Research Article

The effect of endurance training and probiotic consumption on IL-18 gene expression in male rats with steatosis

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Abstract

Background: Fatty liver disease is the most important reason for chronic liver diseases such as liver fibrosis, cirrhosis and liver cancer. The aim of this study was the effect of endurance training and probiotic consumption on IL-18 gene expression in male rats with steatosis.

Materials and Methods: In this experimental study, 32 rats (average weight 200-250 grams) were randomly divided into four groups (healthy control, modeled (steatosis) group, steatosis + probiotic group, and steatosis + probiotic + exercise group). Training groups for 8 weeks, 5 sessions every week, in the beginning week with a speed of 18 meters per minute, time of 10 minutes, and every week the speed of 1-2 meters per minute and the time was increased by 10 minutes, so that In the fourth week, the speed reached 22 meters per minute and the time reached 40 minutes. The groups receiving the supplement received 109 CFU/ml of Lactobacillus rhamnus GG by gavage daily for 8 weeks and 5 days a week. IL-18 gene expression in liver tissue was measured by Real-time PCR method. The data were evaluated using the statistical method of one-way analysis of variance and significance level ($P \geq 0.05$).

Results: The results showed that endurance training along with probiotic consumption significantly decreased IL-18 gene expression in rats with steatosis.

Conclusion: It seems that performing endurance training along with probiotic consumption can have a greater effect than the effect of probiotic alone on IL-18 gene expression.

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1. Introduction

One of the most common metabolic diseases is non-alcoholic fatty liver disease (NAFLD), the prevalence of which is higher in obese and diabetic people and reaches about 70% (1). This disease includes a wide range of disorders that are related to the deposition of fat (more than five percent of liver weight) in hepatocytes and can vary from a simple hepatic steatosis to non-alcoholic cirrhotic osteohepatitis (2). One of the first consequences of liver dysfunction in NAFLD disease is a change in the serum lipid profile and an increase in specific liver enzymes following liver damage. Liver vulnerability is increased by inflammatory cytokines and adipokines, mitochondrial disorder and oxidative stress, which leads to steatohepatitis or fibrosis (2). The mechanism that causes fat accumulation in the liver can be caused by excess dietary fat, increased delivery of fatty acids to the liver, insufficient oxidation of fatty acids, and increased lipogenesis. NAFLD is associated with central obesity, glucose intolerance, insulin resistance, hyperlipidemia, and hypertension and is considered as the hepatic manifestation of metabolic syndrome (3). Obesity and low physical activity are the main risk factors for the spread of this disease. Endurance training reduces the density of liver cells in patients with fatty liver. In fact, regular exercise activity can be an effective treatment for improving fatty liver complications by reducing intrahepatic fat content, reducing hepatic peroxidase oxidative stress, improving liver inflammation by inhibiting inflammatory mediators such as tumor necrosis factor alpha and interleukin 1 beta (4). An increase in the level of total cholesterol in the blood is considered a strong risk factor for coronary heart disease, in general, every 1% reduction in blood cholesterol levels reduces the risk of coronary heart disease by 2.3%. (5,6).

Interleukin-18 (IL-18) is one of the anti-inflammatory cytokines secreted in response to inflammation and is a limiting and reversing factor of the inflammation process and has biological effects similar to IL-12. This cytokine is produced by Kupffer cells, activated macrophages, intestinal epithelial cells, osteoblasts and adrenal cortical cells. An increase in its serum concentration in many diseases such as NAFLD, obesity, insulin resistance, high blood pressure and dyslipidemia has been reported in several studies (7).

