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The Effect of Four Weeks of Continuous Aerobic Training and Starvation on the Expression of Pink1 and Bnibp3 Genes in liver Tissue, liver Enzymes, and lipid profile of Wistar Fatty Model Rats

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Abstract

Background and objectives: Regular exercise and calorie intake promote mitochondrial function by promoting healthy mitochondrial regeneration. The present study aimed to investigate the effects of four weeks of continuous aerobic training and starvation on the gene expression of Pink1 and Bnibp3 in liver Tissue, liver Enzymes, and lipid profile in Wistar Fatty Model Rats.

Material and Methods: Thirty 18-week-old Fatty Model male Wistar rats with an average body weight of 348±25.53 were selected and randomly divided into six groups of 5, control (n=5) and experimental (n=25), including starvation, starvation, and three days of exercise, starvation, and five days of exercise, three days of exercise, five days of exercise groups. Calorimetric methods measured biochemical factors, including lipid profile. Real-time RT-PCR was used to quantify the mRNA expression of genes.

Results: There was a significant decrease in triglyceride (P=0.00), cholesterol (P=0.001), liver enzymes ALT (P=0.001), and AST (P=0.00) in all groups compared to the controls. Furthermore, there was a significant increase in Pink1 gene expression in the starvation group (P=0.00) and starvation groups of 3 and 5 days of training (P=0.00, p=0.00) and in gene expression of Bnibp3 in the starvation group (P=0.00) and starvation groups of 5 days of training (P=0.00) compared to the control group.

Conclusion: Four weeks of continuous aerobic training and starvation combined and alone significantly reduced the status of blood lipids and liver enzymes in fatty model rats. The starvation group and starvation groups, along with exercise, can increase the activity of removing damaged mitochondria by elevating the expression of Pink1 and Bnibp3 genes.

Keywords: Non-alcoholic Fatty Liver Disease [MeSH], Starvation [MeSH], Exercise [MeSH]; Lipid Droplets [MeSH] ; Mitophagy [MeSH]; PTEN-induced putative kinase [MeSH]

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Highlights

- Physical exercise has been proposed as a nondrug treatment against different diseases for people of all ages.
- Four weeks of continuous aerobic training and starvation combined and alone significantly reduced the status of blood lipids and liver enzymes in fatty model rats.
- The starvation group and starvation groups, along with exercise, can increase the activity of removing damaged mitochondria by elevating the expression of Pink1 and Bnibp3 genes.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common cause of liver disease in developed countries (1). NAFLD is a hepatic manifestation of metabolic syndrome and is closely related to dyslipidemia, central obesity, hypertension, and insulin resistance (2). NAFLD affects one-third of the adult population in the western world (3, 4), and rapidly becoming a worldwide public health problem with projected exponential growth in the next 10 years due to high-calorie intake combined with a sedentary lifestyle (4). NASH is also predicted to become the most common sign of liver transplantation shortly (5, 6). NAFLD covers a range of pathologies ranging from simple hepatic steatosis (more than 55 mg triglycerides per gram of liver) to Nonalcoholic Steatohepatitis (NASH), hepatic steatosis with inflammation, cell ballooning, and varying degrees of fibrosis (7). While steatosis is generally considered benign in the absence of inflammation and fibrosis, the more advanced form of NASH, and especially the fibrous NASH, is the leading cause of liver-associated cirrhosis and death (8).

Liver damage due to a high-fat diet is characterized by the accumulation of triglycerides in hepatocytes formed by the esterification of free fatty acids and glycerol (9). In this regard, a slight-to-average increase in hepatic aminotransferases by alanine aminotransferase and aspartate aminotransferase is the most common laboratory sign used in the evaluation and diagnosis of NAFLD (10). NAFLD is the main reason for the increase in liver enzymes in more than 90% of cases. The levels of these enzymes in patients with NAFLD rarely increase to 4 times normal compared to healthy individuals. Recent studies have shown that ALT is even within the normal range as a sensitive marker for the expression of liver cell damage and abnormal function (11). The pathogenesis of the disease is still unclear and has no obvious clinical symptoms. In vitro examination of patients showed an increase in serum ALT and AST of about 1.5 to 3 times (12). In contrast to fatty liver disease, NAFLD often has a serum ALT level higher than AST (ALT to AST ratio is less than one) (13).

