Research Article

The effect of high intensity interval training on CTGF and RXFP1 genes expression of heart tissue and SGPT liver enzyme in rats with fatty liver

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Abstract

This study investigated the effect of high intensity interval training on CTGF and RXFP1 genes expression of heart tissue and SGPT liver enzyme in rats with fatty liver. 48 male Wistar rats (200-250 g) were divided randomly into the following 6 groups: Healthy base group (BH), base Steatosis group (BS), Healthy HIIT group (HIIT), Steatosis HIIT group (SHIIT), Healthy control group (CH), control Steatosis group (CS). Rats in the fatty liver group received oral tetracycline daily for two weeks. Rats in the training groups were also trained for 5 weeks / five days. Both BS and BH groups sacrificed at the end of the 2nd week. CS and training groups sacrificed at the end of 5th week and heart tissue samples were taken to examine CTGF, RXFP1, and SGPT genes expression. The results of the study showed that he amounts of SGPT in BS and CS groups were meaningfully higher than those in the other 4 groups. The level of this enzyme in SHIIT and HIIT groups was significantly lower than that in the fatty liver groups. The RXFP1 gene expression in CS, BS and SHIIT groups were significantly higher than those in the other 3 groups. Thus, it can be claimed that fatty liver increased cardiac fibrosis factors but by reducing these factors HIIT was able to prevent the process of cardiac fibrosis from liver Steatosis; therefore, HITT can be used as a new method to Cardiac rehabilitation of patients.

Key Words: **High intensity interval training, Fatty liver, Cardiac fibrosis, Wistar rats**

Introduction

Performing physical activity and having a healthy body is one of the most essential life needs of people with fatty liver. In recent years, studies have been performed on the relationship between fatty liver and arthrosclerosis, the results of which indicate the relationship between the Non-alcoholic fatty liver and arthrosclerosis of coronary artery disease. Non-alcoholic fatty liver disease is associated with a sedentary lifestyle and poor eating habits around the world (Gaudio et al., 2012). The disease is characterized by elevated levels of triglycerides, liver enzymes, some inflammatory biomarkers, and the rate of liver steatosis (Feldstein et al., 2010).The incidence of steatosis is positively and directly related to BMI non-linearly as increasing every five units in body mass index increases the risk of liver steatosis more than four times (Faienza et al., 2020). Non-alcoholic fatty liver disease includes a wide range of liver disorders ranging from simple steatosis to fibrosis and cirrhosis of the liver, which, if left untreated, can eventually lead to liver cell cancer and death. Also, non-alcoholic fatty liver disease can increase the risk of death from cardiovascular disease (Patil et al., 2017).

Fatty liver is associated with increased serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Studies have shown that the best indicators for assessing the condition of the liver are the aspartate aminotransferase AST, alanine aminotransferase ALT enzymes (Villegas et al., 2011). ALT or SGPT is an enzyme found mostly in liver and kidney cells. Much smaller amounts are also found in the heart and muscles. In healthy people, ALT levels in the blood are low. When the liver is damaged, ALT is usually released into the bloodstream before the more obvious symptoms of liver damage, such as jaundice, occur (Thoma et al., 2012). ALT is often found in the liver and is more specific for liver damage (Patil et al., 2017). In Sookoian et al. studies in Argentina and Karakurt et al. in Turkey, people with nonalcoholic fatty liver disease had increased antima-medial thickness as well as atherosclerotic plaques in the carotid arteries (Sookoian et al., 2008). Studies have also shown that

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fatty liver disease can be effective in causing atherosclerotic changes in arteries and increasing the thickness of the carotid artery as an indicator of atherosclerosis, which can be seen even in mild degrees of fatty liver (Villegas et al., 2011).

