

Research Article

The effect of aerobic training and probiotic intake on gene ICAM-1 expression in rats with nonalcoholic fatty liver

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Abstract

Background: Cell adhesion molecules mediate leukocyte responses to inflammation. The purpose of study was to the effect of aerobic training and probiotic intake on ICAM-1 in rats with fatty liver.

Materials and Methods: In this experimental study, 32 rats (200-250 gr) were randomly divided into four groups (healthy control, steatosis, steatosis + probiotic, steatosis + probiotic + training). To create a fatty liver model, tetracycline at a dose of 100 mg / kg in a volume of 1.5 cc was gavaged daily for two weeks. The training program includes 8 weeks and 5 sessions per week, in the initial week with a speed of 18 meters per minute, time started 10 minutes and every week quickly, 1-2 meters per minute and time was added to 10 minutes. Supplemental groups received 10⁹ CFU / ml of *Lactobacillus ramensus* by gavage daily for 8 weeks and 5 days per week. Liver tissue samples were taken to examine the expression of ICAM-1 gene. 10 ml of blood was collected from the hearts of rats for ALP testing. The data was analyzed using one-way analysis of variance (ANOVA) $p \leq 0.05$.

Results: The results showed that the aerobic training along with probiotic consumption significantly reduced the expression gene of ICAM-1 and ALP in rats with non-alcoholic fatty liver.

Conclusion: It seems aerobic training combined with probiotic consumption improved the expression gene of ICAM-1 and ALP enzyme in the studied samples which can be effective in treating patients with fatty liver disease.

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1. Introduction

Non-alcoholic fatty liver disease is the most common liver dysfunction associated with increased accumulation of visceral fat, blood lipids, hypertension, and diabetes(1). This disease is caused by the deposition and accumulation of large fat particles in the cytoplasm of liver cells at a rate of 5% or more of liver weight and includes a wide range of symptoms from simple steatosis to steatosis with inflammation or without fibrosis and cirrhosis, the incidence of which has increased significantly in recent years as a result of obesity due to sedentary lifestyle and poor eating habits (2, 3). Common enzymes measured in liver disease include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Liver disease is the most important factor in increasing serum transaminases. ALP activity is found in most organs of the body and is particularly associated with cell surface membranes in the small intestinal mucosa and renal tubules, bone, and liver. It also increases slightly in most jaundice disorders caused by liver damage (4). Studies have shown that in NAFLD, impaired lipoprotein metabolism and accumulation of oxidized LDL increase the risk of endothelial cell damage, including adhesion molecules, and cause endothelial dysfunction. One of these molecules is the Intercellular Adhesion Molecule (ICAM-1). ICAM-1 glycoprotein is a cell surface expressed in endothelial, epithelial, fibroblast, and hepatocyte cells (5). A positive correlation has been observed between serum ICAM and the degree of hepatic fibrosis in NAFLD patients. Evidence suggests that ICAM expression is high in patients with NAFLD; several mechanisms, such as increasing oxidative stress, increase ICAM expression in NAFLD patients (6).

ICAM expression in damaged hepatic stellate cells increases the entry of inflammatory molecules into them (7). Studies have shown that aerobic training by improving glucose control, lipid oxidation by increasing glucose transport in striated muscle, glycogen synthase expression and activity in insulin receptors, glycogen storage in muscle and liver, and increased triglyceride synthesis in muscle cells. reducing the accumulation of fatty acid metabolites and suppressing the inflammatory state associated with insulin resistance violate this cycle (8). Researchers have suggested a combination of proper diet and physical activity to prevent and treat this disease (9). In recent years, probiotics have been discussed as a possible alternative to treating various diseases such as NAFLD (10). The World Health Organization describes probiotics as living microorganisms that, when consumed in sufficient quantities, have beneficial effects on the host body. The reported preventive and therapeutic effects of these microorganisms include balance in the intestinal microbial flora; lowering blood cholesterol levels; improve blood pressure; Diabetes, gastrointestinal diseases; Improve the immune system and reduce the risk of various types of cancer (11, 12). Nabavi et al. (2016) reported that consumption of probiotic yogurt improved levels of liver enzymes, total cholesterol and low-density lipoprotein in the studied samples (13). Rafrat et al. (2015) reported that consumption of probiotic yogurt reduced serum ALT and AST concentrations in patients with NAFLD and may be beneficial in improving fatty liver disease (14).