Autophagy-dependent degradation of damaged organs is a cellular pathway critical to maintaining cellular homeostasis. Organopathic degeneration can be selective for mitochondrial (mitophagy), endoplasmic reticulum, and fat droplets (lipophage) (14). Lipophagy is currently considered an alternative pathway for lipid metabolism in liver cells, and lipophage disorder contributes to NAFLD development (2). Previous studies have shown that lipotoxic toxicity induced by Free Fatty Acids (FFAs) disrupts Endoplasmic Reticulum (ER) function by increasing protein miscarriage and inducing ER stress, which can exacerbate NAFLD (15). In addition, autophagy can lead to cell death and is associated with apoptosis-dependent caspase-3 activation (16). Studies show that PINK1 and Parkin usually work together to control mitochondrial quality (2). PINK1 is generally undetectable in healthy mitochondria because it is cleaved by Porcine-Related Protein (PARL) after entering the mitochondrial matrix (17). The cleaved PINK1 fragments are then released into the cytoplasm, where the ubiquitin-proteasome system degrades them via the N-terminal pathway. The cytosolic fragments of PINK1 inhibit parkin-to-mitochondrial transmission by direct interaction with parkin (1). When parkin is located in the mitochondria, it increases the ubiquitination of outer mitochondrial membrane proteins that bind to SARs to further adsorb autophagosomes to

damaged mitochondria (2). This information suggests that two PINK1 cell ponds may regulate parkin transport and mitophagy differently. PINK1 cytosolic fragments inhibit parkin transport, while mitochondrial PINK1 transmits parkin transport and mitophagy (10). Evidence suggests that mitochondria can be removed by parkin-independent autophagy (18).

Several autophagy receptor proteins are also mitochondrial resident proteins, such as BNIBP3, NIX, and FUNDC1, induced under hypoxic conditions. These receptor proteins adsorb autophagosomes to mitochondria through direct interaction with LC3 (19). In BNIBP3 protein, only BH3 has a dual function in regulating cell death and mitophagy (15). Phosphorylation of Ser17 and Ser24 on the BNIBP3 LIR motif positively regulates its binding to LC3 and promotes survival-supporting mitophagy instead of apoptosis in mammalian cells (20). According to various studies, it has been reported that regular aerobic activity can reduce fat and liver vulnerability and reduce inflammation in non-alcoholic fatty liver (21). Some findings suggest that regular endurance training induces fission, mitophagy, and oxidative phosphorylation in human skeletal muscle, regardless of age (22). Recent studies indicate that exercise may improve NAFLD by increasing autophagy (23). On the other hand, aerobic exercise could reduce serum triglyceride, cholesterol and aspartate aminotransferase and alanine aminotransferase and improve the liver condition in patients with fatty liver (24).

In a study, male C57 / BLK6 rats with progressive swimming exercise (6 minutes to 60 minutes per day) with an intensity of 40% max2Vo for 5 days a week for at least 10 weeks over a 22-weeks with a high-fat diet, improved body mass and insulin sensitivity. Also, beneficial changes in exercise were associated with significant suppression and increased lipogenic expression and expression of oxidative genes in the liver. In another study, after endurance training, energy consumption exceeded production and as a result, a higher ratio of AMP to ATP was observed (25). Lifestyle changes such as weight loss and diet modification have long been the first steps in managing NAFTD. Weight loss seems to improve liver function independently in NAHD, although improving intrahepatic lipid profile requires at least 3 to 5 percent weight loss through physical activity and calorie control. Different biochemical activities can make beneficial changes not only in the muscle but also in the liver and fat tissue (16). The main objectives of the study were the effect of 4 weeks of continuous aerobic exercise and starvation as a natural stimulant, the liver mitophagy can provide metabolic protection.

