Research Article

Moderate-intensity continuous training and probiotic consumption on IL-15 gene expression in an animal model of non-alcoholic steatosis

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Abstract

Steatosis is the most important cause for chronic liver disease, from simple steatosis to advanced stages such as liver fibrosis and cirrhosis and liver cancer. In this study, the effect of Moderate Intensity Continuous Training (MICT) and probiotic consumption was examined on IL15 gene expression in an animal model of non-alcoholic steatosis. This study adopted an experimental laboratory design. Thirty-two male Wistar rats were divided into 4 groups of 8: the healthy (normal diet), steatosis, steatosis + probiotic, steatosis + probiotic +MICT groups and were 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. Considering the consumption of probiotics, the relevant groups received 109 CFU / ml of Lactobacillus rhamnosus GG by gavage daily for 5 weeks and 5 days a week. Statistical calculation of this study was performed using SPSS 25 software. The results showed that probiotic consumption and moderate-intensity continuous training significantly increased IL-15 (p =0.000) while they significantly decreased TG (p =0.000) in the non-alcoholic steatosis animal model. It seems moderate-intensity continuous training with probiotic consumption can improve the liver function of non-alcoholic fatty liver patients.

Key Words: **IL-15, Moderate-intensity continuous training, Non-alcoholic steatosis, Probiotics**

Introduction

Life begins in all organisms with relatively healthy intestines. Factors such as unhealthy diet, misuse of antibiotics, stress, environmental pollution, etc., affect the composition and metabolic activity of the gastrointestinal flora. Therefore, they cause an imbalance of the gastrointestinal flora and increase the risk of disease by enhancing the number of destructive microorganisms against beneficial organisms (Browne et al., 2017). In addition, intense activity, endurance and the associated stress lead to a decrease in the level of immunity in people and expose people to all kinds of infections and diseases. Thus, it seems important to use supplements in conjunction with exercise training to improve markers of inflammation (Hedayati et al., 2018; Hosseini et al., 2017). Probiotics are among the supplements that can affect markers of inflammation. The history of using probiotics to prevent diseases and improve the health of humans and animals goes back to several thousand years. Probiotics come into contact with antibiotics or biophysical compounds (Markowiak & Śliżewska, 2017).

Probiotics are defined as a dietary supplement with live microbes and, when consumed by humans and animals, have a beneficial effect on the health of the host to influence the balance of the intestinal microbial flora. On the other hand, the effect of regular daily exercise training on fatty acid oxidation in the prevention of non-alcoholic steatosis disease have been proven in laboratory rats (van der Windt et al., 2018).

Pathogenesis of non-alcoholic steatosis is often based on a two-step process consisting of triglyceride (TG) accumulation followed by the development of oxidative stress and cytokines, mediating inflammation and fibrosis of the liver (Parthasarathy et al., 2020). Aerobic exercise training reduces the density of liver cells in patients with steatosis compared to the control group. In fact, exercise training with regular physical activity can be an effective treatment for steatosis complications by reducing the content of intrahepatic fat, reducing hepatic oxidative stress peroxidase, and improving hepatitis by inhibiti-

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-ng inflammatory mediators such as tumor necrosis factor alpha and interleukin-1 beta (Thyfault & Rector, 2020).

IL-15 is a 14–15 kDa glycoprotein encoded by a 34 kilobase (kb) region on chromosome 4q31. IL-15 mRNA is expressed by a large number of tissues including fibroblasts, keratinocytes, epithelial cells of various tissues, nerve cells, monocytes, macrophages and dendritic cells (Nadeau & Aguer, 2019). Interleukin-15 (IL-15) is essential for the homeostasis of lymphoid cells particularly memory CD8 (+) T cells and NK cells. These cells are abundant in the liver, and are implicated in obesity-associated pathogenic processes (Cepero-Donates et al., 2016). Ofoghi et al. (2018) examined the effect of eight weeks of resistance exercise training on interleukin-15 and found that it increased significantly. In addition, some other studies showed that there was a significant increase in IL-15 after resistance exercise training (Leal et al., 2018; Tolouei azar et al., 2021).

Moreover, previous studies have confirmed the effect of aerobic and resistance exercise training on the role of IL15 on strengthening the immune system. While the body is fighting against the inflammation caused by steatosis damage in its non-alcoholic model, the combination of moderate-intensity continuous training (MICT) and probiotic consumption on the expression of ILI5 gene can be effective. However, no study has conducted so far to investigate the combined effect of MICT and Probiotics consumption on ILI5 gene expression in animal models of steatosis. Therefore, due to the importance of the subject, in this study, the effect of moderate-intensity continuous training and probiotic consumption was investigated on IL15 gene expression in the steatosis animal model.

