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Research Article

Effect of probiotic supplementation with aerobic training on hepatic IL-6 gene expression in non-alcoholic fatty liver rats

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Abstract

Background: non-alcoholic fatty liver disease NAFLD is the most common liver disease in the world, the most important cause of non-alcoholic fatty liver disease is poor eating habits and sedentary lifestyles that cause obesity. The aim of this study was the effect of aerobic training and probiotic consumption on gene expression of IL6 liver tissue in rats with non-alcoholic fatty liver.

Materials and Methods: In this experimental study, 32 male Wistar rats, 200-250 gr, were randomly divided into 4 groups: healthy group, modeled group (steatosis), steatosis + probiotic group, steatosis + probiotic + training group. To create a fatty liver model, tetracycline at a dose of 100 mg / kg at a volume of 1.5 cc per rat was gavaged daily for two weeks. The main training program was for eight weeks/3times per week. In the first week with a speed of 18 meters per minute, the time started 10 minutes and every week quickly, 1-2 meters per minute and time was added to 10 minutes so that in the fourth week the speed to 22 meters per minute and time to 40 minute has arrived. Probiotic groups received 109 CFU / ml of Lactobacillus ramensus by gavage daily for 8 weeks and 5 days a week. 48 hours after the last training session, 10 ml of blood was taken directly from the hearts of rat to test for total cholesterol (TC) and liver tissue samples were taken to test for IL6 gene expression. One-way analysis of variance and Tukey post hoc test was used P≤0.05.

Results: There is a significant difference between the IL-6 gene variable in the groups (P=0/000), Induction of fatty liver in rats was associated with a significant increase in interleukin-6 gene levels, these changes were significantly reduced after taking probiotics (P=0/000), Also probiotic consumption with aerobic training had a significant reduction compared to other groups. However taking probiotic with aerobic training had no significant effect on TC of rats with non-alcoholic fatty liver (P=0/306).

Conclusion: Probably, performing aerobic training along with taking probiotic can have a better effect in controlling non-alcoholic fatty liver disease.

Keywords:
Aerobic training, Probiotic, IL 6, Non-alcoholic fatty liver

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1. Introduction

The liver is one of the important organs of the body. The presence of fat more than 5 to 10% of the total weight of this organ causes fatty liver disease (steatosis) and if not diagnosed on time and proper treatment can lead to an advanced and irreversible liver disease called become "cirrhotic" (1). There are two types of nonalcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD), which have almost the same symptoms. The cause of NAFLD is the excessive accumulation of fat caused by an imbalance between diet and physical activity (2). In the pathogenesis of this disease Factors such as failure in energy metabolism, obesity, metabolic syndrome, insulin resistance and dyslipidemia play a role (1). Cytokines are proteins that produce immune responses (3). Adipose tissue is known as the producer of a number of these active proteins, such as: IL-8, IL-6, and TNF-α, Hs-CRP. Interleukin 6 is a cytokine that has a pro-inflammatory effect (1, 3).

The accumulation of fat in the liver cell causes the production of inflammatory mediators, including interleukin 6. Previous researches have shown the existence of this inflammatory state in patients with fatty liver (4).

Skeletal muscle contraction causes the release of IL-6 and it increases up to 100 times in plasma, which is proportional to the duration, intensity of exercise and muscle mass involved (5). Regular exercise with a lower level of systemic IL-6 in The state of rest is accompanied Reducing the fat mass, increasing the secretion of anti-inflammatory cytokines, decreasing the gene expression of monocytes and macrophages are some of the mechanisms through which the anti-inflammatory effects of exercise are applied (6). While the increase in fat mass increases the production of leptin, IL-6, IL-18, TNFα and other pro-inflammatory factors and decreases the secretion of anti-inflammatory factors (7). Making positive changes in lifestyle and reducing factors related to fatty liver disease such as high blood pressure, diabetes and high cholesterol can help to treat this disease by balancing the diet and using fruits and vegetables. (8)

Probiotics are living organisms that naturally exist in fermented materials (yogurt, milk, cheese). These bacteria greatly contribute to the health of the intestine and control the growth of dangerous bacteria and cause the growth of beneficial bacteria in the digestive tract. Also, beneficial effects such as reducing intestinal inflammation due to surgery, increasing the ability to digest food, improving lactose sensitivity and they help in better absorption of vitamin B (9). Lactobacillus is the most famous type of probiotic bacteria, which is found in dairy products (especially yogurt and fermented foods). This type of bacteria can be useful in cases of gastrointestinal disorders such as diarrhea or indigestion caused by lactose consumption (9).

