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Research Article

Moderate aerobic exercise and probiotic intake on FOXA1 gene expression in non-alcoholic fatty liver animal model: An Experimental Study

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Abstract

Background: Fatty liver disease is also known as fatty liver syndrome and is also called hepatic steatosis the aim of this study was to evaluate the effect of eight weeks of aerobic exercise and probiotic intake on FOXA1 gene expression in rats with fatty liver.

Materials and Methods: This study is an experimental laboratory study. Thirty-two male wistar rats were divided into 4 groups of 8 in the healthy, steatosis, steatosis + probiotic, steatosis + probiotic +aerobic exercise groups and tested for 8 weeks. The exercise protocol was as follows: in the first week from 10 minutes of running at a speed of 18 meters per minute to the eighth week with 60 minutes of running at a speed of 28 meters per minute. Consumption of probiotics; Relevant groups received 109 CFU / ml of Lactobacillus rhamnosus GG by gavage daily for 5 weeks and 5 days a week. RT-pcr method was used to evaluate the expression of FOXA1gene. ANOVA were used for data analysis using SPSS 23 software at a significant level ($p < 0.05$).

Results: The results showed that eight weeks of aerobic exercise with probiotic intake had a significant decrease on the expression of FOXA1 gene and LDL in rats with fatty liver ($P < 0.05$).

Conclusion: According to the results of the study, It seems that aerobic exercise with probiotics intake can improve the liver function of non-alcoholic fatty liver patients.

Keywords:
Aerobic exercise, FOXA1, non-alcoholic fatty liver, probiotics

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1. Introduction

Fatty liver occurs when hepatocytes begin to accumulate fat droplets (mainly triglycerides) which, in turn, lead to the storage of fat in the hepatocytes, leading to nonalcoholic fatty liver disease (1). Fatty liver disease is divided into non-alcoholic fatty liver and alcoholic fatty liver based on its causes. Fatty liver disease is also known as fatty liver syndrome and is also called hepatic steatosis. Non-alcoholic fatty liver disease is the most common chronic liver condition that is emerging in current societies (2). Characteristics of fatty liver include damage to liver cells (enlargement, apoptosis, necrosis or unplanned cell death, and mitochondrial enlargement), inflammation, and turquoise of liver cells. Non-alcoholic fatty liver disease pathogenesis is often based on a two-step process consisting of triglyceride accumulation followed by the development of oxidative stress and cytokines mediated by inflammation and fibrosis of the liver (3).

Foxa1 is one of three members of the FoxA family, a subset of the forkhead family of transcription factors which play vital roles in development (4). Initially Foxa1 was discovered for its role in liver development (5) but has also been implicated in the development of a number of other organs. human and rat livers were analyzed to determine Foxa1 regulation in NAFL. Results demonstrate that Foxa1 is a potent inhibitor of hepatic triglyceride synthesis, accumulation and secretion by repressing the expression of multiple target genes of these pathways (e.g., GPAM, DGAT2, MTP, APOB) (6).

Foxa1 is an antisteatotic factor that coordinately tunes several lipid metabolic pathways to block triglyceride accumulation in hepatocytes (6). However, Foxa1 is down-regulated in human and rat NAFL and, therefore, increasing Foxa1 levels could protect from steatosis. Also, FOXA1 has also been shown to modulate the growth of human lung cancer, brain cancer, and endometrial cancer cells. Genomic distribution analysis showed that FOXA factors and ERα or AR frequently bound to adjacent *cis*-regulatory elements in the genome and the recruitment of ERα or AR to their binding sites was dependent on FOXA factors in breast and prostate cancer cells (7,8)

Probiotics are defined as a live microbial dietary supplement and, if consumed in humans or animals, have beneficial effects on the health of the host by affecting the intestinal microbial fluoride balance (9). Probiotics are mainly located in the end of the small intestine and clone after entering the gastrointestinal tract. These bacteria have a direct impact on the function and life of other microorganisms in the intestine and mainly strengthen the beneficial bacteria in the intestine (10). According to previous research, which has studied the effect of aerobic and resistance training on fatty liver, and the role of FOXA1 in non-alcoholic fatty liver, which increases to prevent the accumulation of triglycerides, and the importance of exercise in this study, effect of moderate aerobic exercise training and probiotic use on FOXA1 gene expression in an animal model of fatty liver was investigated.