Materials and Methods

Animals

In this study, the statistical population included 32 male Wistar rats in 2 healthy and steatosis models. Rats weighting 200-250 were randomly divided into the following 4 groups: control (healthy) group (N = 8), steatosis group (N = 8), steatosis + probiotic group (N = 8), and steatosis + probiotic + MICT group (N = 8). The study protocol conformed to the Declaration of Helsinki and was approved by the animal care and the Committee of Islamic Azad University, East Tehran Branch (Ethics code: IR.IAU.SRB.REC.1399.019).

Creating a steatosis model

Tetracycline with the dosage of 100mg/kg in per 1.5 cc volume was gavaged to every mouse daily for 2 weeks. It should be noted that the weight of the mice was 300 g on average, and100 mg of Tetracycline in every kilogram was used for 3 mice, solved in 4.5 cc and 1.5 cc was gavaged to each mouse.

Culture of Lactobacillus ramensus GG

Lactobacillus ramensus GG (PTCC1637) was purchased in the lyophilized form in standard vials from the Iranian Research Organization for Science and Technology (Tehran, Iran). The bacteria are cultured in MRS environment enriched with l-Cysteine Hydrochloride (rational Biology, Tehran, Iran) and they are incubated for twenty-four hours at 37 degrees Celsius in the incubator.

In order to assess the effect of probiotics consumption, the respective groups received a daily dose of 109 CFU/ml of Lactobasilus Ramensus bacteria in the form of gavage for 5 weeks and 5 days per week (Wang et al., 2011).

Exercise training protocol

The aerobic exercise protocol was performed with 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 (Asgari Hazaveh et al., 2018; Mohammadi et al., 2021).

Biochemical analysis

After the last exercise session and consumption of probiotic and after 12 hours of overnight fasting, the studied rats in each group were anesthetized by intraperitoneal injection of a mixture of 10% ketamine at a dose of 50 mg / kg and xylazine 2% with a dose of 10 mg / kg. Then, about 10 ml of blood was taken directly from the hearts of the mice by syringe through cutting their abdomen and the chest. Blood samples were centrifuged at 1000 g for 20 minutes to separate the serum and were stored at -80 ° C.

Real Time PCR

At the end of the eight week, the rats were taken to the lab, sacrificed and a sample of their livers was extracted for the 1L-15 gene expression. In each group, the tissues evaluation was done by PCR real time technique. At first, the primer design was carried out and then the total RNA was extracted and converted into cDNA which was then amplified by PCR method and evaluated in terms of the mentioned genes.

This technique consisted of major stages: 1. The total RNA of the gathered cells in every group was extracted; 2. It was converted to cDNA using reverse transcription enzyme; 3. The resulted cDNA was treated by DNase I enzyme in order to remove the genomic DNA; 4. It was reproduced by PCR real time method; 5. All the used containers must be void of RNase enzyme respectiv-

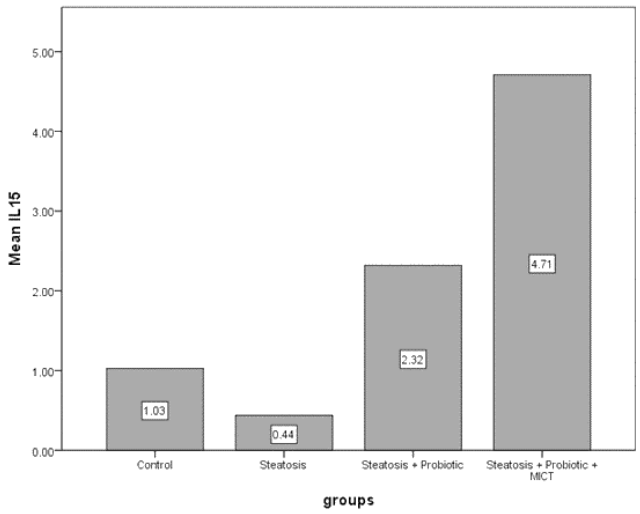

Figure 1. Comparison of IL15 expression in four groups. Data were shown as mean ± standard deviation.

