Bcl-2 in OT group were lower than those in the OC group ($P=0.005$, $P=0.039$, $P=0.001$, $P=0.04$, $P=0.05$,

Table 2. Characteristics of Animals in Different Groups

Variable		Group			
		NOC (n=8)	BOC(n=8)	OC(n=8)	OT(n=8)
Weight (g)	Initial	40±5			
	After obesity induction	158.6±22.2	302.4±15.3*	294.43±11.67	
	After HIIT training	-	-	326.1±18.3	304.2±17.5#
Lee Index	After obesity induction	284.2±35.2	352.0±29.7*	349.47±33.17	
	After HIIT training	-	-	312.7±27.6	304.0±21.1#
Heart weight (g)	After obesity induction	0.61±0.07	0.84±0.06	-	-
	After HIIT training	-	-	0.99±0.11	1.02±0.2
Heart/body weight (g/kg^{-1})	After obesity induction	3.86±0.35	2.91±0.14*	-	-
	After HIIT training	-	-	3.03±0.26	3.5±0.21#

NOC, non-obese control; BOC, basal obese control; OC, obese control; OT, obese with HIIT training.
* Significant Difference with NOC, $P<0.05$; # Significant difference with OC, $P<0.05$.

respectively). However, the Bcl-2 protein in the OT group was significantly higher than that in the OC group ($P=0.004$). There was no significant difference between the two groups of OT and OC regarding Bax protein ($P=0.32$). In other words, 12 weeks of HIIT training significantly reduced the pro-apoptotic proteins, but significantly increased the anti-apoptotic protein in the rat's myocardium (Figure 2).

Discussion

Dietary obesity may be associated with an increased incidence of myocardial apoptosis and various cardiovascular diseases. Therefore, the present study aimed to evaluate the effect of 12 weeks of HIIT training on the intrinsic and extrinsic pathway of myocardial apoptosis in diet-induced obese male rats. As for the initial period (after the obesity induction period), the results of the present study showed that the levels of Bax, Bid, cytochrome-c, caspase-8, and 3 proteins in myocardial of obese rats were about 94%, 227%, 189%, 81%, and 212% higher than the levels of those in non-obese rats, respectively. Also, the Bax/Bcl-2 in the cardiac of obese rats was about 180% higher than that in non-obese rats. In this regard, the level of anti-apoptotic protein Bcl-2 in the myocardium of obese rats was significantly and about 33% lower than the level of that in non-obese rats. Our study results were consistent with the findings from a study by Dungan et al where it was found that dietary obesity increased the critical caspase-3 protein so that the level of this protein became significantly higher in high-fat diet than low-fat diet. However, caloric restriction was able to moderate the adverse changes due to obesity (21). Similar to the results of the present study, some previous studies had suggested that obese diets increased the Bax to Bcl-2 ratio, cytochrome-c, caspase-3, and the number of apoptotic nuclei in rats (22-24). However, there is no conclusive evidence that obesity-induced apoptosis is activated by any intrinsic and extrinsic pathways, particularly in the

