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Review Article

The Effect of 12 Weeks of Resistance Training on Some Micro RNAs Associated with Oxidative/Antioxidant System in Liver of Diabetic rats

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Abstract

Introduction: Type 2 diabetes is an epidemic metabolic disease that is estimated to affect about 5 to 8% of adults in the world. One of the main causes of diabetes is fatty liver. The aim of this study was to evaluate the effect of 12 weeks of resistance training on microRNAs related to oxidation and oxidation of the liver of mice with diabetes.

Materials and Methods: In this study, 27 mice with diabetes were divided into control and resistance training groups. The resistance training group performed 5 sessions of resistance training every week for 12 weeks. Real-time PCR method was used to examine the research variables. Results: The results of this study showed that 12 weeks of resistance training significantly reduced the amount of mir-33 (P = 0.001), mir- 34a (P = 0.001) and mir-122 (P = 0.001) were obtained from liver tissue of diabetic mice. Conclusion: Resistance training can prevent liver diseases such as liver cirrhosis by reducing oxidative microRNAs in the liver.

Keywords: Resistance Training; MicroRNAs; Diabetes; Oxidation; Liver

Introduction

Obesity is now an epidemic that leads to weight gain due to excessive accumulation of fat. Obesity is a chronic disease caused by several factors, which are characterized by the presence of a pro-inflammatory environment in the adipose tissue [1]. This condition can spontaneously lead to the development of harmful processes associated with oxidative damage such as type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and cancer [2]. Type 2 diabetes is an epidemic metabolic disease that is estimated to affect about 5 to 8% of the world's adults. The International Diabetes Federation reported the number of people with type 2 diabetes in 2010 at 285 million worldwide and predicts it will reach 438 million by 2030. Worldwide, direct and indirect costs of diabetes are reported at $ 174 billion a year [3]. The risk of premature death, heart disease, kidney disease, neurosis and blindness in diabetics is twice as high as in non-diabetics. One of the important complications of diabetes is fatty liver, also fatty liver is one of the main causes of type 2 diabetes [4]. In recent years, non-alcoholic fatty liver disease has received much attention as a pathogenic agent of insulin resistance and type 2 diabetes [5]. The prevalence of fatty liver, cirrhosis and liver cancer in the Middle East is the highest in the world. In Iran, the prevalence of non-alcoholic fatty liver is estimated at 43% based on statistical models [6]. Over the past two decades, epigenetic changes in DNA and histone have been studied as important causes of common diseases including non-alcoholic fatty liver [7]. Is Because epigenetic changes can affect the entire genome and are strongly influenced by environmental factors, it is

important to know how the epigenome can be altered in the liver and whether epigenetic patterns can be found [8]. Used to prevent NAFLD and the development of this complication of cirrhosis. Under normal conditions, the liver's aerobic metabolism produces prooxidants, such as reactive oxygen species, which balance by consuming them at the same rate as antioxidants. Oxygenated species with their toxic effects lead to the production of membrane lipid peroxidation such as MDA and can cause liver fibrosis by activating star-shaped cells in the liver that synthesize collagen. The body's antioxidant system protects against this oxidative stress. Today, micro molecules such as microRNA are used to diagnose, treat and identify. miRNAs are promising tools for diagnosis, prognosis or therapeutic guidance that are used as biomarkers of various diseases [9]. MicroRNAs (miRNAs) are small unencoded units of RNAs of approximately 22 nucleotides, which act on the mechanisms of post-transcriptional regulation of gene expression [10]. The most important microRNAs involved in the process of non-alcoholic liver disease are MiR-122, MiR-33, and MiR-34a. One of the reported microRNAs related to lipid metabolism is MiR-122.MiR-122 is the most abundant microRNA in the adult human liver. Inhibition of this microRNA reduces plasma cholesterol [11]. MiR-33 includes two types of MiR-33a and MiR-33b.Both microRNAs are key regulators of cholesterol and fatty acid biosynthesis in the liver [12]. In people with nonalcoholic fatty liver disease, the expression of this microRNA is increased [13]. This microRNA exacerbates the disease by inhibiting SIRT1. On the other hand, regular exercise has been shown to have some effect on improving these risk factors [14]. Therefore, the aim of this study was to evaluate the effect of 12 weeks of resistance training on oxidative and antioxidant-related microRNAs in the liver of diabetic mice.

