OPEN ACCESS

ISSN: 2676-7473

RBMS.2024;29(1):e3

ORIGINAL RESEARCH

Investigating the effect of 8 weeks of moderate resistance training (MRT) and Lactobacillus Reuteri probiotic on the serum concentration of liver enzymes Alanine transaminase and Aspartate transaminase and weight changes in male Wistar rats with non-alcoholic fatty liver disease

Akram Bargir[1], Farshad Ghazalian[2]*, Hossein Keyvani[3], Hossein Abed Natanzi[4], Mandana Gholami[5]

1. Department of Physical Education and Sports Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. *Department of Physical Education Sport Sciences, Science and Research* Branch, *Islamic Azad University, Tehran, Iran.*
3. *PhD, Department of Virology Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
4. *Department of Physical Education Sport Sciences, Science and Research* Branch,*Islamic Azad University, Tehran, Iran.*
5. *Department of Physical Education Sport Sciences, Science and Research* Branch, *Islamic Azad University, Tehran, Iran.*

*Corresponding Author:
Address: Department of Physical Education Sport Sciences, Science and Research Islamic Azad University, Tehran, Iran.
Email: phdghazalian@gmail.com
ORCID: 0000-0002-5805-0559

Date Received: December, 2023 *Date Accepted: January, 2024* *Online Publication: February 15, 2024*

Abstract

Objective: The aim of this study was investigating the effect of 8 weeks of moderate resistance training (MRT) and Lactobacillus Reuteri probiotic on the serum concentration of liver enzymes and weight changes in male Wistar rats with non-alcoholic fatty liver disease (NAFLD).
Materials and Methods: This research was conducted on 32 male Wistar rats aged 8 to 10 weeks with a weight range of 230 to 300 grams. Male rats were randomly divided into 4 groups of 8. All animals were weighed at the beginning and weekly using a digital scale. Serum levels of liver enzymes were checked at the beginning, middle and end of the study.
Results: In general, the findings of this study showed that at the end of the intervention, the level of ALT was significantly reduced in the groups receiving supplements and resistance training, but the serum level of AST was significantly reduced only in the group receiving probiotic supplements ($P<0.05$). Changes in weight also showed a significant increase in all 4 groups at the end of the eighth week.
Conclusion: The findings of the present research showed that 8 weeks of moderate intensity resistance training and Lactobacillus Reutteri probiotic supplement alone improved liver enzyme function (especially ALT serum level) in rats with non-alcoholic fatty liver disease.
Keywords: Moderate resistance training (MRT), Lactobacillus Lactobacillus Reuteri probiotic, Non-alcoholic fatty liver disease (NAFLD), ALT: Alanine transaminase, AST: Aspartate transaminase.