By affecting the composition of intestinal microbial flora and improving its function, probiotics prevent the transfer of bacterial endotoxins to the bloodstream and reduce inflammation by reducing lipopolysaccharides and pro-inflammatory cytokines in the bloodstream (10). Probiotics exert these positive effects without creating a harmful inflammatory response (9). These bacteria in the digestive system reduce digestive disorders and blood cholesterol, and improve the body's immune system and increase the absorption of minerals in the system they are digested (9). Tang et al. (2019) showed that probiotics can reduce weight and body mass, improve liver

function and reduce lipid levels, plasma glucose, and liver fibrosis (11). Considering the prevalence of fatty liver disease among people in the community and the role of immune and inflammatory indicators in the formation process of non-alcoholic fatty liver disease and the effects of training and probiotic consumption in preventing or slowing down the progression of this disease and the lack of research that simultaneously investigated the effect of probiotic consumption and endurance training on steatosis disease, the present study aims to investigate the effect of probiotic supplementation along with aerobic training on IL6 gene expression in the liver tissue of rats with non-alcoholic fatty liver disease.

2. Materials and Methods

The current research is an experimental type. The present research was carried out with the approval of the ethics committee of Islamic Azad University-Research Sciences Unit with code IR.IAU.SRB.REC.1399.019. The statistical population of the samples of the present study included 32 male rats weighing 200-250 grams in 2 healthy and non-alcoholic fatty liver Wistar Dawley models (obtained from Pasteur Research Institute). The rats were randomly divided into the following four groups: healthy group (N=8), modeled (steatosis) group (N=8), steatosis + probiotic group (N=8), steatosis + probiotic+ training group (N = 8).The rats were kept in transparent polycarbonate cages made by Razi Rad Company and in an environment with a temperature of 22 ± 2 degrees Celsius, humidity of 55 ± 5% and a light-dark cycle of 12:12 with standard food and water.

Creation of fatty liver model

Tetracycline was gavage with a dose of 100 mg/kg in a volume of 1.5 cc per mouse daily for two weeks. Lactobacillus rhamnosus GG (PTCC1637) was purchased in lyophilized form in standard vials from the Scientific and Industrial Research Organization of Iran (Tehran, Iran).

Bacteria were cultured in MRS culture medium (Zisti Goya, Tehran, Iran) enriched with L-cysteine HCL and incubated for 24 hours in a 37°C incubator.

Supplement protocol

The supplement groups received daily 109 CFU/ml of Lactobacillus rhamnus GG by gavage for 8 weeks and 5 days a week (12).

Training protocol

In order to get familiar with the activity on the treadmill, the rats in the training and training +supplement groups were trained for five minutes at a speed of 8-10 m/min with zero incline in one week during five sessions. The main training program was for eight weeks, in the first week with a speed of 18 meters per minute, the time was 10 minutes, and every week the speed was increased by 1-2 meters per minute and the time was increased by 10 minutes, so that in the fourth week, the speed It reached 22 meters per minute and the time reached 40 minutes. Five minutes before and after training were considered for warming and cooling the animals (13).

48 hours after the last training session and after 12 hours of overnight fasting, the rats were anesthetized with ether by cutting the abdomen and chest area, about 10 ml of blood was taken directly from the rat's heart by a syringe and transferred to the test tube. After mixing the blood samples in the test tube with saturated EDTA solution, the blood samples were centrifuged for ten minutes at a speed of 3000 rpm to separate the plasma, and the resulting

plasma was frozen at -80 degrees Celsius and for total cholesterol measurement saved.

Liver tissue samples were taken to check IL6 gene expression. Tissues were examined by Real Time PCR technique. First, primer design was done, and then total RNA was extracted from the tissues and converted into cDNA. Then the cDNA was amplified by PCR and analyzed for the expression of the mentioned genes.

Comparative ΔΔCT method was used to quantify IL6 expression (Table 1).

Data analysis

The Kolmogorov-Smirnov test was used for the normal distribution of the data and the Leven test was used to determine the homogeneity of variances. To investigate the significant changes of each of the research variables, between different groups, one-way analysis of variance and Tukey's post hoc test were used in SPSS version 20 software P≤0.05.

Table 1. Primer sequences used for real-time PCR amplification.