Due to recent research and some contradictory results on the effect of aerobic training and probiotic consumption on ICAM-1 gene expression in fatty liver patients, also no research on the synergistic effect of training and probiotics on ICAM-1 gene expression has been studied in patients with fatty liver; the need for further studies was felt. Therefore, the researcher intended to investigate the effect of probiotic use with aerobic training on ICAM-1 in male rats with NAFLD.

2. Materials and Methods

Subjects

This experimental study was conducted as a post-test design with a control group in the laboratory of the Faculty of Humanities of Sari Azad University in 2020. In this study, 32 male Wistar rats aged 10 weeks (weight range 200-250 g) were selected from the Animal Breeding Center of Sari Azad University and transferred to the laboratory. After familiarizing and adapting to the new environment, rats were randomly divided into four groups of 8 healthy, modeled (steatosis), steatosis + probiotic, steatosis + probiotic + training. All rats in standard conditions (average temperature 22 ± 2 ° C and air humidity $55 \pm 5\%$ and light cycle to dark 12:12) with free access to water and food for laboratory animals in transparent polycarbonate cages in number 8. Some were kept. To produce fatty liver, tetracycline at a dose of 100 mg / kg at a volume of 1.5 cc per rat was gavaged daily for two weeks.

Training protocol

Table 1 shows the aerobic training protocol of rats with non-alcoholic fatty liver. Before starting the training and in order to get acquainted with how to work on the treadmill, the rats in the training group trained for five minutes at a speed of 8-10 m / min with zero slope for five sessions in one week. The main training program was done in such a way that 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 22 meters per minute and time reached 40 minutes. It was also intended to warm and cool the animals five minutes before and after training (15).

Table 1: Aerobic training protocol for rats with non-alcoholic fatty liver

	First	second	Third	Fourth	Fifth	sixth	Seventh	Eighth
Activity time (weeks)								
Running time (minutes)	10	20	30	40	50	50	60	60
Running speed (meters per minute)	18	20	22	22	24	26	26	28

The inclination of the device in the exercise was zero degrees and the speed was set in meters per minute (measure of the intensity of the exercise). All stages of the above research were approved by the ethics committee of the Islamic Azad University, Sari branch, with license number 139427.

Lactobacillus ramensus GG (PTCC1637) was purchased as lyophilized in standard vials from the Scientific and Industrial Research Organization of Iran (Tehran, Iran). The bacteria were cultured in MRS medium (Biogooya, Tehran, Iran) enriched with L-cysteine HCL and incubated for 24 hours in an incubator at 37 ° C. Supplementary groups received 10⁹ CFU / ml of *Lactobacillus ramensus* GG by gavage daily for 8 weeks and 5 days per week (16). Animal sampling was performed 48 hours after the last training session (to eliminate the acute effect of training). For this purpose, the animals were first anesthetized by peritoneal injection of ketamine (50-30 mg / kg) and xylazine (3-5 mg / kg) and then killed. For molecular studies on the level of gene expression, tissue RNA extraction was performed in all study groups according to the protocol of the manufacturer (Kiagen, Germany). First, 200-300 Landa chiazoles were added to the Ovums and stored at -80 ° C for 24 hours. After 24 hours, the plaque in the cryotube was crushed by semi-freezing with a sampler, then slightly pipetted. About 100 Landa chloroforms were then added to the sample to lysis the cells. After 1 minute, the solution was centrifuged at 12,000 rpm for 10 minutes.

After centrifugation, the solution was divided into three phases: the upper part of the tube, which was clear and contained RNA, the middle part of the tube, which was white and contained lysed tissue, and the lower part of the tube, which was pink and contained chiazol. The clear liquid at the top of the tube containing the RNA was gently removed and placed in a DEPC microtube. Then 1 cc of isopropranol was poured on clear RNA and stirred by hand for 1 minute. Isopropranol is clear and RNA is clear, but when the two are mixed together they form a turbid liquid. It is better to put the obtained solution at -80 degrees overnight. After adding isopropranol, the samples were centrifuged at 12,000 rpm for 10 minutes. After removing from the centrifuge, the supernatant was drained and 1 cc of 70 alcohol was added. After vortexing, the mixture was centrifuged at 7500 rpm for 10 minutes. The supernatant was then drained with a sampler and then the plaque was dried inside a microtube. After extracting RNA with high purity and concentration from all samples, cDNA synthesis was performed according to the protocol of the manufacturer (Fermentas, USA) and then the synthesized cDNA was used for reverse transcription reaction.

