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High-intensity interval training can inhibit of PPARγ/ OSBPL3 pathway through epigenetic alterations in Non-Alcoholic Fatty Liver Disease

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Abstract

Objectives: Although previous reports show that high-intensity interval training (HIIT) can provide positive effects on NAFLD, However, the mechanism of these effects has not been well demonstrated. On other hands, new findings showed PPARγ/ OSBPL3 pathway could be an important target for the treatment of NAFLD, therefore in this study we investigated the possible effect of HIIT on PPARγ/ OSBPL3 pathway through DNMTs gene expression and activity.

Methods: 30 adult male wistar rats were divided into three groups including normal diet sedentary (ND-SED), high-fat diet sedentary (HFD-SED), high-fat diet & high-intensity interval training (HFD+HIIT), high-fat diet. Firstly, in order to induce fatty liver, high-fat food was used for 12 weeks. Blood samples were collected to determine serum levels of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lipid profile the extracted livers were used for biochemical assays and real time PCR studies. DNMT1, DNMT3A, and DNMT3B activities were assessed in hepatic tissue.

Results: HFD administration significantly increases serum TC, TG and LDL levels (all $p < 0.001$). It also, increases AST and ALP activity, these effects were ameliorated by HIIT treatment. Compared with the ND-SED group, the HFD-SED group showed significantly increased expression and activity of DNMT1, DNMT3A and DNMT3B ($P < 0.01$) and also significantly increased expression of PPARγ and OSBPL3 ($P < 0.01$) in liver tissue. The expression and activity of DNMTs were significantly decreased in HFD+HIIT compared to HFD - SED rats. There were positive significant correction between PPARγ and OSBPL3 with DNMTs activity and gene expression.

Conclusion: HIIT may be helpful in the prevention of NAFLD by modulating some serum liver function markers, lipid profile and glycemic status. In addition, HIIT can inhibit the development of NAFLD by reducing the expression of PPARγ and OSBPL3 through reducing activity and expression of DNMTs.

KEYWORDS: HIIT, DNMT, NAFLD, OSBPL3, PPARγ.

Introduction

The most common liver condition, non-alcoholic fatty liver disease (NAFLD), is brought on by an increased buildup of neutral lipids in liver cells, particularly triglycerides (TG). Chronic liver illnesses such as liver fibrosis, cirrhosis, carcinoma, and non-alcoholic steatohepatitis (NASH) are mostly caused by non-alcoholic fatty liver disease (NAFLD). Numerous genetic and environmental variables interact to develop NAFLD. The development and pathophysiology of many diseases, including diabetes and non-alcoholic fatty liver disease (NAFLD), are greatly influenced by both genetic predisposition and epigenetic changes. NAFLD is linked to transcriptional alterations that impact phenotype and gene expression (1-3). A number of human disorders have been linked to abnormalities in the DNA methylation mechanism. Gene expression is changed as a result of hyper- and hypomethylation of the global genomic DNA, which disturbs cellular homoeostasis. Numerous studies have shown that different environmental and lifestyle factors can modify DNA methylation patterns, which seem to be reversible (4-7). Fat liver illnesses appear to be influenced by a combination of genetic and epigenetic factors, as well as obesogenic exposures, sedentary lifestyles, and high-calorie/carbohydrate diets. Lipid metabolism, mitochondrial damage, oxidative stress, and inflammation are all impacted by epigenetic modifiers. This may lead to a buildup of hepatic lipids and ultimately NAFLD (8, 9).

However, as a transcription factor associated with lipid accumulation, peroxisome proliferator-activated receptor gamma (PPARγ) is known to activate the expression of lipogenic genes, including sterol regulatory element-binding protein (SREBP1c) and carbohydrate-responsive element-binding protein (chREBP). PPARγ expression is highest in adipose tissue, but it is also significantly elevated in the livers of NAFLD model mice and people. Furthermore, it has been determined that the PPARγ gene's DNA hypermethylation status serves as a marker for the advancement of liver disease(10). For the first time, Aibara *et al.* reported that PPARγ activated the expression of oxysterol-binding protein-like 3 (OSBPL3), which in turn accelerated the synthesis of

triglycerides. Furthermore, their research validated the lipid metabolism route unique to NAFLD and indicated that OSBPL3 would be a key target for NAFLD therapy (11).

