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# Comparison of the effects of high-intensity interval training and moderate-intensity continuous training on indices of liver and muscle tissue in high-fat diet-induced male rats with non-alcoholic fatty liver disease

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## Abstract

**Background:** Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver diseases. It is shown that moderate to high physical activities can play a crucial role in improving this disease.

**Aim:** The purpose of this study was to explore the effects of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on the levels of the myonectin in serum and tissue levels and fatty acid transport protein 4 (FATP4) in male rats with NAFLD.

**Materials and methods:** Thirty-three male rats were randomly divided into five groups: high-fat diet to confirm NAFLD induction ( $n = 5$ ), normal diet sedentary ( $n = 7$ ), high-fat diet sedentary ( $n = 7$ ), high-fat diet with HIIT ( $n = 7$ ), and high-fat diet with MICT ( $n = 7$ ). Induction of NAFLD was performed by feeding rats for 12 weeks with a high-fat diet containing 60% fat. The training protocols were performed in five sessions per week for 8 weeks. The HIIT group has performed  $4 \times 4$  min interval running on a treadmill up to 80–95% maximal oxygen uptake ( $VO_{2max}$ ) and then recovered at 50–60%  $VO_{2max}$ . The MICT protocol has performed up to 50–60%  $VO_{2max}$  for 50 min. myonectin and FATP4 were also measured by the animal Elisa kit (Zellbio, Germany) with a sensitivity of 0.02 ng/L. Insulin resistance was evaluated by the insulin resistance homeostasis assessment index using the following formula (HOMA-IR): "fasting glucose (mg/dl)  $\times$  fasting insulin (mg/L)  $\div$  405". One-way ANOVA analysis of variance was utilized for statistical analyses and Tukey's post hoc test at a significant level of  $p < 0.05$ .

**Results:** The 8-week intervention showed that both HIIT and MICT positively influenced the serum myonectin and FATP4 levels ( $p < 0.05$ ). Moreover, there was a significant difference between the trained groups in tissue levels of the myonectin and serum levels of FATP4 ( $p < 0.05$ ).

**Conclusions:** Altogether, both HIIT and MICT can lead to valuable adaptations and recovery of NAFLD in male rats.

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**Keywords:** High-intensity interval training, Moderate-intensity continuous training, Myonectin, Fatty acid transporter protein 4 (FATP4), Non-alcoholic fatty liver disease (NAFLD)

## Background

Early diagnoses and treatments are important for non-alcoholic fatty liver disease (NAFLD). If the NAFLD is not diagnosed and treated at early stages, it destroys hepatic cells. In that case, the NAFLD can lead to an irreversible liver disease called cirrhosis [1]. Studies show that obesity, diabetes, dyslipidemia, insulin resistance, and metabolic syndrome can develop NAFLD [2]. Diet and exercise were stated as the essential non-pharmacological strategies for treating NAFLD [3, 4]. A recent meta-analysis [5] suggested that exercise, regardless of its duration and intensity, could ease NAFLD symptoms and improve patients' physiological condition and metabolism. Accordingly, previous studies have shown that moderate- to high-intensity exercises are viable alternatives to managing fatty liver [6, 7]. It was also recently suggested by multiple groups that high-intensity exercise can effectively recover liver stiffness and the function of liver cells. It can be more effective than moderate-intensity exercise on aerobic power, lipid profile, and reduction of insulin resistance and liver enzymes [8, 9].

Previously, a uniform and long-term aerobic exercise program was reported to reduce fat mass by improving metabolism; however, it did not affect lean body mass, a vital index in metabolism measurements [10]. On the other hand, intense periodic exercises reduce body fat mass and positively affect lean body mass [10]. Moreover, the results showed that intense intermittent exercise has better effects on the two clinically important indices of NAFLD, the treatment of insulin sensitivity and reducing insulin resistance [11].

In general, some researchers consider the intensity of exercise as the main factor in the treatment of NAFLD [12], while others value exercising but suggest the volume to be a key and influential variable in improving fatty liver indices [13]. Consequently, further research is required to elucidate better the effect of different exercise parameters in NAFLD [14].

The liver and skeletal muscles are two crucial and influential tissues in the body's metabolism during exercise. They have unique roles in regulating fat and carbohydrate metabolism upon physical activity through the expression and secretion of specific metabolic factors. Evaluation of intensive interval and moderate continuous exercise leading to alterations in the myonectin and fatty acid transporter protein four (FATP4) in the liver and skeletal muscle tissues may provide insights into

these exercises' role in the treatment of NAFLD. To date, little is known about the effect of high-intensity interval training (HIIT) versus moderate-intensity continuous training (MICT) on the content of the myonectin and FATP4 proteins in serum and tissue of the liver and muscles in patients with the NAFLD.