CTGF is a member of the extracellular matrix (ECM) proteins, also known as CCN2 for short. CCNs control important biological functions, including cell proliferation, differentiation, adhesion, and angiogenesis, as well as several pathological processes such as tumor growth and tissue fibrosis (Gaudio et al., 2012). CTGF is a secretory protein that has been identified as a potent stimulator of extracellular matrix (ECM) synthesis (Ghosh et al.,2002) as well as an important mediator of cardiac fibrosis (Dijke et al., 2007). Cardiac fibrosis is a major global problem expressed by limited CTGF treatment options in cardiac fibroblasts and cardiomyocytes (Wang et al., 2014). CTGF is mainly synthesized by liver cells in the liver and is strongly induced in liver fibrosis (Gressner et al., 2008; Tong et al., 2009). CTGF is induced by fibroblast TGF-β cells and mediates the growth and secretion of extracellular matrix. These results indicate that CTGF is the mediator of many TGF-β-probiotic activities (Varga et al., 2009).

The family of RXFP peptides have been shown to have complex similarities between them and the corresponding insulin role of Relaxin in cardiac reproduction. Relaxin in the cardiovascular system targets vascular systems, and also has powerful signaling effects on the human umbilical cord and smooth muscle vein cells. Special effects of Relaxin in all parts of the body can have different expressions of RXFP1 receptor in different arteries, so in rats this receptor is strongly expressed in aortic endothelial cells and RXFP1 activates cAMP, cGMP and cAMP signaling pathways in ERK1 / 2 endothelial cells (Sarwar et al., 2016). It also activates a wide range of signaling pathways for the production of secondary media, including cAMP and nitric oxide (Bathgate et al., 2013). Since RXFP1 is an extracellular matrix component, it can play a role in inhibiting fibrosis. Fibrosis involves complex and somewhat irreversible biological processes characterized by abnormal and incremental deposition of extracellular matrix components, especially collagen. One of the effects of RXFP1 is that it prevents the proliferation and differentiation of fibroblasts causing collagen to build up in ECM and fibrosis in the heart and other organs, and Relaxin seems to play a protective role in protecting the cardiovascular system (Adham et al.,1993). In general, researchers have shown that relaxin can play an important role in the circulation of blood through the liver at the sinus surface, which is of particular importance in fibrotic liver (Hayden et al., 2009). The anti-fibrotic effects of relaxin are thought to be due to inhibition of TGF-β function. (Masterson et al., 2004; Samuel et al., 2004). Exercise can stimulate lipid oxidation and inhibit lipid synthesis in the liver by activating the AMPK pathway. During exercise, AMPK is activated and its activity remains in muscle, liver and adipose tissue after exercise (Richter et al., 2009).

In a study, Ruiz et al. (2014) concluded that mild to severe activity with 24 minutes per day showed a significant increase in AST and ALT / AST ratio (Ruiz et al., 2014). Keating et al. (2012) showed that exercise, whether aerobic or endurance, has a positive effect on liver fat, but exercise alone does not affect ALT (Keating et al., 2012). In a study of rats, Ovey et al. (2011) found that regular exercise prevented fatty liver disease by improving liver lipid metabolism (Aoi et al., 2011). In a study of the effect of five weeks of intense intermittent exercise on the expression of miR-29a and CTGF genes in the heart tissue of diabetic rats, the results showed that intense intermittent exercise caused a significant decrease in CTGF in both experimental groups compared to the control group (Roozbayani et al., 2016). it is also shown that exercise resulted in cardiac adaptation reactions in Wistar and SHR rats that ultimately reduced the expression of the incompatible TGF-β1, CTGF, and FGF2 genes (Schreckenberg et al., 2017). In limited studies on RXFP1. Kruger (2004) and Heringlake (2009) did not observe a change in RXFP1 levels when exercising the heart patients (Heringlake et al., 2009; Krüger et al., 2004).