It is crucial to follow the right treatment protocol for the prevention or treatment of nonalcoholic fatty liver disease (NAFLD), as it is a chronic liver condition that will worsen if left untreated. It is unknown if medication therapy for NAFLD is effective or immune-boosting. The first recommendations for treatment include antioxidant supplementation, a restricted diet, physical activity, and exercise-related behaviors in addition to a healthy lifestyle. Exercise, whether done alone or in combination, can have a significant impact on the treatment of some diseases, even though it is safer, more secure, healthier, and less expensive when it comes to physical activity. Recently, it has been determined that high-intensity interval training (HIIT) is a unique exercise modality that can effectively control metabolic syndrome, NAFLD, overweight, and type 2 diabetes. Volunteers prefer high-intensity interval training (HIIT) over continuous exercise programs due to its shorter duration, despite prior research suggesting that HIIT can benefit NAFLD patients. High-intensity interval training (HIIT) has been shown in earlier studies to have beneficial effects on non-alcoholic fatty liver disease (NAFLD), but the exact mechanism underlying these effects has not been well explored. However, recent research indicated that the PPARγ/OSBPL3 pathway may be a key target for the treatment of NAFLD. For this reason, we examined in this study the potential impact of HIIT on the PPARγ/OSBPL3 pathway by analyzing the expression and activity of the DNMTs gene.

Martial and Methods

Animals

In this investigation, thirty male Wistar rats (Rattus norvegicus), weighing between 180 and 300 g at eight weeks of age, were utilized. Standard sustenance was provided to the rats on an as-needed basis. The cages were maintained spotless, with 12 hours of light and 12 hours of darkness. Rats were split into two groups at the start of the study: six rats on a conventional diet and eighteen rats on a high-fat diet. The latter group was subjected to a 12-week food course designed to develop NAFLD. Following the examination and death of six confirmed NAFLD groups, 18 rats were split into three groups: ND-SED (normal diet sedentary), HFD-SED (high-fat diet sedentary), and HFD + HIIT (high-fat diet + HIIT). After administering an intraperitoneal dose of 2% pentobarbital (40 mg/kg body weight) to all rats to induce anesthesia at the conclusion of week 8, blood samples were taken from the abdominal aorta. After centrifuging the blood samples for 10 minutes at 4°C at 1,500 × g, the clear supernatants were collected. In order to conduct real-time PCR investigations, liver samples were obtained.

Diet

In this trial, a high-fat, low-protein, and 20%-high-carbohydrate diet (HFD) was given for 20 weeks, supplemented with 1% choline. It was supplied by experts, and rats were fed every day. Normal rodent chow, or ND, contained 70% kcal of carbs, 20% kcal of protein, and 10% kcal of fat (8).

NAFLD induction protocol

Rats were given HFD for 12 weeks in order to cause NAFLD. Five rats were examined following this food regimen to verify the onset of fatty liver disease. In order to do this, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two liver enzymes, were evaluated at serum levels. When compared to the ND+SED group, this measurement revealed a substantial increase in ALT. Furthermore, all samples had hepatic steatosis, according to a histological examination of liver tissue. Five investigated samples of fatty liver groups had reported rates of 15, 10, 20, 30, and 20% steatosis (12).

HIIT

There were even and odd days for the HIIT program. Training began at 40 m/min on odd days and consisted of two repetitions (reps) lasting three minutes each, separated by one minute of active rest (total duration: seven minutes). After each odd day, the reps, speed, and duration were increased. Training began at 54 m/min on even days and consisted of three repetitions of 30 s separated by one minute of active rest (total length = 3.5 min). A prior study found that the speed, repetitions, and duration increased every day after that (13).

Biochemical Assay

An automatic biochemical analyzer (7600-020; Hitachi, Ltd., Tokyo, Japan) was used to measure the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

DNMTs activity

Using DNMT activity kits, the activity of DNMT1 (ab113469), DNMT3a (ab113470), and DNMT3b (ab113471) was determined in accordance with the manufacturer's instructions. There were 48-well microplates used for the assays. A nuclear protein extraction kit (BB18091) was used to prepare nuclear protein. Subsequently, 100 µL of developing solution, 50 µL of detection antibody, and 50 µL of capture antibody were added in succession. After that, the samples were incubated for ten minutes at room temperature and away from light. Ultimately, each well received 100 µL of Stop Solution to put an end to the enzymatic process. In two to ten minutes, the absorbance was determined at 450 nm using a microplate reader.