Materials and Methods

Animals

The design of the present study was experimental. Thirty 18-to-20-week-old fatty Model, male Wistar rats with an average body weight of 348±25.53 purchased from the Pasteur Institute of Iran were selected as the research sample. After one week of familiarity with the laboratory environment, these Fatty Model Rats were randomly divided into 6 groups of 5, control (n=5) and experimental (n=25) including starvation, starvation and 3 days of exercise, starvation and 5 days of exercise, 3 days of exercise, 5 days of exercise groups. They were kept in an environment with an average temperature of 22±1.4 °C, humidity of 55±4%, and the light-dark cycle of 12:12 hours in special polycarbonate cages with a height of 43 cm, a length of 27 cm, and a width of 25 cm with 2 to 3 rats in each cage in the Animal Science Center of Gorgan University of Medical Sciences. The code of ethics of this research with the ID IR.IAU.AK.REC.1399.026 has been approved by the Aliabad Branch of Islamic Azad University.

Experimental design

All fatty model animals had free access to standard pellet water and food (10 g of food per 100 g of rat body weight). All steps of keeping and sacrificing rats were performed according to the instructions for keeping laboratory animals. The starvation protocol was applied to the rats for 14 hours a day during the waking cycle (5:30 p.m. to 7:30 a.m.) for one month. To induce hunger, the rats in the starvation group received the same usual amount (10 g per 100 g of rat weight) of food within 10

hours whereas the rest of the groups received this amount within 24 hours.

Exercise protocol

The whole training period included two stages of introduction and the main training. The purpose of the introduction stage was to adapt to the research environment and the treadmill. For this purpose, the animals were subjected to experimental training for 15 minutes for one week. Then they performed exercise for 4 weeks, 3 and 5 days a week for 45-60 minutes on a treadmill. The main exercise program in this study is shown in Table 1. Rats' training was done on a treadmill with a zero-degree slope at a speed of 14 meters per minute. After training sessions, the treadmill speed reached 16 and 18 meters per minute with a zero-degree slope (26).

Table 1. Details of continuous aerobic exercise protocol

Week	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Exercise characteristics	Speed - Duration	Speed - Duration	Speed - Duration	Speed - Duration	Speed - Duration	Speed - Duration
Week 1 Adaptation to the environment	NO Exercise	NO Exercise	NO Exercise	NO Exercise	NO Exercise	NO Exercise
Week 2 Familiarity with Exercise	4-8	8-10	3-5	3-5	3-5	3-5
First week of exercise	5-7	5-10	20-14	10-7	5-4	5-4
Second week	7-8	7-14	25-16	10-12	6-6	6-6
Third week	5-8	10-14	20-18	10-14	10-10	5-5
Fourth week	5-8	10-14	20-18	10-14	10-10	5-5

Evaluating the biochemical factors and mRNA expression of genes

Biochemical factors including lipid profile (triglyceride, total cholesterol) were measured by using calorimetric method, and liver enzyme levels of Aspartate Transaminase (AST), Alanine Aminotransferase (ALT) by using the kinetic method via Farasa MED and Biorex Fars kits (Iran) and BS480 clinical chemistry autoanalyzer.

To evaluate the gene expression levesl, RNA was first extracted from tissues in all groups of the study, according to the protocols of Azma Yekta-Tajhiz Company (Tehran, Iran; cat. No: FABRK001). Then the quality and quantity of RNA were measured with the nanodrop device of Golestan University of Medical Sciences and the cDNA was synthesized by Pars Tous kit (Mashhad, Iran). The cDNA was then used to evaluate the expression levels of Pink1 and Bnibp3 genes by quantitative real-time PCR method using SYBR green method by Yekta Tajhiz master mix (Tehran, Iran Cat. No: YT2552). Glyceraldehyde-3-Phosphate Dehydrogenase Gene (GAPDH) was used as an internal control gene and the expression of the desired genes were calculated by the $2^{-\Delta\Delta CT}$ formulation. The sequence of primers used is listed in Table 2.