The myonectin is a myokine that is expressed and circulated following a physical activity course [15]. Gamas et al showed in their research that myonectin may prevent the increase of insulin resistance by regulating glucose and lipid metabolism [16]. Lim et al. also reported that myonectin increases AMPK activity and stimulates skeletal muscle glucose transporters and increases glucose uptake in muscle [7]. Studies have shown that exercise training, regardless of its duration, leads to a change in the myonectin quantity and a reduction in insulin resistance [17]. It seems that as a result of weight gain through a high-fat diet, fat tissue increases and deposition of free fatty acids in muscle tissue increases, and as a result, the ability of skeletal muscle to produce myokines, including myonectin decreases [17]. In past studies, it has been reported that myonectin levels decrease in sedentary people and sports activity increases myonectin levels [18].

The FATP4, a member of the fatty acid transporter family, expressed in brain, kidney, muscle, and liver tissues [19], is involved in transporting fatty acids into these tissues. It is thought that exercise training can affect its expression in the liver, fat, and muscle tissues [20]. Moreover, researchers have suggested that muscle contraction may increase the FATP4 level in the plasma members [20]. Consistently, Jeppesen et al. [21] showed that 8 weeks of exercise training increased skeletal muscle FATP4 levels by 33%, and it could lead to an increased fat uptake and lipid oxidation in skeletal muscles. Reports investigating the relationship between exercise and FATP4 protein are limited in the literature [22, 23].

Insulin resistance and decreased plasma glucose consumption are initial symptoms of NAFLD [24]. There are multiple and opposing reports about the effect of intensity of exercises on reducing insulin resistance. Some studies have indicated intensive interval exercises as an effective choice, while others have recommended continuous exercises. Interestingly, a third group has rejected the impact of this parameter on insulin resistance [25, 26]. There are no studies comparing the effects of HIIT and MICT on myonectin and FATP4 in the literature. Therefore, this study aimed to compare the effects of 8

weeks of HIIT and MICT on FATP4 and myonectin levels in male rats with NAFLD.

## Methodology

### Animals

In this study, 33 male Wistar rats (6–8 weeks old) were purchased as research samples from the Animal Room of Mashhad Medical School. Three to four rats were kept in each standard polycarbonate cage (38 × 59 × 20 cm) in control conditions; 12 h of light starting and 12 h of darkness, and the temperature was  $22 \pm 3$  °C. All rats had free access to water and food. At the beginning of the study, rats were divided into two groups, the group with a standard diet ( $n = 7$ ) and the high-fat-diet ( $n = 26$ ) group. This diet course lasted 12 weeks to induce NAFLD in the latter group. After ensuring the induction of NAFLD by examining and killing verified NAFLD groups ( $n = 5$ ), 21 rats were divided into four groups: Normal diet sedentary (ND + SED), high-fat diet sedentary (HFD + SED), high-fat diet with HIIT (HFD + HIIT), and high-fat diet with MICT (HFD+MICT). This treatment was 8 weeks, and the duration of the whole research was 22 weeks.

### Diet

In this study, HFD, enriched with 1% choline, was administered for 20 weeks containing 60% kcal from fat, 20% kcal from protein, and 20% kcal from carbohydrates. It was provided by specialists and rats were fed on a daily basis [27]. The composition of ND (standard rodent chow) was 70% kcal from carbohydrates, 20% kcal from protein and 10% kcal from fat.

### NAFLD induction protocol

An HFD was used for 12 weeks to induce NAFLD in rats [28]. After this diet course, five rats were tested to confirm the development of fatty liver disease. To this end, serum levels of two liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were measured. This measurement showed that ALT increased significantly compared to the ND+SED group. Moreover, histopathological analysis of liver tissue showed hepatic steatosis in all samples. Steatosis was reported at the rate of 10, 7, 10, 10, and 30% in five analyzed samples of fatty liver groups.