Real time PCR

As instructed by the suppliers (Cinnagen, Tehran, Iran), total RNA was extracted from the liver tissues using the Trizol technique. For cDNA synthesis, one microgram of total RNA and arbitrary primers were utilized with the ScriptTM cDNA synthesis kit (Bio-Rad, CA, USA). Three cDNA samples were run for real-time PCR analysis. Using the Applied Biosystems 7500 Instrument, real-time PCR reactions were carried out utilizing Power SYBR® Green (Life Technologies, CA, USA). The standard temperature profile that was employed was 95 °C for 5 minutes, then 45 cycles of 95 °C for 30 seconds, 56 °C for 30 seconds, and 72 °C for 30 seconds. Primers were obtained from the literature, and the ΔCt was computed by deducting the β-actin Ct from each sample Ct following PCR amplification (10). β-actin served as the reference gene.
(9). As a reference gene, the β-actin was used. The primer sets used are in table 1:

Table 1. Specific primers used for real-time quantitative PCR (14-16).

Gene Name	Sequences 5----3	
Dnmt1	F	CCTAGTTCCGTGGCTACGAGGAGAA
	R	TCTCTCTCCTCTGCAGCCGACTCA
Dnmt3a	F	GCCGAATTGTGTCTTGGTGGATGACA
	R	CCTGGTGGAATGCACTGCAGAAGGA
Dnmt3b	F	TTCAGTGACCAGTCCTCAGACACGAA
	R	TCAGAAGGCTGGAGACCTCCCTCTT
PPARγ	F	GGTGAAACTCTGGGAGATCCTCC
	R	AGCAACCATTGGGTCAGCTCT
OSBPL3	F	GTGGCCCTTAAAAGGCTGGC
	R	GAGCCCGACATCAATGCAGC
β-actin	F	GCAAGCAGGAGTATGACGCTAG
	R	GTCACCTTCACCGTTCCAGTGTC

Statistical analysis

If the data are regularly distributed, they are shown as mean (interquartile range); if not, they are provided as mean ± standard deviation (SD). The one-sample Kolmogorov-Smirnov test was used to determine the normality of the data. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test (for regularly distributed data) or the Kruskal-Wallis test followed by the Mann-Whitney U test (for nonnormally distributed data) were used to evaluate statistical differences between various groups of data. $P < 0.05$ was used to indicate statistical significance. Version 25 of the Statistical Package for Social Sciences (SPSS) statistical software (SPSS Inc., IBM, USA) was used for all analyses.

Result

Serum biochemical assement

We assessed the serum levels of ALT and AST to determine liver function (Fig. 2). Following HFD meals, these enzymes rose and dramatically dropped in the HIIT group. Table 2 illustrates how an HFD diet for 12 weeks caused dyslipidemia, as evidenced by significantly higher serum levels of TC, TG, and LDL and lower levels of HDL in comparison to normal control rats ($P<0.05$). The HIIT program has effectively raised HDL levels while suppressing blood TC, TG, and LDL levels ($P<0.05$).

Table 02. Biochemical parameters before and after 12-week of intervention.

Variables	ND-SED	HFD-SED	HFD+HIIT
ALT (U/L)	101.6±13.8	229.84±9.25[a]	160.39±8.17[ab]
AST (U/L)	56.7±7.3	146.38±8.26[a]	98.11±11.33[ab]
TC (mg/dL)	106.15±18.31	237.98±17.74[a]	208.52±17.81[ab]
TG (mg/dL)	90.77±9.11	159.74±8.29[a]	120.39±10.17[ab]
LDL(mg/dL)	74.32±8.46	183.12±7.02[a]	139.87±7.94[ab]

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HDL (mg/dL)	62.32±8.29	34.95±6.74[a]	47.28±5.22[ab]

ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol. *Values are showed as mean ± SD. a: Significantly different compared to ND-SED. b: Significantly different compared to HFD-SED.*

Dnmts gene expression and activity

DNMT1, DNMT3A, and DNMT3B expression in the liver was investigated using quantitative real-time PCR analysis. The DNMT1 (A.1), DNMT3A (B.1), and DNMT3B (C.1) mRNA levels were significantly higher in the HFD-SED group of rats than in the ND-SED group, as Figure 01 illustrates. When comparing HFD+HIIT animals to HFD-SED rats, there was a substantial decrease in the expression of DNMT1. Additionally, the HFD+HIIT group's mRNA levels of DNMT3A and DNMT3B were considerably ($p < 0.05$) lower than those of the HFD-SED group. Figure 01 illustrates that the HFD-SED group exhibited significantly higher specific activity of DNMT1 (A.2), DNMT3A (B.2), and DNMT3B (C.2) in comparison to the ND-SED group ($p < 0.01$). HIIT treatment significantly reduced the activities of DNMT1, DNMT3A, and DNMT3B in NAFLD rats ($p<0.05$). Additionally, compared to HFD-SED rats, HFD+HIIT rats demonstrated a significant increase in all enzyme activity ($p<0.05$).