2. Materials and Methods

This experimental study is of laboratory type and has been done with ethics code IR.IAU.SRB.REC.1399.019. The present study was experimental. After transferring the animals to the laboratory in polycarbonate cages, for one week in an environment with a temperature of 22 ± 2 ° C, humidity of 55 % and light cycle to darkness 12:12 with proper ventilation. This study consisted of 32 male Wistar rats in 2 models, healthy and with fatty liver. Rats weighing 200-250 which were randomly divided into the following 4 groups: healthy group (N = 8), modeled group. (Steatosis) (N = 8), steatosis + probiotic group (N = 8), steatosis + probiotic + exercise group (N = 8). To create a model of fatty liver (steatosis), tetracycline at a dose of 100 mg / kg at a volume of 1.5 cc per mouse was gavage daily for two weeks. The weight of mice was 300 gr on average, 100 mg per kg was used for 3 mice, 100 mg was dissolved in 4.5 cc and 1.5 cc was gavage to each mouse.

cultivation of Lactobacillus ramensus GG bacteria

Lactobacillus ramensus GG (PTCC1637) is purchased as lyophilized in standard vials from the Scientific and Industrial Research Organization of Iran (Tehran, Iran). Bacteria are cultured in MRS medium (Biogeya, Tehran, Iran) enriched with L-cysteine HCL and incubated for 24 hours in an incubator at 37 ° C. To evaluate the effect of probiotics; The respective groups received 109 CFU / ml of Lactobacillus ramensus GG by gavage daily for 5 weeks and 5 days a week (11).

Exercise protocol

The aerobic exercise protocol was moderate intensity for 8 weeks and 5 sessions per week. The duration of the training ranged from 10 minutes of running in the first week to 60 minutes of running in the eighth week. The speed of running on a treadmill started from 18 meters per minute and in the eighth week it reached 28 meters per minute. The slope of the treadmill was also considered to be zero degrees (12).

After the last exercise session and consumption of probiotic and after 12 hours of overnight fasting, the studied rats in each group by intraperitoneal injection of a mixture of 10% ketamine at a dose of 50 mg / kg and xylazine 2% and with Doses of 10 mg / kg were anesthetized. By cutting the abdomen and chest, about 10 ml of blood was taken directly from the hearts of the mice by syringe. Blood samples were centrifuged at 1000 g g for 20 minutes to separate serum and stored at -80 ° C to measure serum LDL. Tissue samples were isolated under sterile conditions and stored at -80 ° C for FOXOA1 gene expression. To measure the FOXOA1 gene in adipose tissue, RT-PCR was performed using the protocol of the manufacturer (Qiagen, Germany). Table 1 shows the pattern of primers.

Table 1: Primers used

Gene	Primer Sequence (5′-3′)	Product Size (bp)	Accession Number
FOXA1	F: GACGTTCAAGCGCAGTTACC	187	NM_012742.1
	R: CAGLDLAGLDLGCGAALDLGAGT		
GAPDH	F: CAAGTTCAAGGGCACAGTCA	102	NM_017008.4
	R: CCCCATTLDLALDLTTAGCGGG		

Kolmogorov-Smirnov test was used to ensure normal distribution of data and Levin test was used to ensure homogeneity of variances. The relative expression of the Delta City gene was measured. Descriptive statistics were used to describe the data and draw graphs, and analysis of variance (ANOVA) was used to compare the groups in the studied variables. Significant level was considered P≤0.05. All statistical analysis was performed using 23 SPSS software.

3. Results

According to Table 2, The results of one-way analysis of variance test with a significance level of less than 0.05 showed that there was a significant difference between the FOXA1 gene variable in the four groups ($p < 0.05$). As a result, eight weeks of aerobic exercise with probiotic consumption has a significant increase effect on the expression of FOXA1 gene and significant decrease LDL in rats with fatty liver and reduces its ($p < 0.05$).