### Exercise training protocols

In this study, exercise training was running on a treadmill, particularly for rodents (Towers-Sanat, Model T.S 8000; Iran) with the ability to control speed and slope. Exercise training was performed for 8 weeks, 5 days a week, with a 10-min warm-up with a speed of 10 m/min per session. MICT was performed for 50 min each day, with a moderate intensity of 50 to 60% VO<sub>2</sub>max with a

0° slope. The HIIT was performed for 4-min speed intervals and the intensity of 80-95% VO<sub>2</sub>max and 4-min slow intervals and the intensity of 50-60% VO<sub>2</sub>max with a -0-degree slope.

### Determination of VO<sub>2</sub>max

After a warm-up, the estimation test, based on the sprint, started at a speed of 10 m/min as a running course on the treadmill, and then, every 3 min, the speed was increased by 5 m/min. This increase in speed continued until animals were no longer able to run. Fatigue is considered when rats stay motionless on the electric shocker at the end of the tidal for 10 to 15 s. Research has shown that for the rats that completed the aerobic power test, VO<sub>2</sub>max is achieved before the final speed of 0.06 to 0.15 m per second [29]. Consistently, in this research, the final speed of running was set to 0.075 m per second, and intensities were adjusted according to this speed. According to the reports, there is a direct relationship between the sprinting of rat and their VO<sub>2</sub>max [29, 30].

### Preparation of blood samples

Forty-eight hours after the last training session and after overnight fasting, all rats were anaesthetized by an intraperitoneal injection of the combination of xylazine (8 mg/kg) and ketamine (75 mg/kg). Their chests were then dissected, and blood samples were collected directly from the heart. The blood samples were kept at room temperature for 30 min and then centrifuged at a speed of 3000 rpm for 10 min to separate the serum. Finally, the samples were stored at -80 °C for laboratory tests. All procedures were performed following the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

### Preparation of tissue and protein samples

Tissue sampling was performed in similar conditions for all groups. For this purpose, the rats were anaesthetized, and after cleaving their chest, liver tissue was immediately isolated. The tissue of soleus muscles was also isolated from their legs as skeletal muscle samples. The tissues were stored at -80 °C for further analysis. One hundred milligram of liver and muscle tissues were combined with protease inhibitor (PMSF) in 500 µl of tissue protein extraction reagent (T-PER) to measure the quantity of the myonectin and FATP4 proteins. Then, the samples were homogenized using the Bioprep-24 model homogenizer for 10 min at a speed of 10,000 rpm. In the end, the obtained translucent supernatants were collected and transferred to the laboratory for subsequent tests [31].

### Biochemical assays

Measurement of serum glucose, ALT and AST concentrations were performed by glucose oxidation and photometric methods using the Pars Azmoon Iran kit. Serum insulin concentration was measured by the Sandwich Elisa kit (Mercodia, Sweden) with a sensitivity of 0.15 mg/L, and serum and tissue levels of the myonectin and FATP4 were also measured by the animal Elisa kit (Zellbio, Germany) with a sensitivity of 0.02 ng/L. Insulin resistance was evaluated by the insulin resistance homeostasis assessment index using the following formula (HOMA-IR): “fasting glucose (mg/dl) × fasting in insulin (mg/L) ÷ 405” [31].

### Statistical analysis

Statistical analysis was conducted using the IBM SPSS software package (version 19.0). Data were reported as means ± standard deviation (SD). Shapiro-Wilk tests were used to evaluate the normality. One-way analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the groups. The significance level was considered at  $p < 0.05$ .

## Results

### Changes in body weight

The data in Table 1 shows that the body weight increased significantly with fatty liver induction, but training significantly decreased it ( $P < 0.05$ ). There was no significant difference between the two group of HFD + MICT and HFD + HIIT ( $p > 0.05$ ).

### Changes in HOMA-IR

The data in Table 1 shows that the difference between the four groups was statistically significant in the HOMA-IR ( $p < 0.001$ ). The post hoc test showed that there was a significant difference between the HFD + SED group with ND + SED ( $p < 0.001$ ), HFD + MICT ( $p < 0.001$ ), and HFD + HIIT groups ( $p < 0.001$ ) in the reduction of HOMA-IR (Fig. 1). There was no difference between the HFD + MICT and HFD+HIIT groups in HOMA-IR ( $p > 0.05$ ).