PPARγ and OSBPL3 genes expression

When comparing HFD-SED rats to the control group, it was found that there was a significant increase in the expression of the PPARγ and OSBPL3 genes ($P<0.001$). HIIT significantly decreased ($P<0.05$ and $P<0.01$, respectively) the HFD-mediated alteration in the expression of the PPARγ and OSBPL3 genes (Figure 02).

Fig 02. PPARγ and OSBPL3 gene expression in NAFLD following treatment with HIIT

Discussion

This study used a widely used rat model of HFD-induced NAFLD (17). our primary funding of financing was the discovery that rats fed a high-fat diet (HFD) experienced dyslipidemia and increased liver damage enzyme markers. Other studies that looked at how exercise affected NAFLD supported our findings.

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Following the HIIT intervention, the blood liver enzymes of NAFLD rats improved; TG, TC, and LDL-C in the Tr groups dropped, while HDL-C increased. These findings are comparable with the research findings of Linden et al., (18). By increasing heat consumption, accelerating lipid oxidation, and reversing lipid deposition, it suggests that regular and appropriate aerobic exercise interventions can inhibit the storage of redundant lipid substances in tissues and cells and improve the disorder of blood lipid metabolism. This provides a theoretical foundation for anti-NAFLD composite targets and joint intervention, as well as a guide for the use of aerobic exercise in conjunction with other polysaccharides in the prevention and treatment of NAFLD. As a result, exercise intervention offers special benefits and potential applications (19). Exercise raises basal metabolic rate, insulin sensitivity, and lipid oxidation; as a result, it's probable that by raising basal metabolic rate and boosting lipid oxidation, exercise can lower levels of ALT and AST (20).

The majority of complex illnesses, including diabetes and obesity, are caused by the interplay between genes and the environment. Heritable epigenetic changes offer an adaptable interface between the living thing and its surroundings (21). There may be many epigenetic changes that coexist with NAFLD. The results showed that, compared to the control group, DNMT gene expression and activity levels were elevated in HDF-induced NAFLD. Changes in the levels of DNMT1 and DNMT3A in the liver were linked to the development of hepatic steatosis in a previous investigation using a mouse model (22). Furthermore, it has been documented that human fibrotic livers exhibit elevated expression of three DNA methyltransferases, specifically DNMT1, DNMT3A, and DNMT3B (23).

In the current study, the DNMTs genes' expression and activity declined in the intervention group following six weeks of HIIT, in contrast to the HDF group. Sølvsten et al. discovered a decreased DNMT3b mRNA expression in the hippocampi of rats that were physically exercising, suggesting that exercise brings about a specific modulation of methylation in the hippocampus (24). Although the exact mechanism of expression of DNMT genes by HIIT is unclear, previous studies have shown that exercise affects the gene expression of DNMTs in different tissues. In support of this theory, Abel and Rissman (2013) discovered that a reduction in the expression pattern of DNMTs (DNMT1, DNMT3A, and DNMT3B) in rats was linked to a week of wheel running. After acute exercise, DNMT3B mRNA levels in skeletal muscle were found to drop by 50% in other

studies (25). However, following acute exercise, Hunter et al. observed an increase in DNMT3A and DNMT3B mRNA in leukocytes (26). On the other hand, as demonstrated by Maugeri et al., stress brought on by high glucose levels can enhance the expression of the DNMT gene (27), and it's likely that HIIT, which has the antiglycemic features previously indicated, also indirectly influences the decrease in DNMT gene expression(27).