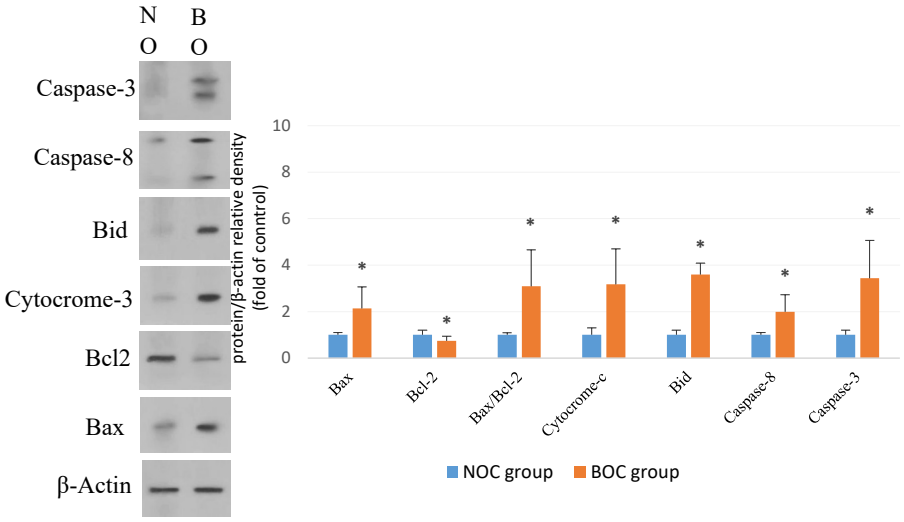

Figure 1. Mean±SD for Myocardial Apotosis Variables After Obesity Induction in NOC and BOC Groups. NOC = Non-obese Control; BOC = Basal Obese Control; * Significant Difference With Non-obese Control, $P<0.05$.

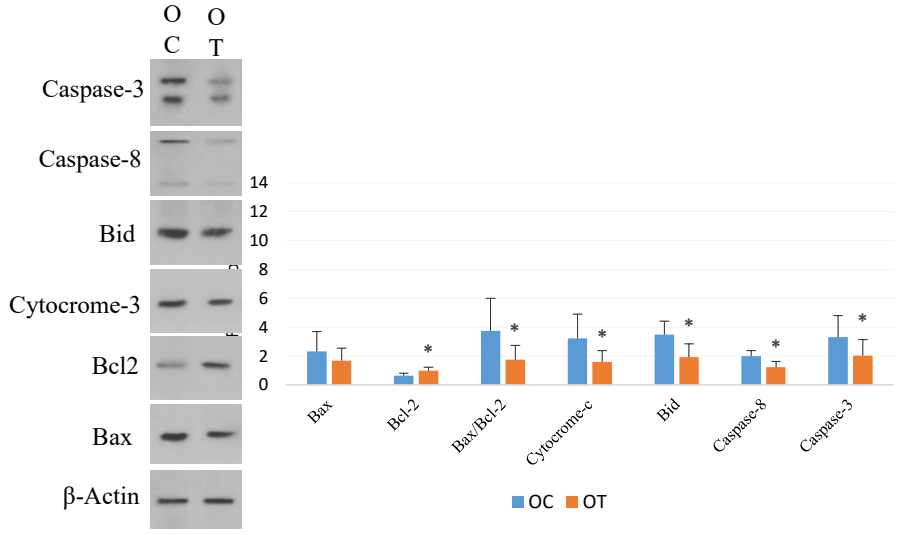

Figure 2. Mean±SD for Myocardial Apotosis Variables After HIIT Training in OC and OT Groups. OC = Obese control; OT = Obese with HIIT training. * Significant difference with OC group $P<0.05$.

myocardium. According to our study results, diet-induced obesity activated proteins of both intrinsic and extrinsic pathways leading to caspase-3. Some studies have linked apoptotic death of cardiomyocytes to the extrinsic pathway only (25), and others have confirmed a further increase in mitochondrial apoptotic pathway activation in the cardiomyocytes of obese rats (26). In the present study capase 8, that activates Bid protein, increased in obese rats indicating the vital role of obesity-induced inflammation and activation of TNF and FAS in myocardial apoptosis. However, the molecular origins of this inflammation are still unknown.