On the other hand, HIIT is an effective and clinical tool for improving aerobic fitness in healthy individuals (Gillen et al., 2014) which includes short periods of high intensity training and low intensity with rest periods that are repeated intermittently (Laursen et al.,2002). One of the benefits of HIIT is that it is not time consuming while providing useful results and comparisons. Therefore, short-term training is a tool that can be combined with training protocols to improve the process of adaptation to training in untrained people (Jones et al., 2012). According to the reports, although in high-intensity exercise the rate of blood stream is faster than moderate-intensity exercise, the rate of oxygen uptake is not slower (Fawkner et al., 2003). HIIT is also a strong stimulant for increasing the enzyme of mitochondrial metabolic pathways and increases muscle buffering capacity, oxidative content of total body fat and aerobic capacity (Jones et al., 2012). Although Meyer et al. (2013) recommended the use of high-intensity intermittent exercise in patients with heart failure whose contractile capacity of the heart muscle is greatly reduced, especially in the left ventricle, and it is much more effective in improving aerobic capacity (Meyer P.et.al..2013), there are still controversies about the application and effects of high-intensity exercise for heart patients. Therefore, considering the importance and necessity of inhibiting cardiac fibrosis, which can be caused by the fatty liver model, and the need to identify the factors affecting the development and inhibition of cardiac fibrosis, and since so far very few studies have been conducted on the effect of fatty liver on CTGF and RXFP1 tissue expression, the aim of this study was to evaluate the effect of five weeks of intense inter-

-mittent HIIT training on cardiac factors RXFP1 and CTGF, which inhibit fibrosis and induce cardiac fibrosis, respectively, and the amount of liver SGPT enzyme in male rats.

Materials and Methods

Animals

The present study was an experimental one performed on an animal model. The statistical population included male Wistar rats and the study sample consisted of 48 rats weighing 200-250 g which were randomly divided into 6 groups as follows:1- Healthy base group (BH); 2- Modeled base group (steatosis) (BS); 3- Healthy HIIT group (HIIT); 4- Modeled HIIT group (steatosis) (SHIIT); 5- Healthy control group (CH); and 6- Modeled control group (steatosis) (CS).

Training protocol

Rats of the training groups were trained for five days and five minutes every day before starting the main protocol in order to get acquainted with treadmill. A fully automatic treadmill was used to control exercise and rest time. The exercise program was already given to the machine.

The number of training sessions was 5 times per week. The slope of the machine in the exercise was zero degrees and the speed was set in meters per minute (training intensity criterion). The total training time was 25 minutes per session. The modified intense intermittent exercise Protocol is the training program of Maleki Joybari et al. (2018).

Fatty liver model (steatosis)

Tetracycline at a dose of 100 mg / kg at a volume of 1.5 cc per rat was used daily for two weeks. The average weight of the rats was 300 g, of which 100 mg per 1 kg was used for 3 rats, and 100 mg was dissolved in 4.5 cc and 1.5 cc was injected to each rat. In the beginning of the study, the control group victimized and they were biopsied. Then, at the end of the second week, two other groups of rats were sacrificed and some groups were sacrificed after 5 weeks. Then, heart tissue samples were taken to check the expression of SGPT, RXFP1 and serum samples for CTGF enzyme evaluation.

Table 1. HIIT training program

Week	*Warm up* *5 min*	*High Intense* *2 min (m/min)*	*Slow Intense* *1 min (m/min)*	*Cool down* *5min*
First	4	16-24	10	***4***
Second	6	26-34	12	***6***
Third	8	36-44	14	***8***
Fourth	10	46-54	16	***10***
Fifth	12	56-64	18	***12***

In each group, tissue analysis was performed by Real Time PCR technique. First, primer design was performed and then total RNA was extracted from tissues and converted to cDNA. Then cDNA was Replicated by PCR and examined for the expression of the mentioned genes. This technique had 4 basic steps:

1. Total RNA was extracted from the collected cells in each group.

2. It was converted to cDNA using reverse replication enzyme.

3. The resulting cDNA was treated with DNase I to remove genomic DNA.

4. Reproduced by real-time PCR.

The first and most important issue when working with RNA is to be careful to prevent RNase contamination. RNase is a nuclease released when cells break down in the tissue and is abundant on the surface of the skin and in fluids such as sweat and saliva. RNase, on the other hand, is highly resistant to prolonged boiling and gentle denaturation due to its in-chain disulfide bonds. Therefore, the best way to prevent the problem is to avoid contaminating glassware, pipes and surfaces with this enzyme.