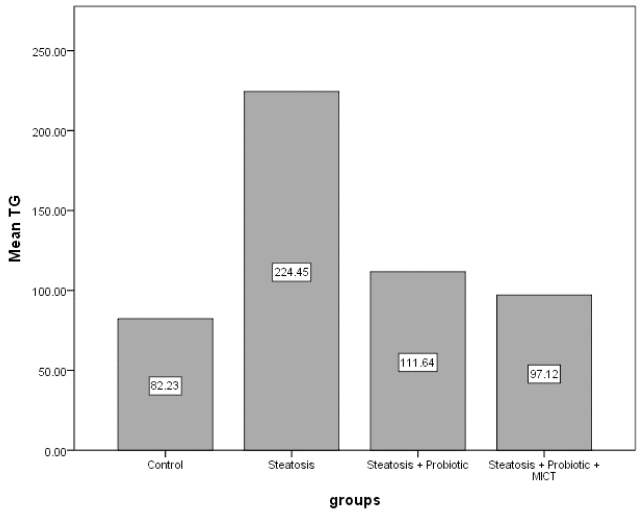

Figure 2. The comparison of TG values in four groups. Data were shown as mean ± standard deviation.

-ely. They first were situated for one hour at 37 degrees Celsius or 24 hours in the room temperature in 0.1% DEPC solution so as to destroy the DEPC which can be a barrier in RNA evaluation and analysis process; 6.Wearing gloves and masks was mandatory in all RNA stages; 7. All buffers and solutions were prepared in DEPC-treated distilled water; 8. The extracting process of RNA was performed under the hood by sterilized UV half an hour before.

In order to study the molecular level of gene expression, first, RNA extraction from tissues in all groups was performed according to the protocol of the manufacturer (Kiagen, Germany). Comparative $2^{\Delta\Delta CT}$ method was used to quantify IL-15 mRNA expression.

Statistical analysis

Mean and standard deviation (SD) values were calculated for each variable. After the normality of data distribution was confirmed by Kolmogorov-Smirnov test, parametric statistics, including one-way analysis of variance (ANOVA), was used to analyze the data using SPSS (version 20, IBM Corp., Armonk, NY, USA). Statistical significance was accepted, a priori, at $p<0.05$.

Results

According to the obtained results from Kolmogorov-Smirnov test, the distribution of data related to research variables is normal.

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 IL15 gene variable in the four groups (P = 0.000). The results showed that, as a result of eight weeks of MICT with probiotic consumption, it has a significant effect on IL15 gene expression in the animal model of steatosis and increases its amount (Figure 1). The results of Bonferroni post hoc test showed that the control group was significantly different from the steatosis group and the steatosis group was significantly different from the probiotic group and the MICT + probiotic group.

Also, 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 TG gene variable in the four groups (P = 0.000). It was revealed that eight weeks of aerobic exercise training with probiotic consumption has a significant effect on TG gene expression in the animal model of steatosis (Figure 2). The results of the Bonferroni post hoc test showed that the control group was significantly different from the steatosis and probiotic groups and the steatosis group was significantly different from the probiotic group and the MICT + probiotic group.

Discussion

With the fat building up in the liver cells and Steatosis, fatty liver

Table 1. Mean and standard deviation of IL15 and TG gene levels.

Variables	Control	Steatosis	Steatosis + probiotic	MICT+Steatosis + probiotic	Sig
IL15	1.02 ± 0.42	0.43 ± 0.33	2.31 ± 0.55	4.7 ± 2.19	0.000
LDL (mg/dL)	82.22±5.18	224.44±25.88	111.64±8.78	97.11±4.47	0.000

disease (FLD) occurs, which in turn causes steatohepatitis and cirrhosis (Alves-Bezerra & Cohen, 2017). Hepatic steatosis may be due to lipid metabolism disorders and an imbalance in lipid production and breakdown (Pei et al., 2020). In the present study, the effect of probiotic consumption as well as MICT was examined on the IL15 gene expression of animal steatosis.

According to the findings, steatosis in rats was associated with a significant decrease in IL15 gene levels, which increased significantly after the consumption of probiotics +MICT compared to the steatosis group. According to the results of the study by Li et al. (2018), the progression of non-alcoholic steatosis disease is the result of a combination of genetic, environmental, and metabolic factors. Fat accumulation in hepatocytes causes a number of cytotoxic events that cause inflammation of the liver. Numerous pathological processes, including: insulin resistance, leptin deficiency, oxidative stress, fat accumulation and inflammation of liver tissue, are associated with steatosis. Genetic and metabolic factors in the pathogenesis of non-alcoholic steatosis indicate that abnormal expression or gene mutations leads to the development of this disease (Li et al., 2018).