Table 2: Changes in FOXA1, LDL levels between different experimental groups

Variables	steatosis + probiotic + Aerobic exercise	steatosis + probiotic group	modeled (Steatosis)	Healthy control	Sig
FOXA1	**2.81±1.23**	**0.43±0.22**	**0.52±0.35**	**1.05±0.32**	**0.000**
LDL (mg/dL)	**36.5±4.52**	**42.01±2.66**	**70.45±5.20**	**32.83±3.14**	**0.000**

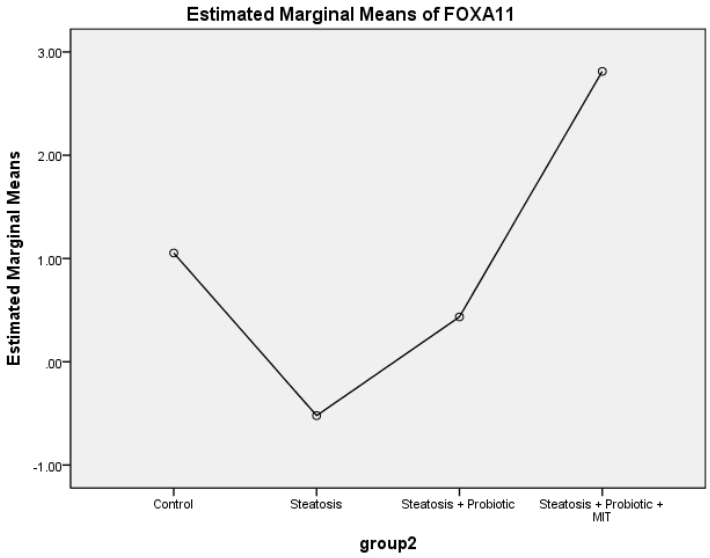

Figure 1: Comparison of FOXA1 expression in four groups of control (healthy), steatosis (patient), patient + probiotic, patient + probiotic + exercise

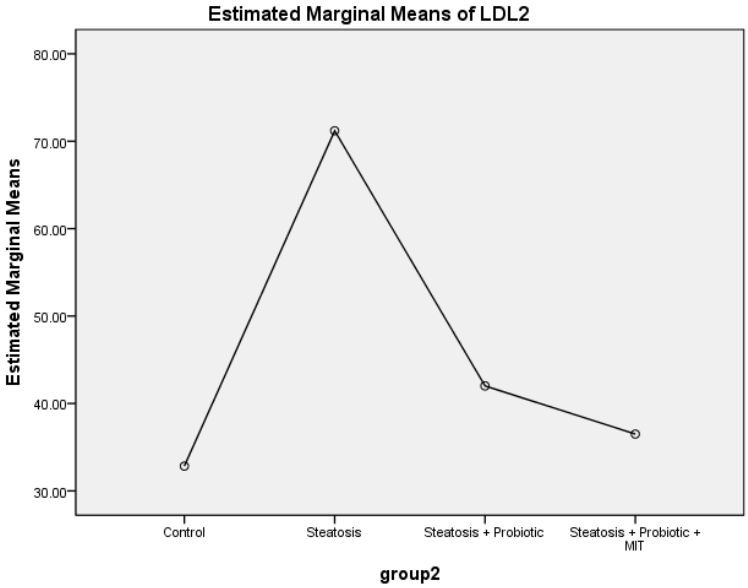

Figure 2: Comparison of LDL in four groups of control (healthy), steatosis (patient), patient + probiotic, patient + probiotic + exercise

4. Discussion

In the present study, the moderate aerobic exercise and probiotic intake on FOXA1 gene expression and LDL in non-alcoholic fatty liver animal model was investigated. The results of this study showed that FOXA1gene expression was significantly increased in the probiotic and aerobic exercise group compared to other groups. This study was consistent with research of the Sturgeon and colleagues, showed that aerobic exercise can improve fatty liver disease (13). Sturgeon and colleagues in their research with Physical activity induced protection against breast cancer risk associated with delayed parity title. data demonstrate patterns of delayed parity induced changes to the breast tissue which are both dependent and independent of exercise training completed between menarche and first pregnancy. While exercise training was beneficial for tumor latency and size, it did not mitigate enhanced collagen levels found in mammary tissue of delayed parity animals. Similarly, exercise training did not mitigate enhanced expression levels of several genes associated with breast cancer. However, there were exercise-dependent changes in the mammary gland. Exercise training prevented the development of inflammation and ductal hyperplasia. Exercise training also led to improved directional regulation of gene expression levels for Cdkn1c and Plau (13). Although this study was on breast cancer, however, some studies have shown that the foxa1 gene reduces triglyceride production in liver patients with a signaling pathway (6,14). In our study, the gene was severely reduced in mice with fatty liver. This is probably due to the increase in fat and improper regulation of this gene. However, with the use of probiotics and aerobic exercise along with probiotics, an increase in foxa1 gene was observed, which could be the effect of reducing the inflammatory pathway and the effect on foxa1 gene to prevent the formation of more fat in liver tissue.