In recent studies, the use of probiotics as a live microbial food supplement in the treatment of fatty liver and reducing the serum levels of liver aminotransferases, total cholesterol and inflammatory markers has been considered. . The mechanism of action of probiotics includes the reduction of circulating pro-inflammatory cytokines, reduction of inflammation and levels of harmful cholesterol and lipoproteins, direct antimicrobial effects, strengthening of the mucosal defense barrier and favorable changes in the body's immune system (8). Most of the probiotics belong to a large group of the main bacteria of the microbial flora of the human intestine. Prescribing probiotics through manipulation of the intestinal microbial flora reduces low-grade intestinal inflammation and improves the integrity of the intestinal barrier (8). West et al. (2012) reported that the concentration of IL-18 did not change significantly after probiotic supplementation (9).

Considering the prevalence of steatosis disease and its increase among all the people of the society and considering the beneficial effects of physical activity and proving the effect of probiotics consumption on strengthening the body's immunity in fighting non-alcoholic fatty liver inflammations and considering that so far there is no research in the field of studying At the same time, the

long-term effect of endurance exercise and taking probiotics on the expression of IL-18 gene in steatosis has not been done and the researches in this direction have reached contradictory results. It seems that a research study aimed at the effect of long-term endurance training and probiotic intake on IL-18 gene expression is necessary.

2. Materials and Methods

The method of the present research was experimental with a post-test design with a control group. For this purpose, 32 Wistar Dawley rats were purchased. And after transferring to the laboratory environment and familiarizing themselves with the new environment for two weeks, they were randomly divided into the following 4 groups (8 heads in each group): Control, supplement, training and training + supplement. The animals tested during the research stages were kept in transparent polycarbonate cages with dimensions of 15 x 15 x 30 cm, made by Razi Rad Company, with a 12:12 light-dark cycle with an ambient temperature of 2 They were kept at ± 22 degrees Celsius and 55 ± 5 percent air humidity, as well as with proper ventilation and free access to water and special food for laboratory animals.

Creating a fatty liver model (steatosis)

Oral tetracycline was administered at a dose of 140 mg/kg of body weight (as a solution in 2 mL of water) for 7 days with fatty liver (9).

Training protocol

Before starting the main training and in order to get familiar with how to work on the treadmill, the rats of the training groups were active for five minutes at a speed of 8-10 m/min with a zero slope 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 was 22 meters per minute and The time reached 40 minutes. Five minutes before and after training were considered for warming up and cooling down the animals (10).

Supplement

Lactobacillus rhamnosus GG (PTCC1637) was purchased in lyophilized form in standard vials from the Scientific and Industrial Research Organization of Iran (Tehran, Iran). The respective groups received 109 CFU/ml of Lactobacillus rhamnosus GG by gavage daily for 8 weeks and 5 days a week (9).

To investigate IL-18, animals are first sampled in a special space (sterile environment) by experienced experts, with a combination of ketamine (30 to 50 mg per kilogram of body weight) and xylazine (3 to 5 mg per kilogram of body weight) fainted. Liver tissue samples were taken to check IL-18 gene expression. In each group, tissues were examined with 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 IL-18 gene expression.

Comparative $\Delta\Delta CT$ method was used to quantify IL-18 mRNA expression.

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

Gene	Primer Sequence (5′-3′)	Product Size (bp)	Accession Number
IL-18	F: TGACAAAAGAAACCCGCCTG	124	NM_019165.1
	R: GGHDLACAGCCAGHDLCHDLTTA		
GAPDH	F:CAAGTHDLAAGGGCACAGHDLA	102	NM_017008.4
	R: CCCCATTTGATGTTAGCGGG		

To investigate the significant changes of each of the research variables, between different groups, the one-way analysis of variance method was used, and if a statistically significant difference was observed, Ben Feroni's post hoc test was used to determine the location of the difference between the groups. The level of significance was considered $P \geq 0.05$ for all calculations. All statistical operations were performed using SPSS version 20 software.