Methods

In this experimental study, based on the method of equalization of resources and based on the ethical guideline for working with laboratory animals and observing the Helsinki Declaration, 28 young male Wistar rats were purchased as a statistical sample from Royan Research Institute and sent to the laboratory pet. Were transferred Rats ranged in age from 35 to 45 days with a mean weight of 110 ± 10 g. The rats underwent a high-fat diet (HFD) after two weeks of exposure to the laboratory environment and reaching an average weight of 197 ± 20. After 20 weeks of high-fat diet prepared by the Embryo Biotechnology Research Institute, which includes 45% for 3 months and 60% high-fat diet for 2 months and free access to food and water, to 2 diabetic control groups (14), resistance training group (14) were divided. Rats were kept in transparent polycarbonate cages with autoclave capability at a temperature of 20 to 24 ° C and a relative humidity of about 55 to 65% in the laboratory animal hall. To induce diabetes, a high-fat diet was used for 20 weeks and then intraperitoneal injection (25 mg/kg) of freshly prepared STZ solution in physiological serum was used. One week after the injection, a small drop of blood was placed on the glucometer with a small injury in the rats' tails, and fasting blood glucose was measured with a glucometer. Blood glucose between 150 and 400 mg/dl as a measure to ensure rats were infected. Was considered diabetes. At the end of the training period and 48 hours after the last training session of the experimental training groups and after 12 hours of fasting in rats by the combination of xylazine (10 mg/kg) and ketamine (75 mg/kg) produced by Alpha Sun Netherlands In traperitoneal injections were anesthetized and sacrificed.

How to remove RNA from microtubules and samplers

All head samples and microtubes used to extract mircroRNAs were placed overnight in DEPC solution at a concentration of one tenth of one percent and then dried using an oven at 45 ° C, then samplers and microtubes were autoclaved twice regularly.

Extraction of mircroRNAs

Liver tissue was isolated and transferred to freezer negative 80 with ice to measure the expression of microRNAs. For the purpose of micro-RNA extraction, from the Irisol RNA extraction kit produced by Rena Biotechnology Company, access code (RB1001) was used according to the following instructions. Also, RT steam-loop housekeeping method was used to check microRNA levels. First, mix 500 µl of the crushed tissue with 1 ml of buffer freezol, mix together to obtain a homogeneous solution, then transfer the mixture to a 2 ml microtube. The mixing solution was placed at constant ambient temperature for 5 minutes. Then 200 microliters of chloroform was added to the set and shaken up and down vigorously for 10-15 seconds and left in the medium again for 5 minutes. The tube containing the mixture was centrifuged at 8000 g for 5 minutes. At the end of this phase, the two phases were clearly visible. Separate the clear phase containing the RNA, transfer it to another tube, add 1000 µl of 100% cold ethanol, and then place it in the freezer for 8 minutes. Was placed. Finally, the tube was removed from the freezer and centrifuged similar to the previous

step, the liquid inside the tube was discarded after centrifugation. At this stage, sometimes but not usually, colorless or whitish spots were observed on the body and bottom of the tube. The centrifuge was repeated by adding 80% ethanol for rinsing, and after removing the ethanol and drying the contents of the tube (air-dry method was used at this stage), 20-50 µl of double-distilled water was added depending on the amount of sediment. And was dissolved in the tube with an RNA pipette. Then 5 microliters of it was analyzed for quality using electrophoresis on 1% agarose gel to be used in cDNA synthesis after confirmation. For DNA replication, DNA strands were removed from the extracted RNA product using DNaseI. For this purpose, after adding DNase enzyme, the reaction mixture was incubated in a 37 ° C incubator for 30 minutes. To confirm the RNA quality, the decomposed DNA sample was analyzed on 1% agarose gel.

miRNA synthesis

CDNA synthesis was performed using RNA biotechnology cDNA synthesis kit. All materials were provided by Rena Biotechnologists Company. After completing the process and obtaining the threshold cycles (ct), the expression of the desired variables was measured using mathematical calculations. ΔCt of each sample was calculated using internal control, (miR-139-5p), and miRNA (by subtracting Cts from internal control) (14). the corresponding ΔΔCt for each sample was calculated by subtracting the ΔCt of that sample from the mean ΔCt of the control group. Finally, a mathematical relation was used to report changes in the quantitative expression of variables