Careful construction and use of buffers and pipettes is also one way to prevent problems. If the buffers are contaminated with microorganisms, the only way is to replace the buffer, because RNase is not destroyed by autoclaving. All containers and pipettes used were RNase-free in the following order:

First, they were placed in 0.1% DEPC solution at room temperature for 24 hours to eliminate DEPC. The presence of DEPC can hinder the process of RNA analysis. The RT-qPCR technique was used to quantitatively confirm the expression of the studied genes. For this purpose, using chiazol solution, the RNA of all cells was extracted according to the synagen protocol and exposed to DNase I (Fermentas) to ensure contamination with genomic DNA. Then, the quality of the extracted RNAs was evaluated by spectrophotometry (DPI-1, Kiagen). To prepare a single-stranded cDNA from Oligo dt primer (MWG-Biotech, Germany) and reverse transcription enzyme (Fermentas) were performed according to the relevant protocol. Each PCR reaction was performed using PCR master mix (Applied Biosystems) and SYBER Green in ABI Step One (Applied Biosystems, Sequences Detection Systems. Focter City, CA) according to the manufacturer's protocol. 40 cycles were considered for each Real-Time PCR cycle and the temperatures of each cycle were set to include 94 ° C for 20 seconds, 60-58 ° C for 30 seconds and 72 ° C for 30 seconds. Melting diagram was performed to

evaluate the accuracy of PCR reactions and was evaluated specifically for each gene and in each reaction with a negative control diagram to check for contamination in each reaction. The expression ratio of the studied genes in this study was evaluated by the comparative threshold cycle method (Thereshold Cycle: CT). Using data placement in formula:

$$R = 2^{-(\Delta\Delta CT)}$$

$$\Delta\Delta CT = (CT_{target} - CT_{refence})_{Time\ X} - (CT_{target} - CT_{refence})_{Time\ 0}$$

The specific standard curve of each gene was plotted using at least 5 logarithmic concentrations in dilute order of positive control of each gene. The expression level of the target gene according to the normalized reference gene and the expression of healthy group genes was considered as a calibrator.

$$Ratio = \frac{(E_{target})^{\Delta CT_{target}}}{(E_{reference})^{\Delta CT_{reference}}}$$

$$(\Delta Ct_{refrence} = Ct_{control}\text{-}Ct_{treatment}\text{-}\Delta Ct_{target} = Ct_{control}\text{-}Ct_{treatment})$$

In the above formula, E represents Efficiency and is obtained by drawing a standard curve for the gene.

Statistical analysis

All statistical operations were performed using SPSS software version 22. Data were described quantitatively using central scattering indices such as mean and standard deviation. Shapiro-Wilk test was used to determine the normality of data distribution and Levene test was used to examine the homogeneity of variances. Also, one-way analysis of variance was used to examine the significant changes in each of the research variables between different groups, and if a statistically significant difference was observed, the Tukey follow test was used to determine the location of the intergroup differences. The significance level was considered P <0.05 in all calculations.

Results

Table 2. The result of Anova test at different variable.

		Sum of Squares	df	F	Sig.
RXFP1	Between Groups	682.420	5	197.035	.0001
	Within Groups	29.093	42		
	Total	711.513	47		
CTGF	Between Groups	657.208	5	75.902	.0001
	Within Groups	72.732	42		
	Total	729.940	47		
SGPT	Between Groups	52759.234	5	27.521	.0001
	Within Groups	16103.344	42		
	Total	68862.578	47		

By examining the groups through Anova test (Table 2), the results of the Sig column show that there is a significant difference between the means of the research groups in all three SGPT, RXFP1 and CTGF variables.