### Changes in serum and tissue myonectin

The results showed that there was a statistically significant difference between the four groups in serum levels of the myonectin ( $p < 0.004$ ; Table 1; Fig. 2A). The post hoc test showed no significant difference between the HFD + MICT with HFD + HIIT group in serum levels of the myonectin ( $p > 0.05$ ; Fig. 2A). The results showed that there was not a significant difference between the HFD + MICT and HFD + HIIT groups in muscle and liver tissue levels of the myonectin. On the other hand,

**Table 1** The results of one-way analysis of variance test related to Body weight, ALT, AST, HOMA-IR, Myonectin and FATP4 among the groups

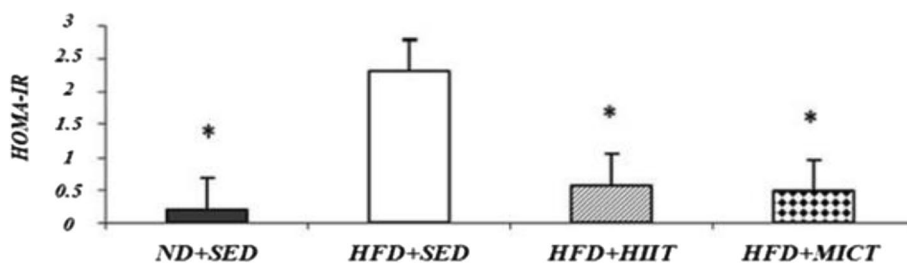
Variables	Groups	Mean ± SD	P value
Final body weight (g)	ND + SED	335 ± 9.34 <sup>§*</sup>	0.001
	HFD + SED	452 ± 16.25	
	HFD + HIIT	402 ± 14.40*	
	HFD + MICT	398 ± 23.81*	
Serum myonectin (ng/ml)	ND + SED	1.37 ± 0.04*	0.004
	HFD + SED	1.53 ± 0.11	
	HFD + HIIT	1.33 ± 1.14*	
	HFD + MICT	1.32 ± 0.10*	
Myonectin muscle tissue (ng/ml)	ND + SED	1.48 ± 0.13*	0.007
	HFD + SED	1.80 ± 0.28	
	HFD + HIIT	1.44 ± 0.03*	
	HFD + MICT	1.67 ± 0.24 <sup>§</sup>	
Liver tissue myonectin (ng/ml)	ND + SED	1.50 ± 0.16 <sup>§#</sup>	0.001
	HFD + SED	2.13 ± 0.11	
	HFD + HIIT	2.01 ± 0.19	
	HFD + MICT	1.92 ± 0.09	
Serum FATP4 (ng/ml)	ND + SED	2.09 ± 0.17*	0.002
	HFD + SED	2.42 ± 0.07	
	HFD + HIIT	2.29 ± 0.04 <sup>#</sup>	
	HFD + MICT	2.16 ± 0.11*	
Muscle tissue FATP4 (ng/ml)	ND + SED	1.95 ± 0.12	0.286
	HFD + SED	2.16 ± 0.44	
	HFD + HIIT	2.02 ± 0.19	
	HFD + MICT	1.91 ± 0.18	
Liver tissue FATP4 (ng/ml)	ND + SED	2.31 ± 0.12	0.127
	HFD + SED	2.57 ± 0.13	
	HFD + HIIT	2.61 ± 0.31	
	HFD + MICT	2.36 ± 0.16	
HOMA-IR	ND + SED	0.20 ± 0.13*	0.001
	HFD + SED	2.30 ± 1.09	
	HFD + HIIT	0.57 ± 0.35*	
	HFD + MICT	0.48 ± 0.29*	
ALT (U/l)	ND + SED	130 ± 0.17*	0.001
	HFD + SED	174 ± 0.14	
	HFD + HIIT	125 ± 0.16*	
	HFD + MICT	138 ± 0.10*	
AST (U/l)	ND + SED	115 ± 0.13*	0.002
	HFD + SED	163 ± 0.25	
	HFD + HIIT	1.37 ± 0.10*	
	HFD + MICT	125 ± 0.16*	

**Abbreviations:** MICT moderate intensity continuous training, HIIT high intensity interval training, HFD high-fat diet, SED sedentary, ND normal diet, SD standard deviation