Statistical analysis

The one-way analysis of variance (ANOVA) was used to analyze data at the level of less than 0.05 and the Bonferroni *posthoc* test was used to further examine the differences.

Table 2. The sequences of primers used

Genes	Primer sequence	Nucleotide count
PINK1	For: 5′- GGAGGAGTATCTGATAGGGCAG -3′ Rev: 5′- AACCCGGTGCTCTTTGTCAC -3′	22 20
BNIBP3	For: 5′- CAGGGCTCCTGGGTAGAACT-3′ Rev: 5′- CTACTCCGTCCAGACTCATGC -3′	20 21
GAPDH	For: 5′- CACTGAGCATCTCCCTC ACAA-3′ Rev: 5′- TGGTATTCGAGAGA AGGGAGG -3′	22 22

Results

Table 3 and Table 4 demonstrate the descriptive data of all variables in each group and represents the means ± SD of each variable in each group. According to the statistical results of one-way ANOVA, there was a significant decrease in triglyceride (P=0.00), cholesterol (P=0.001), liver enzymes ALT (P=.001), AST (P=0.00) in all groups compared to the control group. Furthermore, there was a significant increase in Pink1 gene expression in starvation group (P=0.00) and starvation groups of 3 and 5 days of training (P=0.00, p=0.00) and in gene expression of Bnibp3 in starvation group (P=0.00) and starvation groups of 5 days of training (P=0.00) compared to the control group. Also, an intergroup comparison with Bonferroni test was performed, showing a significant difference in the following groups according to Table 5.

Table 3. Mean and standard deviation of fat groups

Mean and standard deviation of variables	Control	starvation	3 days of exercise	5 days of exercise	starvation + 3 days of exercise	starvation + 5 days of exercise
Final weight	439.6000± 8.17313	295.4000± 16.00937	302.4000± 22.17656	282,60± 9.581	273.2000± 27.96784	261.80± 23.004
Triglycerides	139,00± 50.1098	66.000± 13.39776	70.000± 14.67992	66,200± 44.330	47.200± 11.03177	30,200± 7.19
Cholesterol	178.80 36.092±	64.6000± 5.72713	50.6000± 11.37102	62.600 28.0588±	60.6000± 9.55510	41.8000 19.841±
Alanine transferase	274.22 40.390±	58.4400± 5.87861	119.24 23.9252±	100.88 ±44.542	71.0200± 15.90258	66.980± 10.0542
Aspartate transferase	505.0± 130.7578	221.4800± 40.8545	3.78 63.6146±	223.14± 64.49	237.6800± 52.34498	141.2± 89.77
Pink 1	1.000± .000	32.774± 8.0708	3.8120± 1.80741	.3878± .15514	20.000± 12.00000	14.6780± 5.70118
Bnibp 3	1.0000± .000	17.4860± 8.39657	2.0280± .11713	3.1840± 6.56062	3.3920± 2.59825	16.4080± 7.73678

Table 4. Results of one-way analysis of variance to compare the means of the groups

Variables	Source of variation	Sum of squares	Mean squares	df	F-value	P-value
Weight	Intergroup	107455.767	21491.153	5	58.134	.001*
	Intragroup	8872.400	369.683	24		
	Total	116328.167	--	29		
Triglyceride	Intergroup	34474.967	6894.993	5	8.201	.001*
	Intragroup	20178.400	840.767	24		
	Total	54653.367	---	29		
Cholesterol	Intergroup	18259.100	3651.820	5	6.248	.001*
	Intragroup	14028.400	584.517	24		
	Total	32287.500		29		
Alanine transferase	Intergroup	67622.494	13524.499	5	6.083	.001*
	Intragroup	53362.876	2223.453	24		
	Total	120985.370	--	29		
Aspartate transferase	Intergroup	307081.243	61416.249	5	9.756	.001*
	Intragroup	151087.236	6295.302	24		
	Total	458168.479	--	29		
Pink 1	Intergroup	3869.795	773.959	5	22.419	.001*
	Intragroup	828.528	34.522	24		
	Total	4698.323	--	29		
Bnibp3	Intergroup	1431.965	286.393	5	9.538	.001*
	Intragroup	720.666	30.028	24		
	Total	2152.631	---	29		