Introduction

The rapid increase in the prevalence of obesity in the world and its related metabolic complications, such as non-alcoholic fatty liver disease, dyslipidemia, type 2 diabetes, and metabolic syndrome, have become a threat to human health. Epidemiological studies have shown that there is a significant relationship between physical activity and non-communicable diseases, including metabolic syndrome, cardiovascular diseases, and cancer (1). Increasing fat intake from the diet leads to the accumulation and storage of fat in adipose tissue and hepatocytes (fat cells), and then overweight and obesity (2). Epidemiological studies have shown that non-alcoholic fatty liver disease (NAFLD) is one of the most common diseases of this century and is increasing in parallel with obesity (3, 4). Obesity and overweight are closely related to the increase in the prevalence and severity of liver disease. has non-alcoholic fatty liver disease (NAFLD); Obesity is a global health problem because there is an increasing trend in the prevalence of obesity in children and adolescents in developed and developing countries, which leads to adverse effects in terms of physical and mental health. Obesity leads to metabolic syndrome and chronic liver diseases. NAFLD has now become one of the main causes of chronic liver diseases in the industrialized world. Nonalcoholic fatty liver disease (NAFLD) includes a range of diseases that progress from simple steatosis (NAFLD), nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) (5).So far, no definitive drug strategy has been considered for it (3, 5). However, laboratory studies, especially in the last decade, have introduced lifestyle modification, including appropriate physical activity, a healthy diet, and weight loss, as the most effective treatment method for NAFLD. The global prevalence of non-alcoholic fatty liver disease is 25% (3), which increases to 58% in obese people and is about 44% in obese children and adolescents. Also, this statistic in Iran is about 43%, according to the announcement of the Ministry of Health in 1400. (6) . Liver diseases usually do not have specific symptoms, and enzyme disorders are diagnosed after periodic tests (7). One of the most important biochemical indicators is determining the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in plasma (4). These two enzymes are functional enzymes of the liver and are released when protein is broken, and their increase in plasma means liver cell damage (4). Recently, various studies on useful microorganisms called probiotics have expanded, and their results indicate the effect on the recovery and treatment of some diseases. Probiotic refers to living organisms that are naturally a part of the digestive tract ecosystem, and when taken in a supplemental form, they have sufficient health-giving effects on the host's body (8, 9). Probiotics prevent the absorption of toxins by reducing pH (8) and intestinal permeability (9). It seems that they ultimately lead to a reduction in inflammation and oxidative stress, which is effective in the prevention and treatment of liver cell damage (3). Studies have shown that performing regular sports activities, in addition to having an effect on weight loss, is significantly effective in the recovery and treatment of hepatic steatosis. Exercise improves body composition, blood sugar control, insulin sensitivity, and the immune system (11). So far, most of the studies have been focused on aerobic sports such as walking, running, swimming, cycling, etc., which have an effect on the treatment of steatosis by reducing oxidative stress and inflammation (1). Due to the fact that resistance exercises like working with weights require less cardiovascular power and at the same time provide similar metabolic benefits, it seems that obese, elderly, and busy people who have less physical activity and people who have limited space and place to exercise are also more exposed to the risk of NAFLD (12). Due to the change in lifestyle and the progress of modernity, life in urban societies is progressing with limited time and minimal physical activity and consumption of energy-rich foods, and it seems that there is a need for methods that can prevent diseases by spending the least amount of time and money. did A balanced lifestyle is considered the key and path-breaking treatment for the treatment of NAFLD. Since exercise and physical activity are integral parts of lifestyle, paying attention to this area can be effective in the treatment of NAFLD. (13) Therefore, the purpose of this study is to investigate the effect of 8 weeks of MRT resistance training and a Lactobacillus Reuteri probiotic supplement on the serum concentration of liver enzymes in male Wistar rats suffering from non-alcoholic fatty liver disease.

Materials and Methods

The current research is fundamental and experimental. This research was conducted at the Animal Studies Center of Baqiyatallah University of Medical Sciences. The statistical population of this research consisted of 32 male Wistar rats aged 8 to 10 weeks with a weight range of 230 to 300 grams. The animals were kept in standard laboratory conditions (12 hours of light and 12 hours of darkness) with a temperature of 21 to 23 degrees Celsius. They had free access to water and food. All tests and weighing were done one week after maintenance and adaptation to the new environmental conditions.

Poisoning method: For 4 weeks, all rats were fed a high-fat diet (HFD) with 60% fat and 40% carbohydrates, and in the fifth week, oral tetracycline at a dose of 140 mg per kilogram of body weight (as a solution in 2 ml of water) was fed for 7 days by gavage method by a veterinarian and following the principles of working with animals. Fatty liver (steatosis) was confirmed by measuring liver enzymes and lipid profiles. An increase in the liver enzymes ALT and AST was observed in all groups.

Male rats were randomly divided into 4 groups of 8. The groups were: control group (suffering from fatty liver and receiving enough water and food); supplement group (suffering from fatty liver and receiving probiotic supplement); exercise group (suffering from fatty liver and performing resistance training on a daily basis); and group supplement + exercise (suffering from fatty liver and receiving supplements and performing resistance training on a daily basis).

All animals were weighed at the beginning and weekly using a digital scale. The serum levels of liver enzymes were checked at the beginning, middle, and end of the study. Blood was taken from a vein in the amount of 4 ml in all stages. After the blood collection stage, anesthesia was performed with a 10% ketamine solution at a dose of 5 mg per kg and xylazine at a dose of 2% at a dose of 10 ml/kg. The liver tissue of the rats was then sampled, and after washing with physiological serum, it was placed in 10% formalin for fixation. From the liver tissue samples, 5 micron-thick sections were prepared, and the tissue sections were prepared by hematoxylin-eosin staining. . Stained slides were examined by a pathologist using light microscopy, and all slides of liver tissue sections were recorded and reported for histomorphological changes.