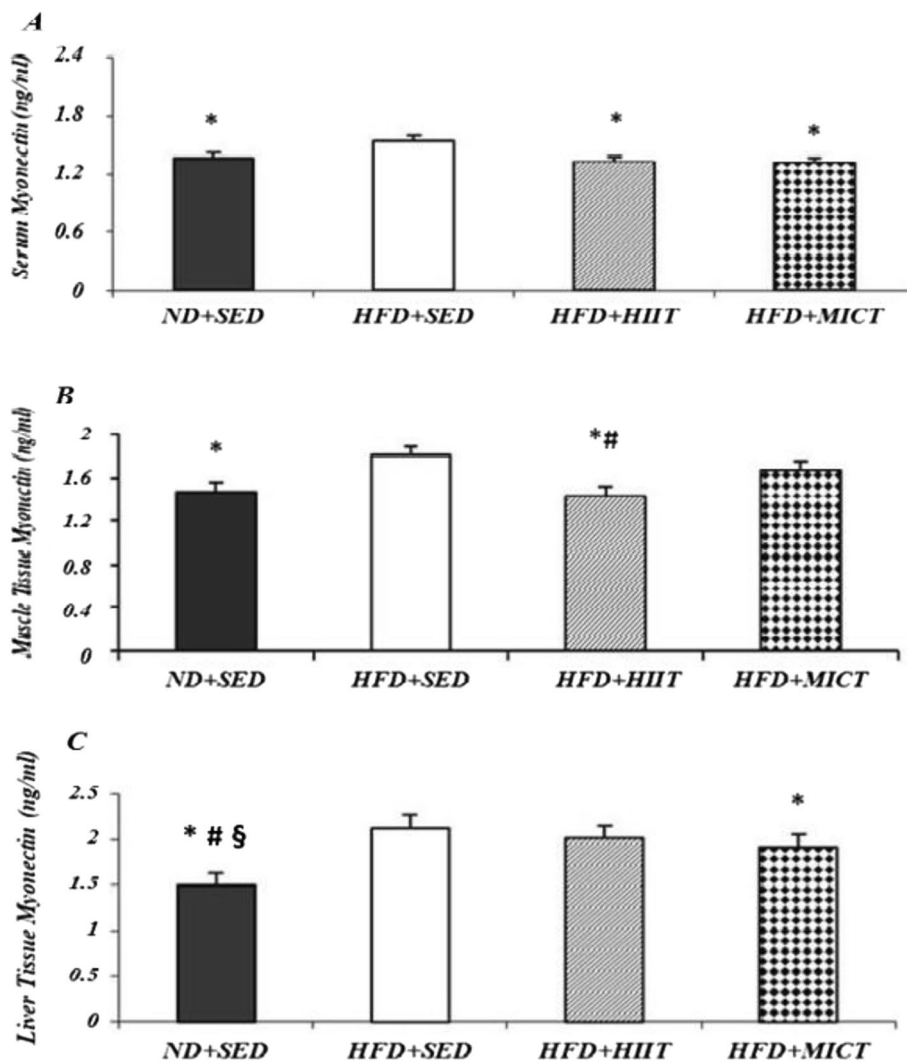
\*  $p < 0.05$  compared with HFD + SED

#  $p < 0.05$  compared with HFD + MICT

<sup>§</sup> Compared with HFD + HIIT in significant level of  $P \leq 0.05$ . Data in the table are presented as mean ± SD



**Fig. 1** The comparison of HOMA-IR among the groups. \* signs of significant difference with the HFD+SED ( $p < 0.001$ )



**Fig. 2** **A** The comparison of serum myonectin among the groups. \* signs of significant difference the HFD + SED with ND + SED ( $p < 0.009$ ); HFD + HIIT ( $p < 0.003$ ); and HFD + MICT ( $p < 0.006$ ). **B** The comparison of muscle tissue myonectin among the groups. \* signs of significant difference the HFD + SED with ND + SED ( $p < 0.04$ ) and HFD + HIIT ( $p < 0.04$ ); # signs of significant difference with the HFD + MICT ( $p < 0.04$ ). **C** The comparison of liver tissue myonectin among the groups. \* signs of significant difference the HFD + SED with ND + SED ( $p < 0.002$ ) and HFD + MICT ( $p < 0.006$ ); # signs of significant difference with the HFD + MICT ( $p < 0.001$ ); § signs of significant difference with the HFD + HIIT ( $p < 0.01$ )

muscle tissue levels of the myonectin decreased significantly only by the HIIT ( $p < 0.04$ ; Fig. 2B). The level of liver myonectin tissue increased significantly with the induction of fatty liver, but exercise could not make a significant change in this field. There was no significant difference between HIIT and MICT ( $p < 0.01$ ; Fig. 2C).