First, all designed primers related to all genes were examined and then gene expression was evaluated using quantitative q-RT PCR.

The RT-qPCR technique was used to quantitatively confirm the expression of the studied genes. For this purpose, using chiazol solution, the RNA of all cells was extracted according to the synagen protocol and exposed to DNase I Fermentas to ensure contamination with genomic DNA. Then the quality of the extracted RNAs was evaluated by spectrophotometry (Kiagen DPI-1). To prepare a single-stranded cDNA from primer (Oligo dt MWG-Biotech, Germany) and reverse transcription enzyme (Fermentas) was performed according to the relevant protocol. To design gene-specific primers, the gene sequence encoding ICAM-1 (as the target gene) and the GAPDH sequence (as the reference gene) were first extracted from the National Center for Biotechnology Information (NCBI) database. Then, primer pairs were designed using Fast PCR software (Table 2).

Serum ALP enzyme activity was measured using Pars Azmoun kits with a sensitivity of less than 3 international units per liter and a coefficient of variation of 1.4% with the help of RA-1000 autoanalyzer manufactured by Techni Com Company by enzymatic metering method. The required collected information was analyzed by SPSS software version 24 at a significant level ($P \geq 0.05$). Kolmogorov-Smirnov test was used to determine the normality of data distribution and Leven test was used to check the homogeneity of variances. To determine the significance of the differences between the variables between the research groups, one-way analysis of variance and Ben Foroni post hoc test were used.

Table 2: Design of specific gene primers

Gene	Primer Sequence (5'-3')	Product Size (bp)	Accession Number
ICAM1	F: CGCCAGAGGAAGATCAGGAT	162	NM_012967.1
	R: AGGTGGGTGAGGGGTAAATG		
GAPDH	F: CAAGTTCAAGGGCACAGTCA	102	NM_017008.4
	R: CCCCATTTGATGTTAGCGGG		

3. Results

Figure 1 shows Comparison of the average ICAM-1 (Pico g / ml) of the four groups. Figure 2 shows Comparison of the average ALP (International Unit per Liter) of the four groups .The results of one-way analysis of variance showed that there was a significant difference between the expression variables of ICAM-1 gene in the four study groups (P = 0.001), which was the difference between the control group with the patient group and the patient group with the training + probiotic group.

The results showed that there was a significant difference between the ALP variable in the four groups (P = 0.000); There was a difference between the control group with all groups and the patient group with the probiotic group and the training + probiotic group.