Resistance training protocol

Resistance training involves climbing a special training ladder with a slope of 80 degrees and attaching a fixed weight to the mouse tail for overload. In this case, a ladder measuring 18 x 110 cm with a distance of two centimeters between the stairs and a compartment Rest was used at the top. These dimensions allowed the mouse 8 to 12 cups of dynamic movement. The weight attached to a narrow rope with adhesive tape and bandage was attached to the end of the tail (2 to 3 cm below the beginning of the tail). And applied overload. The weights consisted of bags containing small lead weights that could be adjusted to the target weight. In the first session, each mouse was first placed in a high ladder compartment for 2 minutes to ensure they were safe. First it is placed at the bottom of the ladder and then in the middle of the ladder and it is known how to climb it. If necessary, light blows of a ruler to the mouse tail are used to stimulate the onset of movement. It took three days to get acquainted with the ladder and climb it. The first session of the exercise was selected as the target weight by considering the weight of 50% of the mouse weight and 4 to 8 repetitions were performed. In the next session, a weight of 20 g was added to this weight. And this process continued until the mice were not able to climb the ladder. In the coming weeks. The desired exercise was used for 6 repetitions. In the fourth session of the exercise, 20 grams of overload was added to the previous weight. This process continued until the end of eight weeks of training. If the mice could not climb the ladder for 10-20 seconds or could not climb, the training would be stopped [15].

Data analysis

Descriptive information will be performed using descriptive statistics (mean and standard deviation and drawing charts and tables) and inferential analysis using inferential statistics. Analysis of variance and post hoc test will be performed using SPSS software. A significance level of $p \leq 0.05$ will be considered. First, the Kolmogorov-Smyonov test is used to determine the normality of the data. Then, the types test will be used to compare the differences between the groups, and if significant, the Tukey post hoc test will be used.

Results

According to the information in table 2, for the experimental group, comparison of pre-test and post-test means shows that the level of mir-33 in the liver of mice with type 2 diabetes had a significant decrease, which according to the value of t2.25 and the significance level is 0.0001, this amount of change is significant. In the case of the control group, the results obtained by comparing the pre-test and post-test of the control group (value of t obtained 1.45 and significance level of 0.756) indicate that there is no significant difference between the two stages. The results of comparison between groups showed that there was a significant difference between the resistance training group and the control group ($p = 0.0001$).

According to the information in Table 3, for the resistance training group, the comparison of pre-test and post-test means shows that the level of mir-34a in the liver of mice with type 2 diabetes had a significant decrease, which according to the value of Ob-

	Weigth		Glucose	
	Pre-test	Pre-test	post-test	post-test
control group	235/1 ±395.25	35/25 ± 445.36	55/16 ± 352.16	55/25 ± 436.41
Resistance training group	15/24 ± 378.25	25/15 ±288.16	235/21 ± 335.17	66/19 ± 141.15

Table 1: Weight and glucose changes in mice before and after the protocol.

	Pretest	Post-test	Percentage of changes./.	Independent t	P-Value
Resistance training group	866/0 ± 4.98	475/0 ±35 .2	-50.54	2.78	0001./0
Control group	1.1± 44.6	1.1±77 .7	+15.1	1.25	./745
Dependent t	P-Value	2.65			

Table 2: Comparison of mir-33 changes in control and experimental groups.

	Pretest	Post-test	Percentage of changes./.	Independent t	P-Value
Resistance training group	866/0 ± 52.5	755/0 ± 3.2	- 49.87	2.66	./0001
Control group	1.1±44 .6	1.1 ± 77.7	+15.1	1.66	./745
Dependent t	P-Value				
	2.65				

Table 3: Comparison of mir-34a changes in control and experimental groups.

tained 2.25 and a significance level of 0.0001, this rate of change is significant. Regarding the control group, the results obtained from the comparison of pre-test and post-test of the control group (value of t obtained 1.15 and significance level of 0.0758) indicate that there is no significant difference between the two stages.

	Pretest	Post-test	Percentage of changes./.	Independent t	P-Value
Resistance training group	866/0 ± 4.98	475/0 ±35.2	-50.54	2.78	0001./0
Control group	1.1±44.6	1.1±77.7	+15.1	1.25	./745
Dependent t	P-Value				
	2.65				

Table 4: Comparison of mir-122 changes in control and experimental groups.

According to the information in Table 4, for the resistance training group, comparison of pre-test and post-test means shows that the level of mir-122 in the liver of mice with type 2 diabetes had a significant decrease, which according to the value of obtained 2.66 and significance level 0.0001, this rate of change is significant. In the case of the control group, the results obtained by comparing the pre-test and post-test of the control group (value of t obtained 1.66 and significance level of 0.0745) indicate that there is no significant difference between the two stages.