As for the second period (after HIIT training), our study results revealed that the levels of Bid, cytochrome-c, Bax/Bcl-2, caspase-8, and caspase-3 proteins in myocardial of trained obese rats were about 45%, 51%, 38%, 39%, and 53% lower than those of untrained obese rats, respectively. However, the level of Bcl-2 protein in trained obese rats was about 56% higher than that in untrained obese rats. Even though the important protein content of Bax pro-apoptotic protein in trained obese rats was about 27% lower than that in untrained obese rats, the difference between the two groups was not significant. It seemed that HIIT training prevented the exacerbation of this process in the myocardium of obese rats by reducing the expression of proteins involved in both intrinsic and extrinsic pathways of apoptosis. Although there was no significant difference between the trained and untrained obese rats in terms of Bax protein, molecular events in the intrinsic (mitochondrial) pathway are mainly defined by the balance between pro and anti-apoptotic specific regulatory proteins. In the meantime, Bax and Bcl2 proteins are involved in the formation of apoptosis and mitochondrial apoptotic signaling as the main proteins. Increasing Bcl2 and, consequently, decreasing the Bax/Bcl2 ratio is one of the most important factors to avoid mitochondrial membrane damage as well as to prevent apoptosis (20). To date, no studies have been conducted to investigate the effect of HIIT training on cardiac apoptosis in obese subjects, and the results obtained by similar studies on non-obese subjects and other tissues are often contradictory. Our study results were in line with the findings from Tolouei Azar et al study which reported that 12 weeks of HIIT reduced the number of apoptotic cells in the testicular tissue of high-fat, diet-induced obese rats (19). Soori et al (27) and Pourrazi et al (20) also determined that HIIT training increased the Bcl-2 gene expression but decreased the Bax/Bcl-2 in the cardiac of young and healthy rats. Lee et al suggested that both Fas-dependent (extrinsic pathway) and mitochondrial-dependent apoptotic pathways were more active in obese Zucker (inherited) rats, and three months of exercise training reduced cardiomyocyte apoptosis through both pathways (28). Although there are several mechanisms for the protective effects of exercise training against apoptosis (e.g., direct changes in the expression of apoptotic genes, decreased release of mitochondrial apoptotic agents, and changes in reactive oxygen species (ROS) production (29)), ambiguities in this regard have not yet removed, especially regarding the effect of HIIT training. Despite the given explanations, the results of some previous studies using interval and continuous exercise were not in agreement with the results from the present study. Krüger et al suggested that short-term HIIT training intensified the process of apoptosis in the immune system (11). Unlike short term exercise, it seems that chronic exercise produces different physiological and metabolic adaptations that can ultimately affect the process of apoptosis.

Contrary to our study results, the findings from the study by Carvalho et al (2020) also indicated that 12 weeks of HIIT had no effect on Bax, SMAC, and caspase-8 proteins expression in the cardiac of healthy rats (30). Although the type, duration, and intensity of exercise in Carvalho et al study were similar to those in the present study, it seemed that the type of subjects was the most important reason behind the difference between the results of Carvalho et al study and those of the present study. As noted in the result section of the first period, diet-induced obesity increased the expression of apoptotic proteins in the myocardium, but this phenomenon rarely occurs in healthy and non-obese rats. Therefore, the lack of change in some apoptotic proteins in healthy subjects following exercise training is not unexpected. However, in obese subjects with high levels of apoptotic proteins, exercise-induced adaptation may prevent the escalation of apoptosis process. The present study had some limitations. First, the sample size was not large enough; therefore, it was recommended that a larger sample size be adopted for future studies. Second, there was a lack of the direct measurement of cardiomyocyte cell death as well as the determination of the mechanism of death. Therefore, changes in the expression of proteins involved in apoptosis may not have confirmed the outcome.

Conclusion

Overall, it was concluded that the diet-induced obesity may have exacerbated the myocardium apoptosis through both apoptosis pathways. Besides the positive functional effects such as weight loss and decreased Lee index, seemingly, high-intensity interval training exerted an anti-apoptotic effect against apoptosis in myocardium of obese rats. However, it was recommended that further studies be conducted to thoroughly understand the effects of high-intensity interval training on cardiac apoptosis indices in obese rats.

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Authors' Contribution

Contribution of the authors was in accordance with the research regulations.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Approval

The present study was approved by the Ethics Committee of Medical Sciences of the Islamic Azad University, Tabriz Branch (IR.IAU.TABRIZ.REC.1398.086).