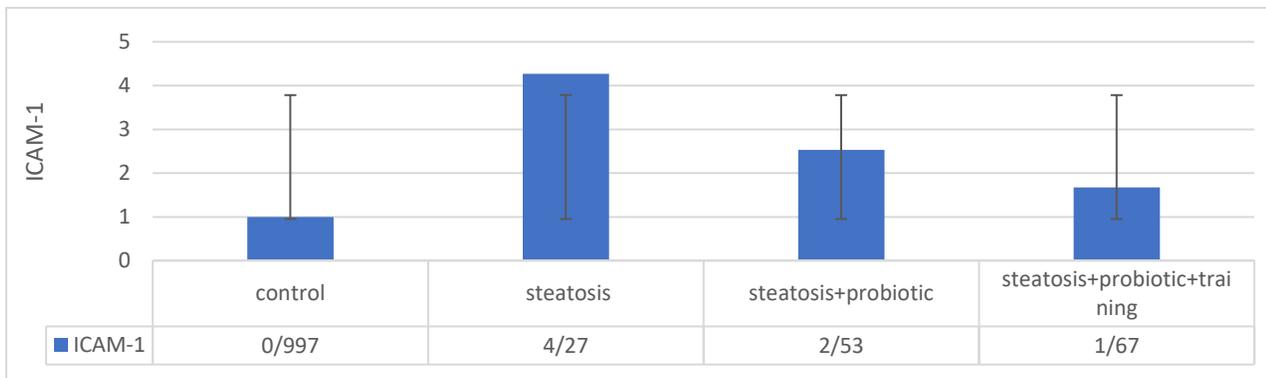


Figure 1. Comparison of the average ICAM-1 (Pico g / ml) of the four groups

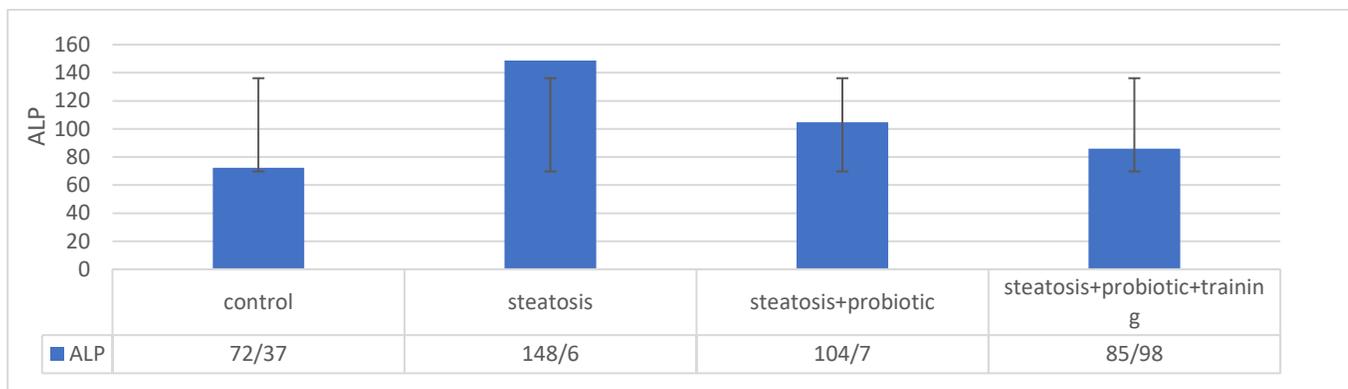


Figure 2. Comparison of the average ALP (International Unit per Liter) of the four groups

4. Discussion

The results showed that eight weeks of aerobic training with probiotics significantly reduced ICAM-1 gene expression in rats with non-alcoholic fatty liver disease compared to the modeled group (steatosis). The results of research by Rezazadeh et al. (2020) showed that probiotic consumption significantly reduced ICAM-1 in patients with metabolic syndrome (17). Sudhakaran et al. (2013) in a study reported a significant decrease in ICAM-1 in rats due to probiotic use (18). Nabi et al. (2016) also reported in a study that probiotic consumption significantly reduced ICAM-1 in atherosclerotic rabbits (19). Riyahi et al. (2017) in their study showed that eight weeks of swimming training and garlic consumption significantly reduced ICAM-1 in obese mice (20). Hosseini et al. (2017) also reported a significant decrease in ICAM-1 in obese postmenopausal rats due to eight weeks of intense intermittent training with curcumin (21), which is consistent with the results of the present study. Probiotic bacteria, along with other beneficial nutrients, appear to alter metabolic pathways and reduce endogenous cholesterol synthesis by fermenting dietary fiber and producing short-chain fatty acids such as acetic acid. Because inflammatory markers increase in heart disease, by controlling the level of inflammation as a risk factor for cardiovascular disease, the complications of the disease can be better controlled (22). Recent studies have shown that probiotics, including lactobacilli, are able to reduce inflammatory responses in animal models, possibly as a result of the effect of probiotics in improving lipid profile levels (23). Mishra et al. (2015) showed that probiotics have antioxidant power (24). These antioxidant effects are effective in preventing or reducing endothelial damage and inflammation by reducing LDL oxidation (25).

Regular training seems to inhibit the release of inflammatory mediators from adipose tissue by decreasing sympathetic stimulation and increasing anti-inflammatory cytokines, followed by a decrease in ICAM-1 concentration (26). Mechanisms of ICAM-1 reduction after training can depend on the amount of training, intensity, duration and frequency of training sessions. Decreased inflammatory index of ICAM-1 may be due to the antioxidant effects of aerobic training. Because free radicals increase ICAM-1 inflammatory mediator expression, Aerobic training by reducing anti-oxidative defenses can lead to a decrease in inflammatory markers (27).