3. Results

Eight weeks of endurance training along with probiotic consumption had a significant effect on IL-18 gene expression in rats suffering from steatosis and caused its reduction.
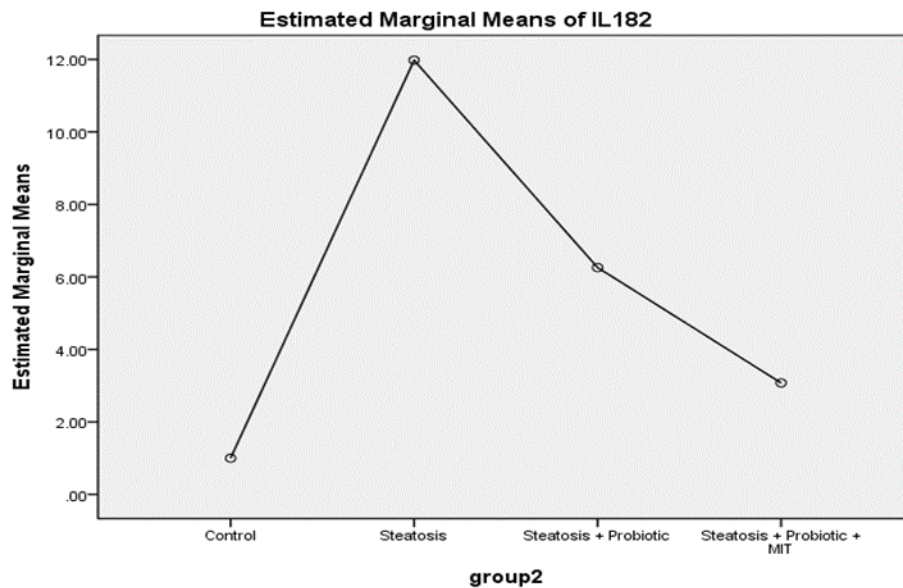
Figure 1. Comparison of IL-18 expression in four groups: control (healthy), steatosis (patient), patient + probiotic consumption, patient + probiotic consumption + training

4. Discussion

The results showed that eight weeks of endurance training along with probiotic consumption caused a significant decrease in IL-18 gene expression in rats with steatosis. Probiotics are living microorganisms that exist in the stomach, bile secretions, and pancreas, attach to epithelial cells, and colonize in the human intestine. Different species of Lactobacillus bacteria are types of lactic acid bacteria. Probiotic products have been recommended as a solution along with a balanced lifestyle and weight loss in the treatment of non-alcoholic fatty liver (11). Probiotics with different mechanisms such as reducing lipopolysaccharides and pro-inflammatory cytokines in the blood circulation and reducing inflammation and as a result reducing insulin resistance, preventing the destruction of pancreatic beta cells and lowering the level of cholesterol and harmful lipoproteins in the blood can be used in liver treatment. Fats are useful (12). The results have shown that lipopolysaccharides activate the nuclear factor kappa B by inducing the signaling pathway in monocytes. As a result, pro-inflammatory cytokines are expressed and secreted. By destroying and deactivating these nuclear factors, probiotic bacteria reduce the production of pro-inflammatory cytokines, and by increasing the number of natural killer T cells, they can be useful in controlling systemic inflammation (12). Mozafari et al. (2015) reported that the use of probiotics caused a significant decrease in IL-18(13). Dargahi et al. (2019) also reported in a study that consumption of Streptococcus thermophilus probiotic caused a significant decrease in IL-18 in human cells (14), which is in line with the results of the present study. IL-18 is an anti-inflammatory cytokine with an important regulatory function in the innate immune system and increases the production of adhesive molecules and nitric oxide synthesis. It is also an indicator for predicting cardiovascular death (15). Sports training reduce body fat reserves by increasing the stimulation of protein synthesis and muscle mass, and following the silencing of the inflammatory cytokine gene in muscle tissue and reducing the serum levels of leukocyte adhesive molecules by inhibiting the reaction of monocytes and endothelial cells, they lead to a reduction in inflammation (16). The reduction of IL-18 due to aerobic training can be a sign of the effect of endurance training on fat tissue, which prevents the release of inflammatory cytokines (17). Based on the findings of this study, the interactive effect of exercise and supplementation led to more changes compared to probiotic consumption alone, which may indicate the strengthening of the effects of endurance exercise combined with probiotic consumption on the reduction of inflammation caused by IL-18 and, as a result, the reduction of other cytokines. be anti-inflammatory that are induced through it. Probiotics strengthen the anti-inflammatory and antioxidant effects of exercise due to their very strong anti-inflammatory and anti-oxidant properties. The results of previous researches have shown that antioxidants can inhibit the expression of IL-18 and modulate oxidative stress through the negative regulation of the expression of the nuclear factor kappa B transcription factor, which plays an important role in regulating IL-18(18). There is a strong relationship between the type and number of microorganisms in the gut with the histological health of the heart, so that Lem et al. (2012) showed that dysbiosis (an increase in the amount of harmful bacteria and a decrease in the amount of beneficial bacteria in the body) in the pathogenesis of many diseases.