Gene	Primer Sequence (5′-3′)	Product Size (bp)	Accession Number
IL-6	F: CCACTGCCTTCCCTACTTCA	191	NM_012589.2
	R: TTCTGACAGTGCATCATCGC		
GAPDH	F:CAAGTTCAAGGGCACAGTCA	102	NM_017008.4
	R: CCCCATTTGATGTTAGCGGG		

3. Results

Fatty liver induction in rats was associated with a significant increase in interleukin-6 levels (P=0.000). Based on the obtained results, the interleukin-6 gene levels in the probiotic consumption and aerobic training groups were significantly decreased compared to the patient control group (P=0.000). The decrease in interleukin-6 gene levels was higher in the subjects of the probiotic + training group than in the subjects of the probiotic group (Chart 1).

Fatty liver induction in rats also caused a significant increase in TC levels, but there was no significant difference in this variable between the four groups after the implementation of the research protocol (P=0.306).

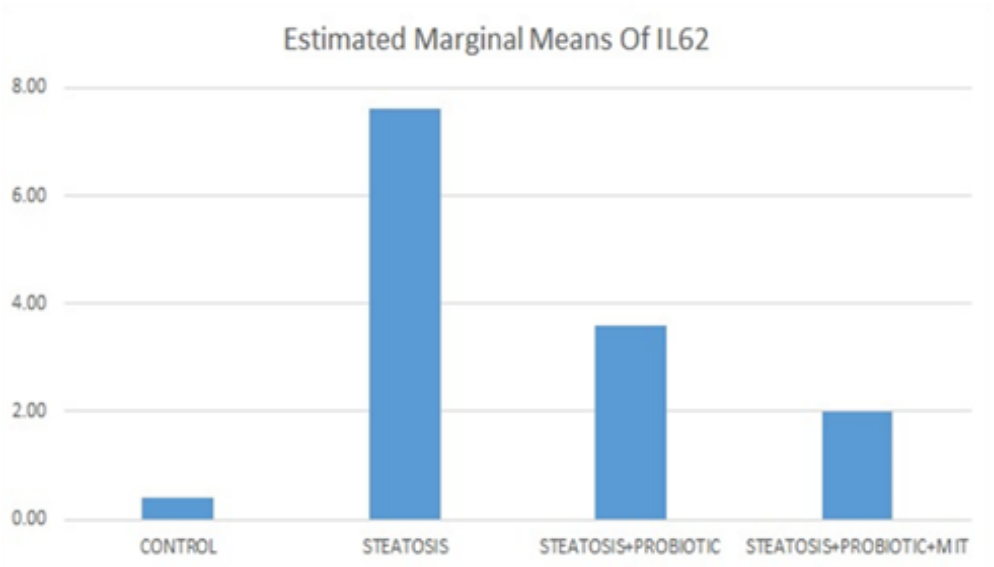

Chart 1. Comparison of IL-6 gene expression average in four groups: control (healthy), steatosis (patient), patient + probiotic

4. Discussion

Based on the results of the research, the induction of fatty liver in rats was associated with a significant increase in interleukin-6 gene levels, which decreased significantly after taking probiotics and aerobic training. The amount of reduction was higher in the subjects of the probiotic + training group. Results have shown that probiotics improve fatty liver, so that taking probiotic supplements for 6 months in patients with non-alcoholic fatty liver led to a significant decrease in IL-6 levels (12). Hodgman et al.'s study (2019) showed that lifestyle modification along with probiotic consumption significantly improves liver tissue damage in patients with non-alcoholic fatty liver disease. In their study, patients in the probiotic group showed a significant decrease in IL-6 levels as a marker of pro-inflammatory mediators (13). Lee et al. (2020) also showed that consumption of probiotics inhibits the production of nitric oxide, TNF-α, IL-6 and IL-10 (14).

Similar studies also showed that probiotic consumption along with aerobic training are effective on IL-6 gene expression and cause its reduction (15, 16, 17). The increase in fat tissue in the body causes the inflammatory process so that inflammatory factors such as IL-6 increase in the body (18). One of the possible mechanisms for the reduction of IL-6 levels following aerobic training is the reduction of fat following these training, adipose tissue is one of the main sources of IL-6 production (19). Researchers have reported that people who are more physically active and have better physical fitness have lower inflammatory index values (19, 20). The results of their research showed that regular physical activity reduces IL-6 in people. Considering that fat cells are the first cells in which the inflammatory response starts and secrete IL-6, aerobic training reduce the amount of IL-6 by reducing the amount of fat (17). On the one hand, training is effective in reducing the concentration of inflammatory indicators by reducing weight and adjusting fat tissue reserves as a source of