The results of Tukey post hoc test comparing the means of the groups showed that the amount of SGPT liver enzyme was not different in BH and CH groups, but this rate was significantly higher in BS and CS groups than those in the other 4 groups. The level of this enzyme in HIIT and SHIIT groups significantly reduced compared to the fatty liver groups, which means that exercise reduced the level of SGPT liver enzyme. RXFP1 gene expression in BS, CS and SHIIT groups was significantly higher than that in the other 3 groups. This rate was about 9 times higher in BS and CS groups than BH, CH and HIIT groups. RXFP1 levels were also different in the exercise groups and were significantly lower in the HIIT group. Rats in the SHIIT group also had significantly lower RXFP1 than the other two fatty liver groups who did not exercise. CTGF in CS group was significantly higher than the other 5 groups. There was no difference between BH, CH and HIIT groups, but both exercise groups had lower CTGF levels than fatty liver groups (CS and BS). CTGF levels in the HIIT group were also significantly lower than the SHIIT group (see figure 1, 2, 3).

Discussion

Our findings showed that with the development of steatosis fatty liver, the amount of SGPT liver enzyme increases in rats while the level of these enzymes remained normal in other groups. The results also showed that in the SHIIT group, i.e., the group with fatty liver who had HIIT exercise, the amount of this enzyme was normal. Increased SGOT and SGPT liver enzymes is a sign of liver cell destruction (Jamali et al., 2008) but SGPT is often more specific for liver damage (Patil & Sood, 2017). Therefore, the incr-

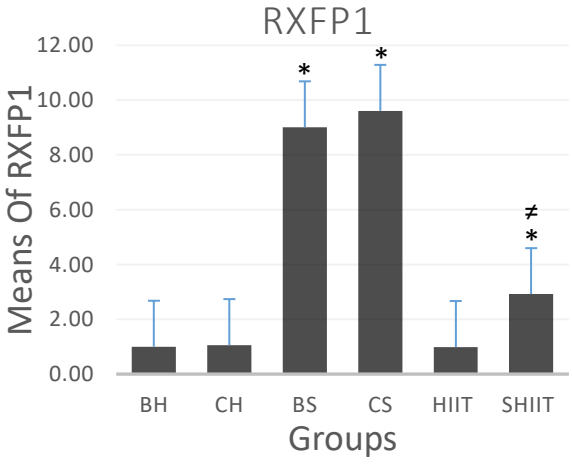

Figure 1. The results of RXFP1 at different groups of study. Data are show as mean±SD. * p<0.001, # p<0.05.

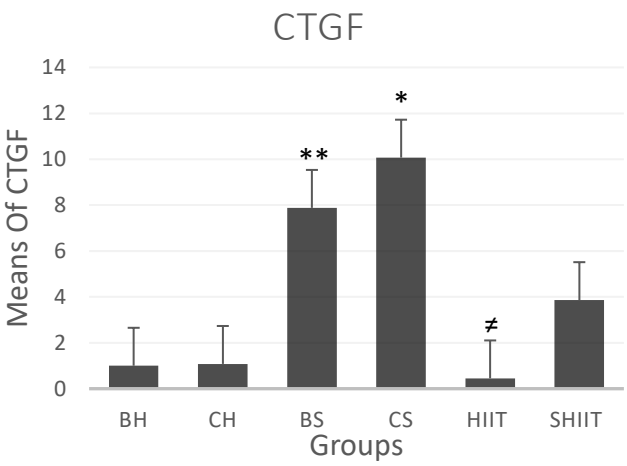

Figure 2. The results of CTGF at different groups of study. Data are show as mean±SD. * p<0.001, ** p<0.01, # p<0.05.