Lactobacillus Reuteri probiotic supplement was purchased in powder form from Faradaro Company. Reuteri probiotic was dissolved in phosphate buffer with a ratio of 1:10, and the number of bacteria was 10^9 cfu in the amount of 0.5 cc for 5 days a week by the gavage method.Training protocol (table 1): In the first week after the induction of non-alcoholic fatty liver, the rats entered the adaptation phase for the implementation of the research project, and they were introduced to resistance training five days a week. Resistance training includes climbing a ladder with a length of one meter and a slope of 50%, and the distance between each step is 2 centimeters. Three days a week, two sets of five ladders were climbed. In the first week without weights, in the second week, the training was done with 15% of the weights. On the first day of the third week, 20% of the mice's body weight was tied under the mouse's chest, and then 50% and 75% of the weight were added for the following weeks in each repetition. The exercises were performed in the form of climbing the ladder for two sets of four repetitions and three days a week (even days) for 8 weeks.

Table 1: Training Schedule

Training week	Number of sessions (per week)	Slope	Weight percent	Repetition	Break between repetition (minutes)
Compatibility	4	50 degrees	0	4	2
1	3	70 degrees	15	4	2
2	3	70 degrees	20	4	2
3	3	80 degrees	50	8	2
4	3	80 degrees	50	8	2
5	3	80 degrees	50	8	2
6	3	80 degrees	75	8	2
7	3	80 degrees	75	8	2
8	3	80 degrees	75	8	2

The weight of the mice was measured at the beginning of the study and at the first of each week (8 weeks) using a scale. The serum concentration of liver enzymes (ALT and AST) at the beginning of the study, after poisoning, the 4th week, and the 8th week was measured by the ELISA method.

Mean (standard deviation) or median (interquartile range) were used to describe quantitative variables according to conditions, and frequency report (percentage) was used for qualitative variables. The Kolmogorov-Smirnov-Smirnov used to check the normality of the data distribution. To compare the mean of the quantitative results between the two studied groups, use the independent t-test or its non-parametric equivalent, i.e., the Mann-Whitney test, and compare the results before and after the intervention within each group using the paired t-test or its non-parametric equivalent, i.e., the Wilcoxon test. The chi-square test, or Fisher's exact test, was used to compare qualitative factors between two groups. A one-way analysis of variance was used to evaluate the difference in quantitative variables. In the comparison of the results between the two study groups, appropriate regression models were used if there was a need to control the baseline variables according to the type of variable. SPSS version 25 software was used for data analysis, and a P-value <0.05 was considered statistically significant.

Results

Figure 1 shows weight changes during the study period in the 4 study groups. The repeated measure ANOVA test showed a significant increase in the weight of all 4 groups (P<0.05). The average of other studied variables at different times between the 4 groups is compared in Table 1. At the beginning of the study, there was a significant difference between the four groups in terms of serum levels of AST (P<0.001). There was no significant difference between the 4 groups in terms of ALT serum level at the beginning of the study (P = 0.385). These differences remained significant after adjusting the effect of weight and the initial level of the desired variable in the ANCOVA model.

After poisoning, there was a significant difference between the four groups in terms of serum levels of ALT (P = 0.004). P = 0). However, this difference in blood sugar lost its significance after adjusting the effect of weight and its initial level in the ANCOVA model. In the 4th week after the start of the intervention, there was a significant difference between the 4 groups in terms of ALT (P = 0.010) and AST (P = 0.003). Of course, in the ANCOVA model, after adjusting the effect of weight and the initial level of the desired variable, the difference between the four groups in terms of ALT lost its significance. In the eighth week, there was a significant difference between the four groups in terms of ALT (P = 0.010) and AST (P = 0.003). Also, in this model, the difference between the 4 groups in terms of AST lost its significance (P = 0.059).

As can be seen in this table, the intra-group difference after poisoning compared to before using the paired sample t-test and at the end of the intervention compared to before the intervention (after poisoning) using the repeated measure ANOVA test has been compared.

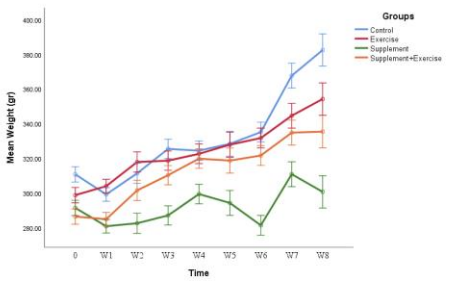

Figure 1: Weight changes in the studied groups during the intervention period. Numbers are shown as mean ± standard error (SE).