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References

1. Archer ZA, Corneloup J, Rayner DV, Barrett P, Moar KM, Mercer JG. Solid and liquid obesogenic diets induce obesity and counter-regulatory changes in hypothalamic gene expression in juvenile Sprague-Dawley rats. J Nutr. 2007;137(6):1483-90. doi: 10.1093/jn/137.6.1483.
2. Wu H, Ballantyne CM. Metabolic inflammation and insulin resistance in obesity. Circ Res. 2020;126(11):1549-64. doi: 10.1161/circresaha.119.315896.
3. Carbone S, Lavie CJ, Elagizi A, Arena R, Ventura HO. The impact of obesity in heart failure. Heart Fail Clin. 2020;16(1):71-80. doi: 10.1016/j.hfc.2019.08.008.
4. Lin KH, Marthandam Asokan S, Kuo WW, Hsieh YL, Lii CK, Viswanadha V, et al. Andrographolide mitigates cardiac apoptosis to provide cardio-protection in high-fat-diet-induced obese mice. Environ Toxicol. 2020;35(6):707-13. doi: 10.1002/tox.22906.
5. Whelan RS, Kaplinskiy V, Kitsis RN. Cell death in the pathogenesis of heart disease: mechanisms and significance. Annu Rev Physiol. 2010;72:19-44. doi: 10.1146/annurev.physiol.010908.163111.
6. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, et al. A mechanistic role for cardiac myocyte apoptosis in heart failure. J Clin Invest. 2003;111(10):1497-504. doi: 10.1172/jci17664.
7. Lorente L, Martín MM, Ortiz-López R, González-Rivero AF, Pérez-Cejas A, Pastor E, et al. Association of serum soluble Fas concentrations and mortality of septic patients. Enferm Infecc Microbiol Clin (Engl Ed). 2020. doi: 10.1016/j.eimc.2020.08.003.
8. Ader NR, Hoffmann PC, Ganeva I, Borgeaud AC, Wang C, Youle RJ, et al. Molecular and topological reorganizations in mitochondrial architecture interplay during Bax-mediated steps of apoptosis. Elife. 2019;8. doi: 10.7554/eLife.40712.
9. Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. 2013;14:32. doi: 10.1186/1471-2121-14-32.
10. George S. A Critical Analysis of Mitochondrial Functioning and Associated Proteins in Obesity-Related Cardiomyopathy [dissertation]. Stellenbosch: Stellenbosch University; 2013. p. 1-23
11. Krüger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, et al. Apoptosis of T-cell subsets after acute high-intensity interval exercise. Med Sci Sports Exerc. 2016;48(10):2021-9. doi: 10.1249/mss.0000000000000979.
12. Liu WY, He W, Li H. Exhaustive training increases uncoupling protein 2 expression and decreases Bcl-2/Bax ratio in rat skeletal muscle. Oxid Med Cell Longev. 2013;2013:780719. doi: 10.1155/2013/780719.
13. Lai CH, Ho TJ, Kuo WW, Day CH, Pai PY, Chung LC, et al. Exercise training enhanced SIRT1 longevity signaling replaces the IGF1 survival pathway to attenuate aging-induced rat heart apoptosis. Age (Dordr). 2014;36(5):9706. doi: 10.1007/s11357-014-9706-4.
14. Lu K, Wang L, Wang C, Yang Y, Hu D, Ding R. Effects of high-intensity interval versus continuous moderate-intensity aerobic exercise on apoptosis, oxidative stress and metabolism of the infarcted myocardium in a rat model. Mol Med Rep. 2015;12(2):2374-82. doi: 10.3892/mmr.2015.3669.
15. Fisher G, Schwartz DD, Quindry J, Barberio MD, Foster EB, Jones KW, et al. Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise. J Appl Physiol (1985). 2011;110(3):730-7. doi: 10.1152/japplphysiol.00575.2010.
16. Gomes Neto M, Durães AR, Conceição LSR, Saquetto MB, Ellingsen Ø, Carvalho VO. High intensity interval training versus moderate intensity continuous training on exercise capacity and quality of life in patients with heart failure with reduced ejection fraction: a systematic review and meta-analysis. Int J Cardiol. 2018;261:134-41. doi: 10.1016/j.ijcard.2018.02.076.
17. Andreato LV. Effects of high-intensity interval training: risk of bias by definition. Int J Cardiol. 2019;279:134. doi: 10.1016/j.ijcard.2018.09.109.
18. Maillard F, Pereira B, Boisseau N. Effect of high-intensity interval training on total, abdominal and visceral fat mass: a meta-analysis. Sports Med. 2018;48(2):269-88. doi: 10.1007/s40279-017-0807-y.
19. Tolouei Azar J, Habibi Maleki A, Moshari S, Razi M. The effect of different types of exercise training on diet-induced obesity in rats, cross-talk between cell cycle proteins and apoptosis in testis. Gene. 2020;754:144850. doi: 10.1016/j.gene.2020.144850.
20. Pourrazi H, Asgharpour-Arshad M, Gholami F, Abbasi S. Effect of high-intensity interval training on apoptotic gene expression in rat myocardial tissue. Gene Cell Tissue. 2020;7(2):e101963. doi: 10.5812/gct.101963.
21. Dungan CM, Li J, Williamson DL. Caloric restriction normalizes obesity-induced alterations on regulators of skeletal muscle growth signaling. Lipids. 2016;51(8):905-12. doi: 10.1007/s11745-016-4168-3.
22. Abrigo J, Rivera JC, Aravena J, Cabrera D, Simon F, Ezquer F, et al. High fat diet-induced skeletal muscle wasting is decreased by mesenchymal stem cells administration: implications on oxidative stress, ubiquitin proteasome pathway activation, and myonuclear apoptosis. Oxid Med Cell Longev. 2016;2016:9047821. doi: 10.1155/2016/9047821.
23. Sishi B, Loos B, Ellis B, Smith W, du Toit EF, Engelbrecht AM. Diet-induced obesity alters signalling pathways and induces atrophy and apoptosis in skeletal muscle in a prediabetic rat model. Exp Physiol. 2011;96(2):179-93. doi: 10.1113/expphysiol.2010.054189.
24. Yuzefovych LV, Musiyenko SI, Wilson GL, Rachek LI. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. PLoS One. 2013;8(1):e54059. doi: 10.1371/journal.pone.0054059.
25. Lee SD, Tzang BS, Kuo WW, Lin YM, Yang AL, Chen SH, et al. Cardiac fas receptor-dependent apoptotic pathway in obese