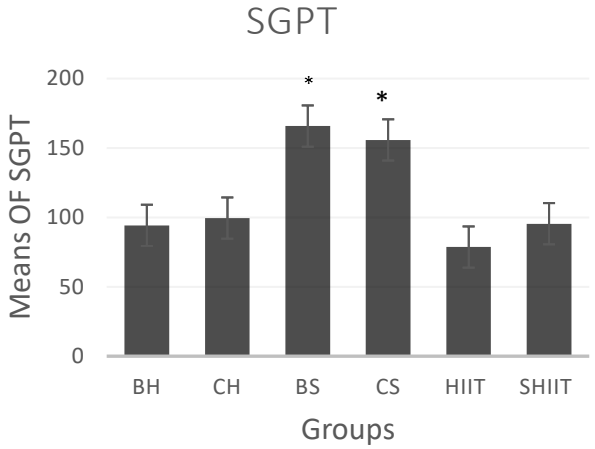

Figure 2. The results of CTGF at different groups of study. Data are show as mean±SD. * p<0.001.

-ease in SGPT enzyme level in this study indicates liver damage and that 5 weeks of HIIT training was able to control this damage. These results are in line with the research results of Roozbayani et al. (2016), Wang et al. (2019) and Yang et al. (2020) while the findings were different from those of Hovanloo et al. (2011), Keating et al. (2012) (Keating et al., 2012) and Guo-qiang et al. (2019).

In healthy people, the level of SGPT in the blood is low. When the liver is damaged, SGPT is usually released into the blood stream before the more obvious symptoms of liver damage, such as jaundice, occur (Thoma et al., 2012). Exercise can stimulate lipid oxidation and inhibit lipid synthesis in the liver, which is mediated by activation of the AMPK pathway. This enzyme is stimulated and activated by increasing the ratio of AMP to ATP in tissues, which is the result of physiological stimulation of exercise. During exercise, AMPK is activated and its activity remains in muscle, liver and adipose tissue after exercise (Richter et.al., 2009). On the other hand, our findings showed that with the induction of liver steatosis, connective tissue growth factor (CTGF) in cardiac tissue increased sharply, such that its rate increased up to 7 times in the first two weeks compared to the control group, and this increase continued until the end of the fifth week, increasing 10 times.

On the other hand, the level of CTGF in the HIIT group after 5 weeks of training was lower than all groups, but was only considerable compared to the BS, CS and SHIIT groups. Also, 5 weeks of high-intensity intermittent exercise in steatosis fatty liver mice significantly reduced CTGF, which causes fibrosis, compared to fatty liver samples, but this amount was still higher than the baseline level. These results are consistent with the results of Roozbayani et al. (2016); Schreckenberg et al. (2017); Wang et al. (2009), and Yang et al. (2020). CTGF is a secretory protein known as a potent stimulator of extracellular matrix (ECM) synthesis (Shi-Wen et al., 2008) and also as an important mediat-

-or mediator of Cho-Kai Wu2014 heart fibrosis (Chen et al., 2000). CTGF is seen in many fibrosis, such as liver fibrosis, lung fibrosis, heart fibrosis, and skin fibrosis (Wu et al., 2014), but researchers have shown that increasing CTGF after heart tissue damage is a compensatory activity for heart regeneration (Chaqour, 2020) and can play a protective role (Sciarretta et al., 2009). Some believe that CTGF can be used as a new method for further clinical studies (Wu et al., 2014). CTGF can stimulate the activation of the Akt / GSK3β and p70S6 rescue pathways, which in turn protects the heart against ischemia-reperfusion injury (Ahmed et al., 2011)

However, this method should be considered with more caution because high expression of CTGF increases the level of fibronectin, collagen I and III proteins in the extracellular matrix, followed by myocardial infarction (Deswal et al., 2001). On the other hand, blocking the function of CTGF protects the heart from damage (Szabó et al., 2014). The expression level of CTGF in sick rats model is proportional to the rate of fibrosis and this increase in expression leads to migration, proliferation and production of extracellular matrix (Lichtman et al., 2016). CTGF exerts its effect on the TGF-β signaling pathway through the tyrosine kinase (TrkA) which is a cell surface receptor (Wahab et al., 2001). It has been discovered that CTGF is secreted by both cardiomyocytes and fibroblasts when heart tissue is damaged, but only fibroblast secretion of this factor affects fibroblast activity and fibrosis induction (Dorn et al., 2018).