Table 2: Comparison of Serum Concentrations of Liver Enzymes (ALT and AST) in Four Groups During the Intervention Period

Variables	Time	Control Group	Exercise Group	Supplement Group	Exercise + Supplement Group	P-value[1]	P-value[2]
ALT	Before Intervention (Study Start)	26.31 ± 7.44	23.50 ± 9.92	20.95 ± 14.76	13.69 ± 13.03	0.385	0.627
	After Poisoning	96.21 ± 11.28	75.39 ± 26.66	130.02 ± 17.63	85.72 ± 22.20	0.004	0.041
	4th Week	105.69 ± 20.46	113.84 ± 12.28	82.44 ± 6.54	94.92 ± 8.99	0.01	0.084
	8th Week	89.85 ± 23.83	56.66 ± 7.95	54.07 ± 5.81	75.38 ± 4.88	0.002	0.003
	P-value[3]	<0.001	<0.004	0.001	0.001	-	-
	P-value[4]	0.535	0.006	<0.001	0.205	-	-
AST	Before Intervention (Study Start)	279.55 ± 33.73	124.58 ± 64.74	198.54 ± 18.22	251.25 ± 51.94	<0.001	0.001
	After Poisoning	179/04± 50.13	161.97± 52.05	211.54 ± 60.60	144.17 ± 37.51	0.229	0.195
	4th Week	112.26 ± 9.69	137.06 ± 6.91	125.88 ± 7.65	127.11 ± 9.42	0.003	0.013
	8th Week	132.19 ± 21.18	157.86 ± 15.30	116.58 ± 34.51	98.78 ± 19.85	0.008	0.059
	P-value[3]	0.021	0.088	0.641	0.030	-	-
	P-value[4]	0.083	0.424	0.014	0.129	-	-

Abbreviations:

ALT: Alanine transaminase
AST: Aspartate transaminase

This table presents a statistical comparison of liver enzyme levels (ALT and AST) across different intervention groups before and after poisoning, as well as during a subsequent intervention period. The P-values indicate statistical significance within and between the groups. Numbers are shown as mean ± standard deviation (SD).

1. calculated by a one-way ANOVA test
2. Calculated by the ANCOVA test by adjusting the effect of weight and the initial level of the target variable (items marked with * are only adjusted in terms of weight).
3. Calculated by a paired sample t-test to compare baseline and post-poisoning levels.
4. Calculated with repeated measures ANOVA to compare variable changes during the study compared to the time after poisoning

Discussion

In epidemiological studies, it was shown that exercise is effective in improving non-communicable diseases such as fatty liver disease, obesity, and metabolic syndrome (11). In addition, in various studies, a direct relationship between the prevalence of fatty liver, physical activity, and sitting time has been reported (1). However, there is currently no definitive and effective treatment for NAFLD. But it has always been stated in various studies that lifestyle changes such as physical activity, healthy eating, and weight loss can control this disease (14 and 15). Aerobic, resistance, and combined training protocols are all effective on liver steatosis in patients with NAFLD (1). In general, the activity of liver enzymes changes under the influence of the duration, intensity, type, and method of exercise, but what is clear is that exercise helps reduce hepatic steatosis (16).

In the present study, weight gain was observed in all groups. In line with the current study, Yagao et al. showed in a systematic review that in the random effects model, BMI does not decrease significantly in fatty liver patients with high BMI after long-term exercise intervention (17). After studying rats with non-alcoholic fatty liver disease, Yuyang et al. showed that only aerobic exercise is effective in reducing weight in sick rats (11). In addition, the results of the present study showed that feeding rats with a high-fat diet increased the serum levels of ALT and AST and caused NAFLD in the rats under study. However, after receiving a normal diet and performing resistance exercises along with probiotic supplementation, a decrease in the serum level of liver enzymes ALT and AST was seen in the researched rats, but this decrease was only in the amount of ALT in the recipient of the supplement alone and moderate resistance exercise to The loneliness of the group and AST in the control group and the group receiving the probiotic supplement were statistically significant.