synthesis and secretion of IL-6 and CRP cytokines, and on the other hand, by reducing sympathetic stimulation and reducing the release of pro-inflammatory cytokines (21). Regarding the possible mechanism of the effect of probiotics on the reduction of IL-6 levels, it seems that probiotic bacteria together with other beneficial food components by fermenting dietary fiber and producing short chain fatty acids such as acetic acid, change metabolic pathways and reduce cholesterol synthesis are endogenous. Also, by inhibiting hydroxyl and superoxide radicals and reducing the expression of IL-6 in adipocytes, they can prevent the increase of hs-CRP and reduce inflammation (16, 17). The results of the present study are not consistent with the results of some previous studies. In the study Gilolo et al. (2018) showed that no change was observed in IL-6 gene expression after using isoflavone supplement along with exercise (22). Barzegar et al. (2016) also showed that plasma levels of IL-6 and IL-10 after 8 weeks training were not significantly different in any of the training groups compared to the control group (23). The reason for the discrepancy between the results of these studies and the current research can be attributed to different training methods (combined use of aerobic and resistance exercises or the use of weight loss programs), the use of different supplements, the use of healthy subjects, and the non-similarity of the subjects. Fatty liver induction in rats caused a significant increase in TC levels, but probiotic consumption and aerobic training had no significant effect on TC. Loman et al.'s research (2018) showed that prebiotics and probiotics have no effect on total cholesterol, HDL-c and LDL-c (24). It seems that the sphingolipids present in the cell membrane of probiotics are effective on the metabolism and transfer of total cholesterol in the body and can increase or decrease the cholesterol level (25).

The lack of change in the total cholesterol concentration in this study could be due to the difference in the response of the samples, the insufficient amount of probiotic consumed, or the longer duration of consumption to reveal the effect of the probiotic. For this reason, it is recommended to conduct more extensive research with a longer period of time and a higher dose of probiotic bacteria.

Different mechanisms have been expressed in reducing cholesterol by probiotics. Deconjugation of bile acids by hydrolase enzyme and stopping the intestinal-hepatic cycle of bile acids can be one of the possible mechanisms. When bile acids are unconjugated, their solubility and absorption in the intestine decreases and they are excreted through feces. As a result, the body uses cholesterol again to make new bile acids, and this can lower the concentration of serum cholesterol (24, 25).Another mechanism is the ability to bind cholesterol to the cell wall of probiotics and combine cholesterol with the cell membrane of bacteria and prevent absorption of dietary cholesterol. Living and growing bacteria have more ability to remove and expel cholesterol (23).

Wang et al.'s research (2018) showed that probiotic consumption can significantly reduce TC and TG in type 2 diabetes patients (26). It seems that the short chain fatty acids produced by probiotics during fermentation in the digestive system play a role in reducing cholesterol. Butyrate prevents the synthesis of cholesterol in the liver and propionate is also probably effective in reducing the rate of cholesterol synthesis in the liver (27). Wu et al. (2017) found that Lactobacillus probiotic consumption significantly reduced TC (28). Porerjab et al. (2020) showed that probiotic yogurt can significantly reduce total cholesterol

and LDL-c in subjects with mild to moderate hypercholesterolemia without having a significant effect on HDL-c and triglyceride levels (27). . Sivamaruti et al. (2019) showed that probiotic consumption significantly improves the health status of patients with hypercholesterolemia by reducing total cholesterol, low-density lipoprotein, triglyceride and increasing high-density lipoprotein (29). These results are contrary to the results of the present study, which can be the result of the difference in the type and dose of probiotic bacteria consumed, the duration of the intervention, the sample volume, and the clinical characteristics of the studied samples.

On the other hand, Stein Jensen et al. (2020) aimed to investigate the effect of exercise on Alzheimer's patients and showed that after 16 weeks of exercise, there was no change in the level of total cholesterol, TG or LDL-C (30). Zhang et al. (2018), in the study of the effect of aerobic exercises on the serum levels of free fatty acids, IL-6, and total cholesterol, found that the amount of these indicators increased significantly in the high-fat diet group compared to the control group. However, in the exercise group compared to the fatty liver group, it decreased significantly (31). The reason for the non-alignment of the results with the results of the present research is probably due to the different duration and intensity of the exercises, different samples of subjects and the variable measuring method.

Conclusion

According to the results of the current research, the use of probiotics within the range of healthy amounts, along with training, can have a positive effect on obtaining better results and adjusting the complications of fatty liver disease. However, it is recommended to conduct more research on human samples so that along with prescribing aerobic activity to patients with non-alcoholic fatty liver disease, probiotic consumption is also recommended.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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