Regarding the effect of exercise on CTGF, in a study of spontaneous hypertensive (SHR) Wistar rats, 6 months of aerobic exercise resulted in cardiac adaptation responses that were reflected in increased mitochondrial respiration, decreased heart rate, and improved systolic function. Exercise also had antioxidant effects and reduced the expression of TGF-β1, CTGF and FGF2 incompatible genes (Schreckenberg et al., 2017). In a

similar study, Guo-qiang et al., 2019, found no significant changes in TGF-β1, CTGF, and TIMP-2 protein expression in SHR rats after 6 months of prolonged aerobic exercise. However, aerobic exercise is effective in improving myocardial fibrosis and can inhibit the differentiation of fibroblasts into myofibroblasts. This suggests that other mechanisms are also involved in the inhibitory effect of aerobic exercise on myofibroblast differentiation (Guo-qiang et al., 2020). Also, 5 weeks of moderate aerobic exercise on the treadmill reduced the expression levels of various fibrosis factors, such as CTGF and TGF-β1, which were increased by doxorubicin injection (Yang et al., 2020). In a study by Wang et al. (2019), diabetic rats received moderate-intensity treadmill training for 8 weeks. The results showed that exercise greatly reversed diabetes and suppressed myocardial fibrosis factors, including expression of MMP-2, CTGF, TGF-β1. Therefore, exercise may be an alternative treatment for diabetic cardiomyopathy (Wang et al., 2019). But the effects of HIIT exercise have been less studied. Roozbayani et al. (2016) examined the effect of two types of aerobic exercise and HIIT on CTGF of male rats with diabetes. Exercise protocol was implemented 5 days a week for 5 weeks. The results showed that HIIT training significantly increased the level of miR-29a compared to the aerobic group, which decreased the level of CTGF and thus reduced cardiac tissue fibrosis in diabetic patients. Therefore, performing HIIT exercise reduces cardiac fibrosis without drug tolerance, and acceptance of HIIT is more compatible than aerobic exercise for diabetic rats and has better effects on heart function (Roozbayani et al., 2016).

Our other findings showed that with induction of fatty liver, the level of RXFP1 in heart tissue increased from the beginning and did not change until the end of the fifth week. The expression level of RXFP1 gene in steatosis fatty liver groups (BS, CS and SHIIT) was significantly higher than that in the other 3 groups. This rate was about 9 times higher in BS and CS groups than BH, CH and HIIT groups that did not exercise. Regarding that RXFP1 acts in contrast to CTGF, the simultaneous increase of them in this study may be due to the high degradation of the extracellular matrix because atherosclerotic changes in blood vessels can be seen even in mild degrees of fatty liver (Tahereh Fakharian et al., 2017). CTGF (CCN2) in response to tissue damage initiates signaling pathways of connective tissue regeneration (Chaqour, 2020); therefore, the increase in connective tissue growth factor (CTGF) in this study was probably to produce extracellular matrix and compensate for their degradation, and the increase in RXFP1 was to prevent collagen deposition in ECM (Samuel, 2005) and cardiac tissue fibrosis. RXFP1, which is a component of the extracellular matrix and is most commonly expressed in aortic endothelial cells (Alleva et al., 1997), exerts most of its physiological effect on the cardiovascular system through NO which includes : Inhibition of lipopolysaccharide-induced adhesion (LPS) in coronary endothelial cells (Nistri et al., 2003), inhibition of neutrophil activation by proinflammatory agents through induction of NO synthase expression, increased coronary blood flow in the heart of rats and guinea pigs (Bani-Sacchi et al., 1995), and increased hyperfiltration and dilation of renal vessels in mice via ETB receptor (Danielson et al., 2000).