Result of our study show level of PPARγ gene expression was significant higher in HFD group than control group. Some studies showed feeding of high fat and high fructose diet increased the mRNA and protein level of PPARγ in liver, which was in accordance with high fat diet fed mice (28). In an animal model of non-alcoholic fatty liver disease (NAFLD), Lee et al. discovered that PPRAγ is substantially expressed. They also demonstrated for the first time that PPARγ increased the synthesis of triglycerides through the expression of MGAT1(29). According to Pettinelli and Videla, obese NAFLD patients with either steatosis or steatohepatitis have hepatic PPAR-γ mRNA levels that are considerably higher than lean control values (30). Our findings in this study showed that exercise reduced the expression of the PPARγ gene in NAFLD rats. According to Zhang et al., combining a ketogenic diet with aerobic exercise decreased the expression of PPARγ in the liver as well as the genes that it targets, such as SREBP-1C, ACC1, SCD-1, and FAS. In addition to lowering fat buildup, blocking PPARγ gene expression in the liver of HFD-fed mice also decreased the expression of inflammatory genes, a sign of the advancement of NASH (31).

PPARγ is nonetheless essential for liver function, even though it is less prevalent in the liver than PPARα, and the PPARγ gene's DNA methylation status has been found to be a marker of the advancement of liver disease. Increased hepatic methylation of the promoter of the PPARγ coactivator one-alpha (PGC1-α) gene, a crucial transcriptional regulator of mitochondrial fatty acid oxidation, was found to be substantially linked with both fasting insulin levels and peripheral IR status in a case-control study of NAFLD patients. Therefore, taking into account: 1) the development of IR-dependent higher mobilization of nonesterified FAs from the adipose tissue to the liver; and 2) the up-regulation of lipoprotein lipase, FAT/CD36, and FATP5, affording enhanced uptake and intracellular binding/transport of nonesterified FAs, thereby leading to increased de novo FA biosynthesis, it is possible that the up-regulation of PPAR-γ in the livers of obese NAFLD patients may have prosteatotic effects (30, 32).

In the current study, exercise enhanced the substantially greater expression of gene OSBPL3 in group HDF compared to group control; however, the expression of gene OSBPL3 in the intervention group was significantly lower than in group HDF. According to an experimental investigation by Stein et al., OSBPL3 expression is elevated in NAFLD but low in healthy livers (33). According to some research, cancer cells have a higher amount of OSBPL3 than normal cells. Oxysterol-binding protein-like 3 (OSBPL3) is a member of the oxysterol-binding protein (OSBP) family, which includes both humans and mice (34). Intracellular lipid-binding/transport proteins, or OSBP family proteins, are essential for lipid transport and the preservation of the body's cholesterol homeostasis. According to reports, animals with SUMOylation-defective liver receptor homolog 1 (LRH-1) exhibit elevated expression levels of OSBPL3 in their livers. Moreover, OSBPL3 activates de novo lipogenesis, which increases the processing of sterol regulatory element-binding protein 1 (SREBP1) and encourages the storage of fat in the liver (33-36). These findings imply that OSBPL3 is essential for the development of hepatic fat. Furthermore, the accumulation of OSBPL3-related inflammation and fibrogenesis in NAFLD mouse models and people suggests that the LRH-1-OSBPL3 signal may initiate the pathophysiology of NASH (33-36).

Positive and substantial connections between PPARγ and OSBPL3 and DNMT gene expression were observed in this investigation. According to Hajri et al., PPARγ promoter DNA methylation is altered by both HFD and palmitic acid, which results in markedly elevated PPARγ expression and improved lipid retention in the liver, ultimately contributing to the development of NAFLD. Furthermore, it was discovered that there was a strong correlation between the degree of liver fibrosis and PPARγ methylation levels in both NAFLD patients and rat models (37, 38). Furthermore, Tian et al. demonstrated that OSBPL3 expression was elevated by hypermethylation in liver cancer (38). Our findings suggest that exercise causes hypomethylation of the PPARγ promoter and decreases the production of the PPARγ gene by lowering the expression of DNMTs. Conversely, there was a noteworthy direct correlation found between PPARγ and OSBPL3. Aibara et al. have demonstrated that PPARγ binds to the two functional PPARγ-responsive sites found in the 5′ upstream region, thereby favorably regulating Osbpl3 transcription (11).

Conclusion

By modifying a few serum liver function indicators, lipid profiles, and glycemic status, HIIT may be useful in the prevention of nonalcoholic fatty liver disease. Furthermore, by lowering the expression of PPARγ and OSBPL3 through DNMT activity and expression reduction, HIIT can prevent the development of NAFLD.

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