In particular, it is directly related to the level of inflammatory cytokines and cardiac ischemia (18). Researchers recommend that supplementing with probiotics can improve heart damage by maintaining a balanced ratio of intestinal microflora (18).

Among the limitations of this research was the nocturnal activity of rats and the lack of control over the effect of anesthetic drugs, which is suggested to be controlled in future research.

Conclusion

By examining the results of the researches and the present study, it can be said that regular endurance exercises reduce IL-18 serum levels. The findings of the present study showed that eight weeks of endurance training with probiotic consumption caused a further decrease in IL-18 gene expression in rats with steatosis. Therefore, it can be concluded that the intervention of endurance training along with the consumption of probiotics plays an important role in improving the inflammatory condition in patients with steatosis. Useful probiotic compounds can be used as adjunctive and possibly alternative treatment for patients with this type of disease and be beneficial in managing steatosis risk factors. Among the limitations of this research was the nocturnal activity of rats and the lack of control over the effect of anesthetic drugs, which is suggested to be controlled in future research.

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

References

1. Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology. 2008 May;134(6):1641-54. doi: 10.1053/j.gastro.2008.03.002. PMID: 18471544; PMCID: PMC2553363.

2. Arabzadeh E, Shirvani H, Ebadi Zahmatkesh M, Riyahi Malayeri S, Meftahi GH, Rostamkhani F. Irisin/FNDC5 influences myogenic markers on skeletal muscle following high and moderate-intensity exercise training in STZ-diabetic rats. 3 Biotech. 2022 Sep;12(9):193. doi: 10.1007/s13205-022-03253-9. Epub 2022 Jul 26. PMID: 35910290; PMCID: PMC9325938.

3. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM. 2010 Feb;103(2):71-83. doi: 10.1093/qjmed/hcp158. Epub 2009 Nov 13. PMID: 19914930; PMCID: PMC2810391.

4. Riyahi Malayeri S, Kaka Abdullah Shirazi S, Behdari R, mousavi Sadati K. Effect of 8-week Swimming training and garlic intake on serum ICAM and VCAM adhesion molecules in male obese rats. JSSU 2019; 26 (10) :867-878. URL: http://jssu.ssu.ac.ir/article-1-4695-en.html.

5. Moroti C, Souza Magri LF, de Rezende Costa M, Cavallini DC, Sivieri K. Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. Lipids Health Dis. 2012 Feb 22;11:29. doi: 10.1186/1476-511X-11-29. PMID: 22356933; PMCID: PMC3305430.

6. Riyahi Malayeri, S., Mirakhorli, M. The Effect of 8 Weeks of Moderate Intensity Interval Training on Omentin Levels and Insulin Resistance Index in Obese Adolescent Girls. Sport Physiology & Management Investigations, 2018; 10(2): 59-68. https://www.sportrc.ir/article_67070.html?lang=en.