Table 5. Results of Bonferroni post hoc test to compare the means of intervention and control groups

	Variables interventions	Final weight	Triglycerides	Cholesterol	Alanine transferase	Aspartate transferase	Pink1	Bnibp 3
Control	Starvation	P=.000*	P=.008*	P=.025*	P=.001*	P=.001*	P=.000*	P=.001*
	3 days of exercise	P=.000*	P=.014*	P=.002*	P=.221	P=.001*	p=1.000	P=1.00
	5 days of exercise	P=.000*	P=.009*	P=.018*	P=.052*	P=.001*	P=1.000	P=1.00
	Starvation+ 3 days of exercise	P=.000*	P=.001*	P=.013*	p=.004*	P=.002*	P=.008*	P=1.00
	Starvation+ 5 days of exercise	P=.000*	P=.000*	P=.001*	P=.003*	P=.000*	P=.018*	P=.003*

Discussion

In the present study, non-alcoholic fatty liver disease was induced in Wistar rats (without genetic intervention) by feeding a high-fat, high-energy diet. Non-Alcoholic Fatty Liver Disease (NAFLD) is a chronic liver disease that can eventually lead to liver cirrhosis and hepatocellular carcinoma. The disease is associated with elevated levels of the liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in the blood. Increase in fats, cholesterol, and triglycerides was more than the increase seen in metabolic syndrome (25). Due to the increasing prevalence of this disease in the world, especially in our country, the importance of studies in this field is becoming more and more apparent. Since the non-alcoholic fatty liver disease is directly related to lifestyle and especially people's diet, creating such a model that is very similar to human disease (diet) can be one of the most suitable models for research and treatment studies of this disease. The ideal animal model in fatty liver induction should reflect all aspects of pathology in humans and the various stages of the disease (14). The animal model must be reversible, reliable, simple, cost-effective, and practically feasible with minimal disadvantages (6). In order to induce fatty liver in fatty liver groups, 30% of vegetable oil and 10% sugar mixture were added to the standard diet, and 10 g of food per 100 g of body weight per rat was given to the rats daily. The rate of maximum weight gain in people with NAFLD was significantly higher than in those without NAFLD (15). Rapid improvement in nutrition can lead to significant weight gain in the short term, which is often with excessive body fat (27). The results showed that there was a significant relationship between 4 weeks of continuous aerobic exercise (3 days and 5 days) and 14 hours of starvation on the rate of weight change in male rats of the fatty liver model. This may be due to the mechanism underlying fat deposition in rat viscera resulting from the rapid improvement of nutrition after malnutrition, which may be associated with increased gastrin secretion and increased gastrin receptor expression in adipocytes, the latter of which may develop resistance to insulin. Observing an increase in the incidence of NAFLD with increasing weight in the groups studied in this study suggests that adult weight gain is an independent risk factor for NAFLD. Comparison of the findings of the current study with those of previous studies leads us to the hypothesis that a rapid increase in the incidence of NAFLD may be associated with a rapid improvement in nutrition (16). Therefore, it can be said that 4 weeks of continuous aerobic exercise (3 days and 5 days) and 14 hours of starvation will reduce the weight of male rats with fatty liver (6, 28), and (14) reported in their studies that starvation causes reduction in intracellular nutrients and in sensation by brain-sensing signaling pathways such as the mTOR and AMPK pathways, which eventually stimulates autophagy. Moreover, the combination of fasting diet, acute resistance training and protein consumption (immediately or 1 hour after exercise stimulation) increases the serum levels of leucine, insulin and glucose, as well as the protein content of autophagy in skeletal muscle, but it reduces other autophagic pathway proteins in the liver (28). NAFLD has also been shown to increase with weight gain, both of which are controlled by exercise and a period of starvation (fasting) and return to normal (29).