Several studies confirm the effectiveness of IL-6 in increasing ICAM-1. Studies have shown that training, especially aerobic training, as well as probiotic supplementation have an effective role in reducing tumor necrosis factor alpha and interleukin-6; Therefore, it seems that reducing the levels of these inflammatory cytokines due to training is also effective in reducing ICAM-1. Reduction of proinflammatory factors also reduces the release of chemical mediators such as NF-KB, which can be effective in modulating inflammation. NF-KB is inactive in the cytoplasm and mediates ICAM-1 translation (26). Brevetti et al. (2001) reported an increase in ICAM-1 after maximum intensity training, which is not consistent with the results of a recent study (28).

Differences in the results of this study may be due to differences in the type of subjects, training protocol and the length of the research period. The Brevetti specimens had intermittent tremor and their training protocol was acute. Another result of the present study was a significant decrease in ALP due to eight weeks of aerobic training with the use of probiotics in rats with non-alcoholic fatty liver compared to the modeled group (steatosis). Consistent with the present results, a study conducted by Dubey et al. (2015) on mice showed that probiotic consumption reduced blood ALP levels (29).

Rostamizadeh et al (2017) reported in a study that probiotic consumption significantly reduced ALP in patients with non-alcoholic fatty liver disease compared to the modeled group (steatosis) (30). Famouri et al (2017) in a study of obese adolescents with non-alcoholic fatty liver showed that the levels of liver enzymes and cholesterol in the group that took probiotic capsules (*Bacillus acidophilus*) were much lower than in the control group (31). Probiotics improve liver function by reducing the activity of the bacterial urease enzyme in intestinal absorption of ammonia and toxins and reducing oxidative stress due to ammonia absorption, and possibly lower liver ALP enzyme levels because probiotics balance intestinal. Increasing the digestibility of nutrients and increasing the immune response reduces intestinal toxins and therefore decreases the serum level of this enzyme by increasing its functions (32).

Probiotics can also be used to treat fatty liver by lowering circulating pro-inflammatory cytokines and lipopolysaccharides and reducing inflammation and thus reducing insulin resistance, preventing the destruction of pancreatic beta cells and lowering the level of cholesterol and harmful blood lipoproteins. On the other hand, aerobic training and muscle endurance training can improve NAFLD by activating lipolysis, increasing the regulation of UCP-1 (Uncoupling protein-1) and PPAR γ (Peroxisome proliferator-activated receptor γ) and changes in adipocytokines (33). Guo et al. In a review article stated that aerobic training can be effective in improving NAFLD by reducing visceral and peripheral fat reserves and increasing the uptake of free fatty acids by the liver and inhibiting and modulating oxidative stress, inflammation and hepatic apoptosis (34). Training can also help reduce liver steatosis and prevent the progression of cirrhosis, and can be effective in improving insulin sensitivity, reducing insulin resistance, and cardiovascular health, which is the leading cause of death in these patients (35). Hosseini et al (2017) reported a significant decrease in ICAM-1 in obese postmenopausal rats due to eight weeks of intense intermittent training (21). The results of Khalesi et al. (2018) showed that there was no significant change in ALP in adults due to probiotic use (36), which is inconsistent with the results of the present study. The cause of this mismatch can be attributed to the type of subjects. Mousavi Nezhad et al. (2015) reported in a study that the use of probiotics significantly increased ALP rats with non-alcoholic fatty liver (8). The reason for this discrepancy can be due to differences in species and dose of probiotic bacteria consumed, intervention length, sample size and clinical characteristics of the subjects.

Conclusion

The results of this study showed that aerobic training and probiotic consumption in rats with non-alcoholic fatty liver disease can affect the inhibition of adhesion molecules and significantly reduce ICAM-1 and ALP. Therefore, aerobic training can be a good solution to reduce fat stores, prevent inflammation and reduce non-alcoholic fatty liver disease, and the use of probiotics in the range of health, along with training can be effective in achieving better results and as an adjunctive therapy to be used for patients with NAFLD.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval The Ethics Committee of Islamic Azad University East Tehran Branch approved the study.

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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