One of the mechanisms of probiotic lactobacillus is the stimulation of the activity of cytokines in the immune system. They also boost the immune response, which is consistent with the results of the present study. Soleimany et al. (2018) investigated the parasporal cytocidal effect of gram-positive bacterium, Bacillus thuringiensis on the stimulation of peripheral blood mononuclear cells and the ability to produce cytokines interleukin-2 and interleukin-5. Their results showed that the toxin of gram-positive bacterium Bacillus thuringiensis stimulates the immune system, production of interleukin-2 and stops stimulation of inhibitory cytokine production of interleukin-5 (Soleimany et al., 2018).

Numerous studies have investigated the effect of exercise training in terms of type, intensity and duration on IL15. It has been reported that endurance and resistance exercise is associated with increased levels of IL15 (Leal et al., 2018; Tolouei azar et al., 2021) which is consistent with the results of the present study. This study showed that the combined effect of probiotics and exercise caused an increase in interleukin 15. Probably, the mechanism of this increase in interleukin 15 can be due to the effect of exercise on reducing inflammation in the liver and the effect of probiotics on inflammation.

Zhang et al. investigated the effect of high-intensity aerobic exercise training and MICT in mice with diethyl nitrosamine-induced liver carcinoma. They found that MICT compared with high-intensity aerobic exercise training inhibited the onset of liver cancer in mice under treatment with diethyl nitrosamine. Therefore, their findings showed that MICT can prevent tumor a--ccess by fat atrophy (Zhang et al., 2020). However, the results are contradictory regarding the effect of the types of exercise training used on the IL15, depending on the intensity, duration and type of exercise training used.

Based on the findings of the present study, induction of steatosis in rats caused a significant increase in TG levels that decreased significantly compared to the steatosis group after taking probiotics + MICT for 8 weeks. In order to investigate the effect of Lactobacillus probiotics on laboratory animals (mice), certain amounts of these compounds were included in the diet containing alcohol. In this study, the main mechanism of Lactobacillus brevis probiotics was attributed to its ability of preventing increased levels of AST and ALT in serum and total cholesterol and TGs in liver tissue following alcohol consumption. AST and ALT enzymes are commonly found in liver cells and enter the bloodserum following damage to liver cells. Lactobacillus brevis also inhibits the overexpression of mRNA of the TNF-α molecule, the hepatic SREBP-1 and SREBP-2 signal proteins, and increases the expression of heat shock protein 25 in the small intestine. The hepatic SREBP-1 and SREBP-2 signaling proteins act to regulate the transcription of genes involved in TG and cholesterol synthesis, respectively, which inhibits the expression of these proteins to help reduce TGs and cholesterol in the liver (Jeong et al., 2022; Moslehi & Hamidi-Zad, 2018). In the present study, Post hoc analysis of the results of comparing TG levels between the probiotic group and the control group showed that there is a significant difference between these groups, but there was no significant difference between the control and probiotic + MICT groups. In addition, comparing the TG levels of the probiotic group and probiotic + MICT group showed that the rate of decrease in TG levels in subjects in the probiotic + MICT group was greater than in subjects in the probiotic group, but no significant difference was observed between the two groups.

Regarding the possible mechanism of the effect of probiotics, researchers believe that probiotic bacteria, along with other useful food components alter metabolic pathways and reduce the synthesis of lipid profiles by fermenting dietary fiber and producing short-chain fatty acids such as acetic, propionic and butyric (Parvez et al., 2006) or is attributed to the reduction of inflammatory cytokines and activation of Toll-4 receptors. The production of inflammatory cytokines due to the activation of the TLR4 membrane protein leads to an increase in the innate immune response, which is involved in insulin resistance, diabetes, and atherosclerosis. In addition, hydrolase produced by some types of probiotics can reduce cholesterol absorption by further excretion of bile salts (Patel et al., 2010).

Other possible mechanisms are reduced inflammation and insulin resistance, TG storage in the liver, novo lipogenesis from carbohydrate-binding protein and binding protein of sterol regulatory elements, and very low-density lipoprotein secretion (Leung et al., 2016). The secretion of fasting induced adipose factor (FIAF) by probiotics consumption inhibits endothelial lipoprotein lipase and subsequently controls the secretion of TGs from chylomicrons and VLDL (Leung et al., 2016). Other factors such as probiotic dose, duration and intensity of different exercises, age of the participants, study conditions, and different bacterial strains may also be effective factors in the effect of probiotics on lipid profile (As'Habi et al., 2020). The results show that moderate-intensity continuous training with the use of probiotics in patients with non-alcoholic fatty liver improves their immune function and reduces their body fat.