Discussion

The aim of this study was to evaluate the effect of 12 weeks of resistance training on oxidative and antioxidant-related microRNAs in the liver of diabetic mice. The results of this study showed

that mir-33, mir-34a, mir-122 and glucose increased in the liver of mice with diabetes due to diabetes and high fat diet consumed by mice. The results of previous research also showed that 12 weeks of resistance training significantly reduced the levels of mir-33, mir-34a, mir-122 and glucose in the liver of mice with diabetes. Importantly, these findings contradict the findings of Goedeke., *et al.* (2021) Who showed that miR-33 is involved in triglyceride metabolism and reported that elimination of miR-33 in mice increases circulating fat content, especially triglyceride levels, leading to accumulates in the liver lipids. Overall, there is ample evidence of a fat-reducing effect in various exercise programs, but the present study examined this finding in more detail at the molecular level by analyzing the effect of exercise on miR-33 expression in high-fat diet mice. Inhibition of miR-33 increases plasma HDL, decreases LDL, TGs, and promotes reverse cholesterol transfer [16]. The results of this study with Gharghani., *et al*. (2017) who showed that aerobic endurance training improves the characteristics of nonalcoholic fatty liver disease (NAFLD) by inducing miR-33-dependent autophagy in mice fed a high-fat diet [17]. The exercise mechanism involved in how resistance training reduces mir-33 is not yet known, but the role of the liver receptors Liver X Receptor and Sterol regulatory element binding protein (SREBP) in this process can be noted. ABCA1 is influenced by mir-33, which in turn is regulated by liver receptors [18]. The results of this study also showed that 12 resistance exercises significantly reduced the levels of mir-34a in the liver of mice with diabetes. Expression of miR-34a in animal models or human patients with alcoholic liver injury, non-alcoholic fatty liver disease (NAFLD), Hepatic fibrosis or HCC is increased and its expression level is associated with the severity of the disease [19]. miR-34a is the direct target gene of p53 and one of the targets of miR-34a is sirtuin 1 (SIRT1), which can inhibit p53-dependent apoptosis by de-acetylating all major sites of p53 acetylation [20]. Therefore, the signaling pathway miR-34a/SIRT1/p53 forms a positive feedback loop in which p53 induces miR-34a and miR-34a and then activates p53 by inhibiting SIRT1 and plays an important role in cell proliferation and apoptosis. MiR-34a/SIRT1/p53 signaling is activated in NAFLD and is involved in hepatocyte apoptosis [21]. miR-34a is transcriptionally regulated by the p53 tumor suppressor protein and regulates a host of target proteins involved in cell cycle, apoptosis, differentiation, and cell growth [22]. It has recently been reported that the miR-34a/SIRT1/p53 signaling pathway is involved in human NASH and is strongly associated with NAFLD severity, resulting from the association between hepatocyte apoptosis and the miR-34a/SIRT1/p53 signaling pathway [23]. The results of this study also showed that 12 weeks of resistance training significantly reduced mir-34a levels in the liver of mice with type 2 diabetes. The results of previous studies showed that mir-34a levels are consistent with ROS levels. Several studies have shown that serum miR-34a levels were higher in participants with NAFLD. MiR-34a, apoptosis and acetylated p53 increased with disease severity [24]. Resistance training inhibited the miR-34a/SIRT1/p53 pathway. Regulation of miR-34a mutation was observed in the liver of streptozotocin-induced diabetic rats, indicating that miR-34a is highly abnormal in NAFLD [25]. The results of previous studies have shown that mir-34a inhibits the AMPK pathway, which is one of the most important metabolic proteins. AMPK stimulates glucose uptake and FAA oxidation [26]. Therefore, resistance training by activating protein kinase B (AMPK) reduces mir-34a in the liver of mice with diabetes. Resistance training and exercise increase ATP intake, and muscle ATP intake decreases the ATP/AMP ratio and increases AMPK activity. The result of these reactions is an increase in the transport of GULT4 from inside the cell to the membrane surface, which ultimately results in glucose uptake and reduced underuse of fat cells as fuel [26]. The results of this study also showed that 12 weeks of resistance training reduced mir-122 in the liver of mice with diabetes. MiR-122 was one of the most abundant miRNAs in the liver. It was significantly adjusted in serum and was suggested as a potential biomarker for NAFLD in patients. In people with fatty liver, mir-122 is suppressed by suppressing the SIRT1 protein [27]. Resistance training may increase oxidation of free fatty acids by increasing the SIRT1 protein.

Conclusion

In mice with type 2 diabetes, mir-33, mir-34a, mir-122 increase, and these microRNAs can cause liver damage by reducing fat oxidation. According to the results of this study, Aerobic exercise in mice with type 2 diabetes can prevent resistance training to prevent fatty liver damage. Further research is needed to reach a definitive conclusion.

Conflict of Interest Statement

None declared.

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