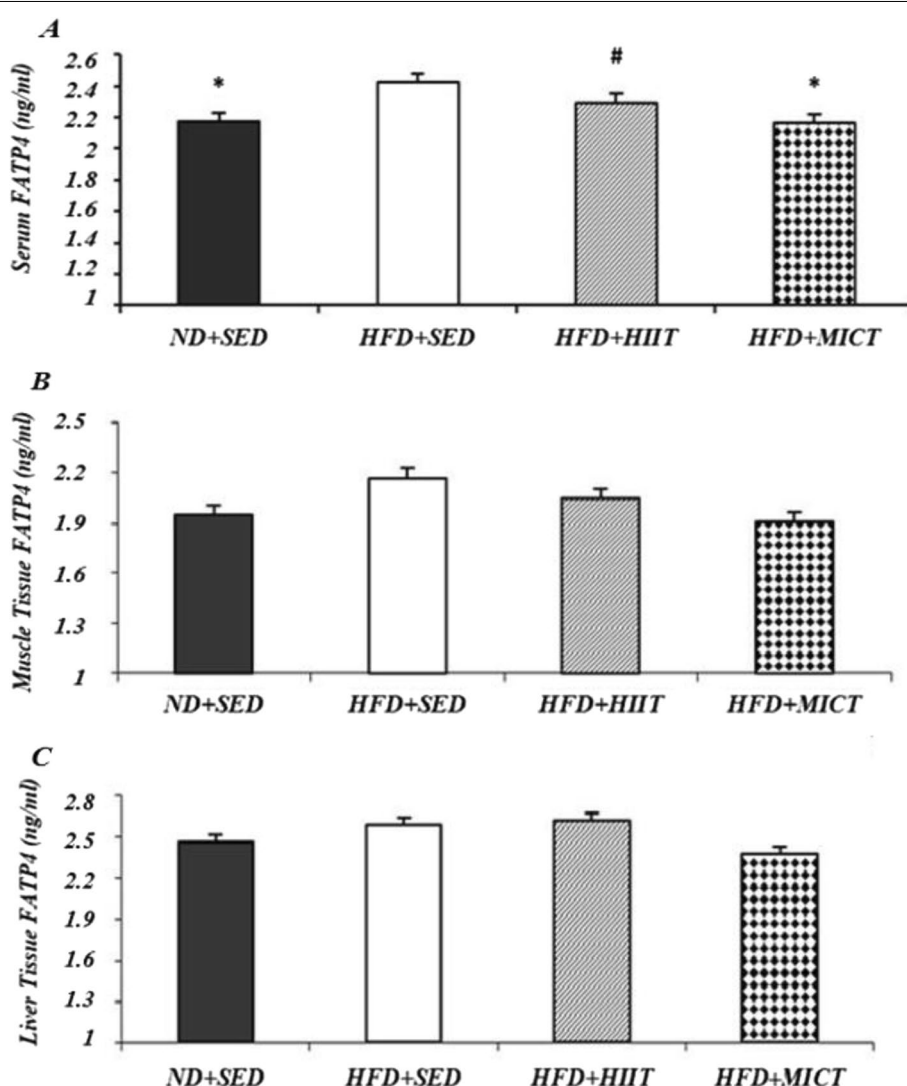
**Changes in serum and tissue FATP4**

There was a statistically significant difference between the four groups in the serum level of FATP4 ( $p < 0.002$ ). The serum level of FATP4 decreased significantly only in the MICT group ( $p < 0.001$ ; Table 1, Fig. 3A), and there was a significant difference between the HFD + MICT and HFD+HIIT groups in serum levels of FATP4 ( $p < 0.004$ ). However, there were no significant differences

between the four groups in skeletal muscle and the liver tissue of FATP4 ( $p > 0.05$ ; Fig. 3B, C).

**Discussion**

The current study indicated that 8 weeks of HIIT and MICT can significantly decrease the serum level of the myonectin in the rats with NAFLD. In contrast, a significant decrease in muscle the myonectin was observed only after HIIT. A recent study showed that the myonectin gene expression was affected by environmental factors, exercise, and nutrition, while nutrition had a larger impact compared to a period of exercise. Myonectin expression was also shown to increase in genetically obese rats, while after exercise, its level decreased in both genetically obese and healthy rats [17]. The authors



**Fig. 3** **A** The comparison of serum FATP4 among the groups. \* signs of significant difference with the HFD + SED in  $p < 0.001$ ; # Signs of significant difference with the HFD+MICT in  $p < 0.004$ . **B** The comparison of muscle tissue FATP4 among the groups. No significant difference was observed among the groups. **C** The comparison of liver tissue FATP4 of among the groups. No significant difference was observed among the groups

suggested that leptin could regulate the myonectin expression, and this decrease might be due to the leptin resistance in this breed of cardamom rat. Moreover, it was stated that an HFD, regardless of obesity status, might lead to changes in circulating levels of the myonectin.