Conclusion

Induction of Steatosis in rats was associated with a significant decrease in IL15 gene levels and a significant increase in TG levels, and with probiotic consumption compared to steatosis group, IL15 gene levels increased while TG decreased significantly. In addition, the comparison of IL15 and TG gene expression levels between the probiotic groups combined with MICT and the steatosis group showed that the use of probiotics with the intervention of MICT increased IL15 gene levels and decreased TG levels significantly. It seems moderate-intensity continuous training with probiotic consumption can improve the liver function of non-alcoholic fatty liver patients.

What is already known on this subject?

Although the number of studies on IL15 measures has increased in the last years, to date, it remains an open question whether the existing effect of exercise and probiotic consumption on immune system measures are appropriate for non-alcoholic fatty liver.

What this study adds?

Moderate-intensity continuous training with probiotic consumption can improve the liver function of non-alcoholic fatty liver patients

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol conformed to the Declaration of Helsinki and was approved by the animal care and Committee of Islamic Azad University East Tehran Branch (Ethical code IR.IAU.SRB.REC.1399.019).

Informed consent Animal study.

Author contributions

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

References

Alves-Bezerra, M., & Cohen, D. E. (2017). Triglyceride metabolism in the liver. Compr Physiol, 8(1), 1-8. doi: https://doi.org/10.1002/cphy.c170012

As'Habi, A., Nazari, M., Hajianfar, H., Arab, A., & Faghfoori, Z. (2020). A systematic review and meta-analysis of probiotic consumption and metabolic status of athletes. International Journal of Food Properties, 23(1), 941-954. doi: https://doi.org/10.1080/10942912.2020.1772286

Asgari Hazaveh, D., Riyahi Malayeri, S., & Babaei, S. (2018). Effect of eight weeks high intensity interval training and medium intensity interval training and aloe vera intake on serum vaspin and insulin resistance in diabetic male rats. Journal of Arak University of Medical Sciences, 20(11), 67-75.

Browne, H. P., Neville, B. A., Forster, S. C., & Lawley, T. D. (2017). Transmission of the gut microbiota: Spreading of health. Nat Rev Microbiol, 15(9), 531-543. doi: https://doi.org/10.1038/nrmicro.2017.50

Cavallini, D. C. U., Suzuki, J. Y., Abdalla, D. S. P., Vendramini, R. C., Pauly-Silveira, N. D., Roselino, M. N., . . . Rossi, E. A. (2011). Influence of a probiotic soy product on fecal microbiota and its association with cardiovascular risk factors in an animal model. Lipids in Health and Disease, 10(1), 126. doi: https://doi.org/10.1186/1476-511X-10-126

Cepero-Donates, Y., Lacraz, G., Ghobadi, F., Rakotoarivelo, V., Orkhis, S., Mayhue, M., . . . Ramanathan, S. (2016). Interleukin-15-mediated inflammation promotes non-alcoholic fatty liver disease. Cytokine, 82, 102-111. doi: https://doi.org/10.1016/j.cyto.2016.01.020

Hedayati, S., Riyahi Malayeri, S., & Hoseini, M. (2018). The effect of eight weeks of high and moderate intensity interval training along with

aloe vera consumption on serum levels of chemerin, glucose and insulin in streptozotocin-induced diabetic rats: An experimental study. Journal of Rafsanjan University of Medical Sciences, 17(9), 801-814.

Hosseini, M., Eftekhar, B., & Riyahi Malayeri, S. (2017). Effect of interval training with curcumin consumption on some adipokines in menopausal obese rats. Journal of Rafsanjan University of Medical Sciences, 16(6), 505-516.