The results also showed that the probiotic intake and aerobic exercise is able to significantly reduce the LDL in rats with fatty liver. It was reported Aerobic exercise, on average, HDL cholesterol increased by 4.6 % while triglyceride levels fell by 3.7 % and LDL cholesterol fell by 5 %. Total cholesterol remained unchanged, although the HDL: LDL cholesterol ratio improved considerably, suggesting that the increased intensity and structure normally associated with aerobic exercise has a more consistent impact upon triglycerides and LDL cholesterol than moderate levels of physical activity (15). Our study showed that the probiotic intake and aerobic exercise is able to significantly reduce the LDL in rats with fatty liver. The results of this study are not consistent with Shirpoor et al (16). They examined effect of moderate exercises and curcumin on hepatic transcriptional factors associated with lipid metabolism and steatosis in elderly male rat and concluded that a significant increase in FAT/CD36, PTP1B, significantly decreased HNF4α genes expression, increase in LDL and cholesterol in the aged group compared to the young control. Compared to those in the young control group, no significant changes in HDL and TG amounts in the aged control were observed. Moreover, compared to the young control, the aged group showed liver histological changes such as fibrosis and mild or grade 1 steatohepatitis. Moderate aerobic exercise and curcumin alone or in combination completely masked this effect (16). One of the reasons for the contradiction with the present study could be the use of the young group versus the old group in the study of Shirpoor et al Considering that only the old group is considered, LDL has decreased compared to it, or possibly using curcumin instead of probiotic.

This study was consistent with research of the Kazeminasab and colleagues, showed that There was a significant decrease in the concentrations of LDL-C after 4-week aerobic exercise (17). They consider Effects of A 4-Week Aerobic Exercise on Lipid Profile and Expression of LXRα in Rat Liver. Their experimental intervention study included twelve adult Wistar male rats (12-14 weeks old, 200-220 g) which were divided into the control (n=6) and training (n=6) groups. The training group received exercise on a motor-driven treadmill at 28 meters/minute (0% grade) for 60 minutes a day, 5 days a week for 4 weeks. Rats were sacrificed 24 hours after the last session of exercise. A portion of the liver was excised, immediately washed in ice-cold saline and frozen in liquid nitrogen for extraction of total RNA. Plasma was collected for high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) measurements. That result showed A significant increase in LXRα transcript level was observed in trained rats. Plasma HDL-C concentration was also significantly higher in trained rats. There was a significant decrease in the concentrations of LDL-C and TC, and the ratios of TC/HDL-C and LDL/HDL-C in trained rats. However, the TG concentration was unchanged. Which had the same result as this study (17).

Consistent with our research there is ample evidence in animal models and human studies that probiotics improve fatty liver. Patients with nonalcoholic fatty liver disease have been reported to improve liver enzymes after three months of treatment with Lactobacillus, Bulgaricus and Streptococcus thermophilus (18). In this regard, a study by Duseja et al. (2019) on the effect of High potency multistrain probiotic improves liver histology in non-alcoholic fatty liver disease (NAFLD): a randomised, double-blind, proof of concept study showed that lifestyle modifications combined with probiotic preparations significantly damaged liver tissue. Improves in patients with non-alcoholic fatty liver (19). In their study, patients in the probiotic group showed a improve in hepatic.

5. Conclusion

In the present study, it was found that the moderate aerobic exercise and probiotic intake on FOXA1 gene expression significantly increased and LDL significantly decreased in non-alcoholic fatty liver animal model. Therefore, according to the results of the study, It seems that aerobic exercise with probiotics intake can improve the liver function of non-alcoholic fatty liver patients.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles (IR.IAU.SRB.REC.1399.019).

Informed consent Informed consent was obtained from all participants.

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Author contributions

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

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