On the other hand, the myonectin may act autonomously and regulate itself. In addition, Jing and Zeyuan reported a decrease in serum myonectin levels after 10 weeks of endurance exercise in HFD-fed rats, suggesting that myonectin levels may increase with increased insulin resistance [29]. In this regard, Mi et al. [30] linked the growth in the myonectin levels to key metabolic components in patients with metabolic syndrome and insulin resistance and stated that obesity might lead to the myonectin induction, as well as insulin resistance. These changes could be the result of a compensatory adjustment of the myonectin to obesity. In contrast, an increase in serum myonectin was also reported following 2 weeks of endurance activity on the wheel. The authors attributed the myonectin induction to increased calcium and cyclic adenosine monophosphate (cAMP) levels in the myonectin protein. An explanation for these inconsistent results can be the variation in the type of studied rats. Moreover, the molecular mechanisms underlying the expression, secretion, and function of the myonectin are not precise yet; cAMP makes it challenging to explain the effect of exercise on the molecular mechanisms in the observed real-time changes.

Myonectin is known to phosphorylate AMP-activated protein kinase (AMPK), trigger signals in glucose carrier protein, increase glucose transporter density in the cell membrane, improve glucose intake, stimulate oxidation of fatty acids, and finally increase glucose harvesting [15]. Myonectin has a similar function as insulin, except that insulin induction can occur immediately upon feeding, whereas circulating amounts of the myonectin increase 2 h after glucose or lipid intake [31]. Recent studies have confirmed the dual role of the myonectin, but due to the existing inconsistencies in reports, the expression profile of the myonectin following nutrition needs further investigations. Furthermore, due to the limited number of studies on the effect of physical exercises on the myonectin in rats with obesity and non-alcoholic liver disease, a general and accurate conclusion on this issue is missing. According to the current study, changes in insulin resistance may be an influential factor in increasing the myonectin levels under an HFD. In the present study, the initial increase in the myonectin due to high fat intake and the transfer of fatty acid from the blood appears normal. However, a significant increase after the training period can

indicate the adaptation to regular exercise training and desirable changes in the body's metabolism.

Additionally, a significant decrease in the muscle the myonectin only after HIIT indicated a high muscle involvement during this type of exercise. HIIT was performed in short but highly intense periods, a situation that resulted in extensive changes to fat metabolism in the pure body mass. In contrast, MICT could only modulate the myonectin in liver tissue. Long-term aerobic exercises are associated with multiple changes in fat metabolism. Consequently, an adaptation in liver tissue (which is the focus of major interactions), changes and conversion of substrates, oxidation of fatty acids, and the conversion and change of fats into sugar are expected. Further investigations are necessary to assess these interactions.

Besides these findings, a significant decrease in serum levels of FATP4 in the MICT group but not in HIIT was also observed. Feng and Chen [32] had previously shown that protein and transcript levels of FATP4 in rats with NAFLD increased after 12 weeks of an HFD. It was suggested that high expression of FATP4 and prolonged application of an HFD could lead to an imbalance in fatty acids and ultimately NAFLD in rats. Jeppesen et al. [21] showed that 8 weeks of endurance training led to a 33% growth in the quantity of FATP4 protein in muscle tissue. An increase followed this change in lipid oxidation, which supported the hypothesis that key mitochondrial enzymes experience an increase upon exercise. It was also stated that FATP4 could play an essential role in entering fats into the muscle and the orientation of long-chain intracellular fatty acids. These reports are, however, in contrast to our findings. In this study, FATP4 protein quantity dropped only in the serum samples of the MICT group, and there was no significant change in muscle and liver tissues. FATP4 has been introduced as a transporter of fatty acids and metabolites in muscle and liver tissues, while in the present study, there was no significant change in its amount in these two tissues. There are two explanations for this observation. First, the duration and intensity of the exercise were probably not enough to bring about changes in these two tissues. It is necessary to perform the exercise for a longer period and with higher intensity. Second, following an HFD and in NAFLD, other fatty acid transporters were probably expressed in muscle and liver tissues, quantification of which was not possible due to the limitations in this study. On the other hand, in the present study, serum levels of FATP4 decreased only after MICT, which shows that other tissues such as adipose tissues should be studied as well. The present study is one of the few studies on muscle and liver FATP4 in NAFLD, and further

investigations are necessary to determine the functional mechanisms of FATP4 protein.