The results of ALT description revealed that the highest mean was observed in the fatty control group (274.22) and the lowest mean was observed in the group (fat + starvation) (58.4400) and fat + hunger + 5 days of exercise (66.980). The results of AST description also indicated that the highest mean was observed in the fatty control group (505.0) and the lowest mean was observed in the group (fat + starvation + 5 days of exercise) (141.42). Excess lipid consumption leads to excess energy intake and accumulation of fat in the body. Increased visceral fat increases the flow of free fatty acids to the liver and leads to hepatic steatosis (30). Excessive consumption of saturated fatty acids is thought to lead to insulin resistance and type 2 diabetes (2). Therefore, it can be assessed that 4 weeks of continuous aerobic exercise (3 days and 5 days) and 14 hours of starvation have a significant effect on changes in liver enzymes in male rats of fatty liver model and will reduce hepatic ALT. This means that exercise and proper diet can regulate the expression of liver enzymes and prevent damage to liver tissue (15) showed that a low-fat diet combined with exercise can significantly reduce liver fat, while in other animal groups, a high-fat diet causes liver steatosis and inflammation, insulin resistance, and increased (TNFα). These changes may be related to the activation of γ receptors activated by peroxisome proliferators (PPARγ). Furthermore, some findings showed that both resistance training and intense intermittent training are suitable training strategies to reduce plasma ALT plasma concentrations, lipid profile, insulin resistance and liver fat content. Some findings indicate that exercise has no effect on the liver enzymes of obese women. This may be related to the lack of physical fitness and both groups' having high weight and body mass index even in the exercise group after twelve weeks (31). One of the reasons for the non-significant increase in ALT in the training group is the long half-life of this enzyme. Hence, more than 48 hours can be considered for recovery. The results of TAG description disclosed that the highest mean was observed in the fatty control group (139.000) and the lowest mean in the group (fat + starvation + 5 days of exercise) (30.200). The liver, by glycogen catabolism, delivers energy substrates to peripheral tissues. Thus, exercise also affects glycogen metabolism and reduces gluconeogenesis and helps maintain glycogen on the liver to maintain glucose homeostasis during exercise (15). Liver glycogen in obese and diabetic individuals with active Hepatic glycogen synthase kinase 3β is reduced, which suppresses glycogen synthase (9). In addition, increased hepatic glycogen synthesis improved the metabolic phenotype of rats fed a high-fat diet (32). Overall, elevated liver glycogen may be one of the mechanisms by which exercise improves hepatic insulin resistance and NAFLD. Increasing glycogen helps reduce the AMP / ATP ratio, which activates AMPK (12, 33). Therefore, according to the results, we can say that exercise reduces the amount of triglycerides in the liver by affecting energy metabolism and prevents fibrosis or liver steatosis.

The results of cholesterol description showed that the highest mean was observed in the fatty control group (178.80) and the lowest mean was observed in the group (fat + starvation + 5 days of exercise) (41.8000). Exercise reduces liver fat and increases VLDL clearance, but does not increase VLDL production in NAFLD (4). Aerobic exercise is consistently associated with favorable changes in blood triglycerides and HDL-C. However, data from multiple intervention studies and several meta-analyses also support a less pronounced and variable LDL-C-reducing response to exercise (7, 14). Weight loss (5% to 10%) through diet, with or without exercise, reduces hepatic steatosis (12). A number of randomized and controlled trials have also shown that regular exercise, even without calorie restriction, reduces liver steatosis (33). Some studies have revealed that 16 weeks of supervised exercise in men and women with NAFLD has no effect on the overall kinetics of VLDL (27). Weight loss in obese men following a low-calorie diet also reduces the rate of VLDL-apoB production without any effect on VLDL-apoB FCR (34). Similarly, in obese women, the low-calorie diet has no effect on VLDL-TG or VLDL-apoB FCR (35). Additionally, both endurance and resistance exercise reduce liver fat with and without weight loss (25). Therefore, it

should be noted that to achieve this goal and reduce plasma TG and VLDL production, longer or more intense exercise intervention, or a calorie-restricted combination approach may be required.