Jeong, J. J., Park, H. J., Cha, M. G., Park, E., Won, S. M., Ganesan, R., . . . Suk, K. T. (2022). The lactobacillus as a probiotic: Focusing on liver diseases. Microorganisms, 10(2). doi: https://doi.org/10.3390/microorganisms10020288

Leal, L. G., Lopes, M. A., & Batista, M. L. (2018). Physical exercise-induced myokines and muscle-adipose tissue crosstalk: A review of current knowledge and the implications for health and metabolic diseases. Frontiers in Physiology, 9. doi: https://doi.org/10.3389/fphys.2018.01307

Leung, C., Rivera, L., Furness, J. B., & Angus, P. W. (2016). The role of the gut microbiota in NAFLD. Nat Rev Gastroenterol Hepatol, 13(7), 412-425. doi: https://doi.org/10.1038/nrgastro.2016.85

Li, L., Liu, H., Hu, X., Huang, Y., Wang, Y., He, Y., & Lei, Q. (2018). Identification of key genes in non-alcoholic fatty liver disease progression based on bioinformatics analysis. Mol Med Rep, 17(6), 7708-7720. doi: https://doi.org/10.3892/mmr.2018.8852

Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients, 9(9). doi: https://doi.org/10.3390/nu9091021

Mohammadi, S., Rostamkhani, F., Riyahi Malayeri, S., & Shirvani, H. (2021). High-intensity interval training with probiotic supplementation decreases gene expression of NF-κβ and CXCL2 in small intestine of rats with steatosis. Sport Sciences for Health. doi: https://doi.org/10.1007/s11332-021-00829-5

Moslehi, A., & Hamidi-Zad, Z. (2018). Role of SREBPs in liver diseases: A mini-review. J Clin Transl Hepatol, 6(3), 332-338. doi: https://doi.org/10.14218/jcth.2017.00061

Nadeau, L., & Aguer, C. (2019). Interleukin-15 as a myokine: Mechanistic insight into its effect on skeletal muscle metabolism. Appl Physiol Nutr Metab, 44(3), 229-238. doi: https://doi.org/10.1139/apnm-2018-0022

Ofoghi, M., Isanejad, A., & Samadi, A. (2018). Investigating the effect of eight-weeks resistance training with Thera-band on the serum levels of IL-15, balance and muscle strength in elderly women. Metabolism and Exercise, 8(1), 43-60. doi: https://doi.org/10.22124/jme.2018.3563

Parthasarathy, G., Revelo, X., & Malhi, H. (2020). Pathogenesis of nonalcoholic steatohepatitis: An overview. Hepatol Commun, 4(4), 478-492. doi: https://doi.org/10.1002/hep4.1479

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. J Appl Microbiol, 100(6), 1171-1185. doi: https://doi.org/10.1111/j.1365-2672.2006.02963.x

Patel, A. K., Singhania, R. R., Pandey, A., & Chincholkar, S. B. (2010). Probiotic bile salt hydrolase: Current developments and perspectives. Appl Biochem Biotechnol, 162(1), 166-180. doi: https://doi.org/10.1007/s12010-009-8738-1

Pei, K., Gui, T., Kan, D., Feng, H., Jin, Y., Yang, Y., . . . Li, Y. (2020). An overview of lipid metabolism and nonalcoholic fatty liver disease. Biomed Res Int, 2020, 4020249. doi: https://doi.org/10.1155/2020/4020249

Soleimany, M., Moazamian, E., & Rasouli, M. (2018). Effect of bacillus thuringiensis parasporal toxin on stimulating of IL-2 and IL-5 cytokines production. Biological Journal of Microorganism, 7(25 #M005).

Thyfault, J. P., & Rector, R. S. (2020). Exercise combats hepatic steatosis: Potential mechanisms and clinical implications. Diabetes, 69(4), 517-524. doi: https://doi.org/10.2337/dbi18-0043

Tolouei Azar, J., Shabkhiz, F., & Khalafi, M. (2021). The effects of eight weeks of resistance training on serum levels IL-15, IL-6, TNF-α and insulin resistance in older type 2 diabetic men. Journal of Sport Biosciences, 12(4), 391-406. doi: https://doi.org/10.22059/jsb.2018.251040.1241

van der Windt, D. J., Sud, V., Zhang, H., Tsung, A., & Huang, H. (2018). The effects of physical exercise on fatty liver disease. Gene Expr, 18(2), 89-101. doi: https://doi.org/10.3727/105221617x15124844266408

Wang, Y., Kirpich, I., Liu, Y., Ma, Z., Barve, S., McClain, C. J., & Feng, W. (2011). Lactobacillus rhamnosus GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. Am J Pathol, 179(6), 2866-2875. doi: https://doi.org/10.1016/j.ajpath.2011.08.039

Zhang, X., Cao, L., Ji, B., Li, L., Qi, Z., & Ding, S. (2020). Endurance training but not high-intensity interval training reduces liver carcinogenesis in mice with hepatocellular carcinogen diethylnitrosamine. Exp Gerontol, 133, 110853. doi: https://doi.org/10.1016/j.exger.2020.110853