Gan-Schreier et al. [33] have pointed out that the expression of FATP4 was induced in human cases with central obesity, and FATP4 polymorphism was associated with insulin resistance. They also showed that serum FATP4 level went up in rats with an HFD, but it declined after 8 weeks of continuous training. The initial increase in FATP4 can be attributed to high fat intake, as it was shown that after an HFD, FATP4 expression could be induced [34]. On the other hand, the subsequent reduction of FATP4 after adaptation to continuous training with moderate intensity can also be explained by the dominance of fats as the fuel source during training, as a result of which the need for FATP4 was decreased. Instead, HIIT is performed over short periods, and consequently, the body's primary fuel is sugar (glucose and glycogen). Likewise, it can be the main reason for the lack of significant changes in FATP4 level by the interval training, although it had a yet more significant impact than continuous training.

Another remarkable observation in the present study was a significant decrease in insulin resistance in the rats with NAFLD following 8 weeks of HIIT. It was improving insulin resistance after regular physical activity is rooted in the modifications in insulin function. Desirable changes in insulin, insulin resistance, and serum glucose in patients with NAFLD as a result of physical activity were indicated before [6]. Zheng and Cai [35] reported a decrease in insulin resistance in rats with fatty liver following 12 weeks of aerobic exercise. They stated that this decrease in insulin resistance is likely due to the effect of exercise on activation of peroxisome proliferator-activated receptor  $\gamma$  in liver tissue, which can increase fatty acid oxidation, glucose uptake, and glucose catabolism. Abdelbasset et al. [6] also proposed that regular MICT could be an effective training strategy to reduce insulin resistance. Despite these results, Cauza et al. [36] reported that compared to resistance training, 4 months of continuous training could not significantly reduce insulin resistance in patients with type 2 diabetes. They reported larger baseline levels of fasting glucose and insulin in the resistance training group than close-to-normal baseline glucose values in the endurance training group.

In contrast, Vosadi et al. [37] reported no significant change in insulin resistance following endurance training, probably due to the short training period of only 4 weeks. Altogether, observations revealed that resistance and endurance exercises could be effective strategies in restoring glucose in skeletal muscles, fat absorption, and metabolic regulation, which were likely by induction of glucose-carrying proteins such as glucose transporter

type 4 (GLUT4). This can finally result in reduced insulin resistance. Besides, it is expected that by activation of AMPK, which increases the density of glucose transporters on the surface of the cell membrane, glucose harvesting increases, and eventually, the patient's condition improves.

## Conclusion

The reduction in insulin resistance in rats with NAFLD following HIIT and MICT was observed. This can result from an increase in GLUT4 protein in skeletal muscle tissue. Regarding the myonectin and FATP4, our observations proposed that these indices could adapt to HIIT and MICT in serum, liver, and muscle tissue for at least 8 weeks. However, it appeared that changes in the myonectin levels in muscle and liver tissues depended on the type of exercise; HIIT, with stressful nature of muscle tissue, could only moderate the myonectin in muscles tissues, while MICT, with a long-term nature, is having a high impact on changing the metabolism of the body and substrates during training and modulating myonectin in liver tissue. Collectively, both types of exercises and a combination of HIIT and MICT could be effective in managing NAFLD.

## Abbreviations

NAFLD: Non-alcoholic fatty liver disease.

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## Authors' contributions

All authors have assumed responsibility for data integrity and accuracy of the data analysis. Study concept and design: ZKS, MEA, MMA, MAS. Data acquisition: ZKS, MEA, MMA, MAS, SGF, HK. Data analysis and interpretation: ZKS, MEA, MMA, MAS. Drafting of the manuscript: ZKS, MEA, MMA, MAS, SGF, EA, AA. Critical revision of the manuscript for important intellectual content: ZKS, MEA, AA, MH, EA. Statistical analysis: ZKS, MEA, MMA, MAS, HK. Writing—review and editing: MH. Study supervision: MEA, MAS, EA. All authors read and approved the final manuscript.

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No

## Availability of data and materials

Please contact author for data requests.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committees of the School of XXX and conformed to the Guide for the Care and Use of Laboratory Animals (IR.MUMS.MEDICAL.REC.1397.065).

### Consent for publication

Not applicable



**Competing interests**

The authors declare that they have no competing interests.

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