The results of PINK1 gene expression showed that the highest mean was observed in the starvation group (32,774), the starvation group +3 days of training (20.000) and the group of 3 days of training (15.8000). The results of BNIBP3 description indicated that the highest mean was observed in the group of starvation (17.480) and starvation + 5 days of training (16.408). PINK1 levels are normally high and decrease in the blood during liver injury (25). Our results showed that the PINK1 level, which was low before exercise, increased with exercise and a 14-day starvation period, thus preventing liver damage. Mitochondrial damage is also caused by other liver disorders (36). Due to the presence of damaged mitochondria, parkin-induced mitophagy plays a protective role against NAFLD (13) and prevents cell death and tissue damage.Mitophagy is activated in response to alcohol consumption by ROS, mitochondrial depolarization, and hypoxia mediated by induction of BNIBP3 and NIX. However, the initial adaptive induction of mitophagy fails over time, leading to chronic maladaptive changes that cause ALD (37). Apart from their role in cell death, BNIBP3 and NIX are also involved in inducing autophagy (2). In erythroid cells, NIX is required for the specialized type of autophagy that targets mitochondria for destruction (mitophagy). Similarly, BNIBP3 regulates mitophagy in response to hypoxia. Following liver injury, the expression of BNIBP3 in liver tissue increases to cause autophagic death of damaged cells (37).The results showed that the amount of PINK1 increased after the development of the fatty liver model in rats, and subsequently, with the onset of exercise and starvation, the amount of BNIBP3 increased to control the increased expression of the PINK1 gene, which resulted in controlling excessive liver tissue damage.BNIBP3 and NIX have been shown to induce cell death and also participate in the induction of autophagy. Induction of autophagy by BNIBP3 or NIX has a protective effect in some settings, while in others it is associated with autophagic cell death (25). With the prevalence of NAFLD increasing over the decades, it has become one of the most common chronic liver diseases worldwide. However, effective treatments for this liver disease are still limited. So far, only exercise and diet modification have been recommended by the FDA. Recently, there is growing evidence that mitochondrial dysfunction is closely linked to the development of NAFLD. Mitochondrial damage can exacerbate hepatic lipid accumulation and ROS production, leading to inflammation and fibrosis, which contributes to the pathogenesis and progression of NAFLD. Therefore, drug therapies that target mitochondria can be a promising way for NAFLD intervention in clinics. In fact, many naturally occurring mitochondrial targeting agents have been extensively studied and have shown good drug activity in combating NAFLD (12). The most important limitations of the study are: changes that may occur in different markers due to aging during the study, as well as failure to study metabolic changes during training, lack of 100% cooperation of animals during training and lack of control over stress and anxiety. It is suggested that the same research be done by examining different periods in terms of time and different training styles. Similar research should be done with more statistical samples on human sample.

Conclusion

The liver can act as a central regulator of lipid metabolism in the organism, regulates the availability of the substrate to other tissues. Autophagy is essential in controlling the quality of mitochondria and homeostasis of lipids in the liver. No drug strategy is currently available to reduce hepatic steatosis, but exercise Along with starvation to improve the clinical outcome of chronic liver disease, particularly Nonalcoholic Fatty Liver Disease (NAFLD).

Four weeks of continuous aerobic training and starvation combined and alone significantly reduced the status of blood lipids and liver enzymes in fatty model rats. The starvation group and starvation groups, along with exercise, can increase the activity of removing damaged

mitochondria by elevating the expression of Pink1 and Bnibp3 genes.

Conflict of interest

The authors declare that there is no conflict of interest.

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