Zucker rats. Obesity (Silver Spring). 2007;15(10):2407-15. doi: 10.1038/oby.2007.286.
26. Lu MC, Tzang BS, Kuo WW, Wu FL, Chen YS, Tsai CH, et al. More activated cardiac mitochondrial-dependent apoptotic pathway in obese Zucker rats. Obesity (Silver Spring). 2007;15(11):2634-42. doi: 10.1038/oby.2007.315.
27. Soori R, Ghram A, Zare Shahneh M, Choobineh S, Costa PB, Voltarelli FA. Effects of high intensity interval training and aging on cardiac muscle apoptosis markers in C57BL/6 Mice. Sport Sci Health. 2021;17(1):173-9. doi: 10.1007/s11332-020-00670-2.
28. Lee SD, Shyu WC, Cheng IS, Kuo CH, Chan YS, Lin YM, et al. Effects of exercise training on cardiac apoptosis in obese rats. Nutr Metab Cardiovasc Dis. 2013;23(6):566-73. doi: 10.1016/j.numecd.2011.11.002.
29. McMillan EM, Graham DA, Rush JW, Quadrilatero J. Decreased DNA fragmentation and apoptotic signaling in soleus muscle of hypertensive rats following 6 weeks of treadmill training. J Appl Physiol (1985). 2012;113(7):1048-57. doi: 10.1152/japplphysiol.00290.2012.
30. Carvalho MR, Mendonça MLM, Oliveira JML, Romanenghi RB, Morais CS, Ota GE, et al. Influence of high-intensity interval training and intermittent fasting on myocardium apoptosis pathway and cardiac morphology of healthy rats. Life Sci. 2021;264:118697. doi: 10.1016/j.lfs.2020.118697.