Relaxin also increases the activity and expression of three types of NOs: endothelial NOS (eNOS; NOS III), induced NOS (iNOS; NOS II) (Baccari et al., 2007), and neural NOS (nNOS; NOS I) (Baccari et al., 2004). RXFP1 reduces liver steatosis and further reduces liver fibrosis by activating intraliver eNOS in rats with fatty liver. Thus, human RXFP1 is a potential treatment for NAFLD (Lee et al., 2019). Relaxin activates the Pi3 kinase pathway (Bryant-Greenwood et al., 1993) and potentially mediates TGF-β inhibitory activity in various organs and cells (Banerjee et al., 2010). These findings also showed that 5 weeks of HIIT training did not change the level of RXFP1 in healthy rats, but significantly reduced the expression level of this gene in the SHIIT group, although it did not reach the level of healthy rats. Very little exercise studies have been done on relaxins. This is the first exercise study to be performed on samples with fatty liver. Kruger et al. examined the effect of an exercise session on chronic heart failure (CHF) samples and found no change in Relaxin levels at the beginning of exercise as well as maximum exercise in heart failure patients compared to the healthy group (Krüger et al., 2004). In a similar study on CHF patients, no significant difference was observed between patients and the control group after exercise (Heringlake et al., 2009; Krüger et al., 2004), but in these studies, the amount of relaxin in the blood was measured, which is different from our method. It seems that the common pathway of RXFP1 and CTGF activity is related to TGF-β, which is one of the most important cytokines in the progression of fibrosis (Massagué, 1998). CTGF produced by hepatocytes is regulated by TGFβ and, to some extent, accelerates fibrogenesis by increasing TGFβ activity (Gressner et al., 2007). TGF-β injection alone causes temporary skin fibrosis, whereas serial CTGF injection after TGF-β causes stable fibrosis. Therefore, CTGF maintains TGF-β-induced skin fibrosis by activating the collagen promoter and increasing the number of active fibroblasts (JINNIN, 2010). Elevated CTGF has been observed in many fibrosis cases such as liver fibrosis, lung fibrosis, heart fibrosis and skin fibrosis (Varga & Pasche, 2009). On the other hand, researchers have shown that the anti-fibrotic effects of relaxin are due to the inhibition of TGF-β function (Masterson et al., 2004). In human renal fibroblasts, TGF-β increases the expression of -SMA α (a marker of fibroblast differentiation), type I collagen, and fibronectin, and these effects are reversed by relaxin. The inhibitory effects of relaxin are due to the inhibition of Smad2. However, relaxin treatment reduces TGF-β-induced Smad2 phosphorylation and eliminates Smad2 alone (Heeg et al., 2005). Thus, relaxin inhibits TGF-β and fibroblast differentiation by a NO

-dependent pathway.

Conclusion

Overall, the findings of this study showed that with the onset of fatty liver disease, all three connective tissue growth factors, liver enzyme SGPT and relaxin, increased in the heart, indicating damage to heart tissue that could lead to fibrosis of the heart tissue. HIIT training, performed in a short time, was able to reduce the heart fibrosis caused by liver steatosis by reducing these factors. Therefore, the use of HIIT exercise can be used as a new method for cardiac rehabilitation of patients.

What is already known on this subject?

Studies have been performed on the relationship between fatty liver and arthrosclerosis, the results of which indicate the relationship between the Non-alcoholic fatty liver and arthrosclerosis of coronary artery disease.

What this study adds?

HIIT training, performed in a short time, was able to reduce the heart fibrosis caused by liver steatosis by reducing these factors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The NIH “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 80-23, revised 1996) and the professional governmental guidelines, in compliance with the Institutional Animal Care and Use Committee (IACUC), had been observed in all experiments.

Informed consent Animal study.

Author contributions

Conceptualization: M.H., M.R.; Methodology: M.H., M.R.; Software: M.H., M.R.; Validation: M.H., M.R.; Formal analysis: M.R.; Investigation: M.H., M.R.; Resources: M.H.; Data curation: M.R.; Writing - original draft: M.H., M.R.; Writing - review & editing: M.R.; Visualization: M.H.; Supervision: M.H.; Project administration: M.R.; Funding acquisition: M.H.

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