7.Wang Z, Gao ZM, Huang HB, Sun LS, Sun AQ, Li K. Association of IL-8 gene promoter– 251 A/T and IL-18 gene promoter– 137 G/C polymorphisms with head and neck cancer risk: a comprehensive meta-analysis. Cancer management and research. 2018 Aug 10:2589-604. https://www.tandfonline.com/doi/full/10.2147/CMAR.S165631.

8. Bonyadi F, Tukmechi A, Mohebalian H. An overview of probiotics and their role in cancer management. J Mazandaran Univ Med Sci 2014; 24 (112) :128-140

URL: http://jmums.mazums.ac.ir/article-1-3122-en.html

9. West NP, Pyne DB, Cripps AW, Christophersen CT, Conlon MA, Fricker PA. Gut Balance, a synbiotic supplement, increases fecal Lactobacillus paracasei but has little effect on immunity in healthy physically active individuals. Gut Microbes. 2012 May-Jun;3(3):221-7. doi: 10.4161/gmic.19579. Epub 2012 May 1. PMID: 22572834; PMCID: PMC3427214.

10. Hosseini M, Ghasem Zadeh Khorasani N, Divkan B, Riyahi Malayeri S. Interactive Effect of High Intensity Interval Training with Vitamin E Consumption on the Serum Levels of Hsp70 and SOD in Male Wistar Rats. Iranian J Nutr Sci Food Technol 2019; 13 (4) :21-28. URL: http://nsft.sbmu.ac.ir/article-1-2689-en.html.

11. Roberfroid M. Prebiotics: the concept revisited. J Nutr. 2007 Mar;137(3 Suppl 2):830S-7S. doi: 10.1093/jn/137.3.830S. PMID: 17311983.

12. Thomas CM, Versalovic J. Probiotics-host communication: Modulation of signaling pathways in the intestine. Gut Microbes. 2010 May-Jun;1(3):148-63. doi: 10.4161/gmic.1.3.11712. PMID: 20672012; PMCID: PMC2909492.

13. Mozaffari Namin B, Daryani NE, Mirshafiey A, Yazdi MKS, Dallal MMS. Effect of probiotics on the expression of Barrett's oesophagus biomarkers. J Med Microbiol. 2015 Apr;64(Pt 4):348-354. doi: 10.1099/jmm.0.000039. Epub 2015 Feb 9. PMID: 25666837.

14. Dargahi N, Johnson JC, Apostolopoulos V. Immune modulatory effects of probiotic Streptococcus thermophilus on human monocytes. Biologics. 2021 Nov 19;1(3):396-415. doi: https://doi.org/10.1101/2020.08.27.271346

15. Zirlik A, Abdullah SM, Gerdes N, MacFarlane L, Schönbeck U, Khera A, McGuire DK, Vega GL, Grundy S, Libby P, de Lemos JA. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. Arterioscler Thromb Vasc Biol. 2007 Sep;27(9):2043-9. doi: 10.1161/ATVBAHA.107.149484. Epub 2007 Jul 12. PMID: 17626902.

16. Colbert LH, Visser M, Simonsick EM, Tracy RP, Newman AB, Kritchevsky SB, Pahor M, Taaffe DR, Brach J, Rubin S, Harris TB. Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study. J Am Geriatr Soc. 2004 Jul;52(7):1098-104. doi: 10.1111/j.1532-5415.2004.52307.x. PMID: 15209647.

17. Bruun JM, Stallknecht B, Helge JW, Richelsen B. Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. Eur J Endocrinol. 2007 Oct;157(4):465-71. doi: 10.1530/EJE-07-0206. PMID: 17893261.

18. Lam V, Su J, Koprowski S, Hsu A, Tweddell JS, Rafiee P, Gross GJ, Salzman NH, Baker JE. Intestinal microbiota determine severity of myocardial infarction in rats. FASEB J. 2012 Apr;26(4):1727-35. doi: 10.1096/fj.11-197921. Epub 2012 Jan 12. PMID: 22247331; PMCID: PMC3316900.