Many studies have shown the role of intestinal microbiota in the pathophysiology of NAFLD, which is one of the most important pathways, through its effect on bile acids (18). Microbiota can cause NAFLD through different mechanisms, including altering the capacity to extract energy from the diet, affecting denovo lipogenesis and the synthesis pathway of short-chain fatty acids (SCFAs), altering choline and bile acid metabolic signaling pathways, and increasing intestinal permeability. and affect inflammation (3). Dysfunction of the intestinal microbiota and bile acid dysfunction play an important role in the pathogenesis of NAFLD (18). New research shows that manipulation of the intestinal microbiota can be a suitable therapeutic strategy for the treatment of NAFLD, and probiotics in particular have shown a special potential to restore homeostasis and improve diseases related to the intestinal microbiota (3).

In contrast to the present study, Ambrin et al. did not show a significant reduction in serum ALT levels in a study on performing resistance exercises in patients with NAFLD (19). But in contrast, Park et al. observed beneficial effects on the ALT enzyme and improved liver function independent of weight loss (20). In several studies, it has been shown that, because of sports activities, the amount of Bifidobacterium increases and improves intestinal-liver function and decreases the ALT enzyme (11). In addition, 24 weeks of moderate-intensity aerobic training improved liver function in NAFLD patients by reducing the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Understanding 2020 No. 25). In another study, it was also shown that consumption of probiotic supplements is independently effective in reducing the level of ALT in the blood (21).

In the study of Park et al., where acidophilus was given 3 times a day for 1 month to patients with NAFLD, serum levels of ALT and AST were significantly reduced (22). In a study by Monem et al., probiotics reduced oxidative stress and, as a result, decreased serum ALT and AST levels (23). The study by Raf et al. showed that consumption of probiotic yogurt in patients with NAFLD caused a decrease in liver enzymes (24).

In Wang et al.'s study, it was observed that the consumption of probiotic tablets for 3 months reduced the serum levels of ALT and AST (25).

Shahmohammadi et al. found in research that 5 weeks of intense intermittent exercises along with the consumption of probiotics (Lactobacillus rhamnus GG) caused a decrease in the serum level of liver enzymes and can be effective in improving the condition of this disease (26).

The findings of the present research showed that 8 weeks of moderate intensity resistance training and Lactobacillus roteri probiotic supplement alone improved liver enzyme function (especially ALT serum level) in rats with non-alcoholic fatty liver disease. The possible mechanism of liver function improvement can be explained by the fact that exercise training with weight improvement and Lactobacillus Reuteri probiotic with improvement in intestinal-liver function and improvement in bile acid function can improve liver function, which was evident in the form of a decrease in serum ALT and AST levels. Therefore, it can be concluded from this The study concluded that resistance training style and probiotic supplement consumption can probably be useful for people with NAFLD, but we need more studies and human samples in this field to obtain more complete and definitive results.

Acknowledgments

This research is taken from the student thesis of the Islamic Azad University, Science and Research Unit that was done with the cooperation of Animal Studies Center of Baqiyatullah University of Medical Sciences. The author hereby expresses his gratitude to the Islamic Azad University, Research Sciences Unit, and the staff of the Laboratory Animal Studies Center, Baqiyatullah University of Medical Sciences and special thanks to Miss Samaneh Ansari for her sincere cooperation in this project.

Conflict of interest

Author declares no conflict of interest.

References:

1. Hirokazu Takahashi, kazuhiko katani ,kenichi Tanaka,guichiro egucih and keizo anzai.Thrapeutic Approaches to Nonacoholic Fatty Liver Disease : exercise intervention and related Mechanisms . Frontiers in Endicrinoligy 2018;588
2. Atilla Engin. Non-Alcoholic Fatty Liver Disease. Obesity and Lipotoxicity Advances in Experimental Medicine and Biology 2017: 960, DOI 10.1007/978-3-319-48382-5_19
3. Mingfei Yao,Lingling Qv,JanmengLu, Baohong Wang ,Bjorn Berglund,Lanjuan Li . AnUpdate On the Treatment of Non-Alcolic Fatty Liver Disease. Engineering 2021(7).979 686.doi: org/10.1016/j.eng.2020.01.017
4. Dr farokh,ar , farokh,h,aghazadegan p,farokh ,mr .Liver enzimes. Journal of Laboratory Diagnosis2021.195.
5. Lebeaupin, C., Vallée, D., Hazari, Y., Hetz, C., Chevet, E., Bailly-Maitre, B., Endoplasmic Reticulum stress signaling and the pathogenesis of Non-Alcoholic Fatty Liver Disease, Journal of Hepatology (2018), doi: https://doi.org/10.1016/j.jhep.2018.06.008
6. Marta Alonso-Peña, M.; Del Barrio, M.; Peleteiro-Vigil, A.; Jimenez-Gonzalez, C.; Santos-Laso, A.; Arias-Loste, M.T.; Iruzubieta, P.; Crespo, J. Innovative Therapeutic Approaches in Non -Alcoholic Fatty Liver Disease: When Knowing Your Patient Is Key. Int. J. Mol. Sci. 2023, 24, 10718. https://doi.org/10.3390/ijms241310718
7. Mohammad Ranaei, Ali Yaqoubi, Sadegh Cheragh Birjandi. The effect of intense intermittent exercise with the consumption of purslane extract on FGF-21 and insulin resistance in rats with non-alcoholic fatty liver disease (NAFLD). scientific magazine, Shahid Sadoughi University of Medical Sciences, Yazd 1402.
8. Shirin Mal Ganji, Mohammad Javad Ivani, Sara Sohrab Vandi, Seyed Amir Mohammad Mortazavian. Probiotics and their health-giving properties. Iranian Journal of Nutrition Sciences and Food Industries, 7,5. special issue of winter 2013, 579-590
9. Moin Hamidi Hessari, Miyad Bani Tarabi. Probiotics and therapeutic applications. Laboratory and diagnosis quarterly. 2017:42, 61-66
10. Judith Aron-Wisnewsky, Chloé Vigliotti, Julia Witjes, Phuong Le, Adriaan G. Holleboom, Joanne Verheij, Max Nieuwdorpand Karine Clément. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. Nature reviews GastroenteroloGy & HepatoloGy.2020
11. Yong Zou and Zhengtang Qi. Understanding the Role of Exercise in Nonalcoholic Fatty Liver Disease: ERS-Linked Molecular Pathways. Hindawi Mediators of Inflammation Volume 2020, Article ID 6412916, 15 page. doi.org/10.1155/2020/6412916

12. HallsWotth K et al . resistance exercise reduces Liver fat and its mediators in non-alcholic fatty liver disease independent of weight loss .gut 2011;60(9):1278-83

13. Atefeh Barikani, Shahzadpashaipour. Lifestyle and non-alcoholic fatty liver disease: a review study. Iran Nursing Research Journal. 2017; (13) 6: 6. Doi: 10.21859/ijnr-130606
14. .Barikani A, Pashaeypoor S. Lifestyle in non-alcoholic fatty liver: A review. Iranian Journal of Nursing Research. 2019;13(6):39-47.
15. .Lazo M, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, et al. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. Diabetes care. 2010;33(10):2156-63.
16. 36. Sheki, Massoud, Hosseini, Ghorbani, Rezaei Shirazi, Parsian, & Heshmat Elah. (2020). The effect of 8 weeks of intermittent aerobic training along with silymarin supplementation on tissue antioxidant, liver damage and atherogenic indices of male Wistar rats fed with high-fat diet. Metabolism and sports activity, 10(2).
17. Ye Gao1,2, Jiandong Lu, Xinhong Liu, Jingqi Liu, Qirui Ma, Yajun Shi and Hao Su . Effect of Long-Term Exercise on Liver Lipid Metabolism in Chinese Patients With NAFLD: A Systematic Review and Meta-Analysis. Front. Physiol. 12:748517. doi: 10.3389/fphys.2021.748517
18. Minmin Luo , Junbin Yan , Liyan Wu , Jinting Wu , Zheng Chen ,Jianping Jiang ,Zhiyun Chen , and Beihui He. Probiotics Alleviated Nonalcoholic Fatty Liver Disease in High-Fat Diet-Fed Rats via Gut Microbiota/FXR/FGF15 Signaling Pathway. Hindawi Journal of Immunology Research Volume 2021, Article ID 2264737, 10 pages doi.org/10.1155/2021/2264737
19. Ambrin Farizah Babu, Susanne Csader , Johnson Lok , Carlos Gómez-Gallego , Kati Hanhineva , Hani El-Nezami and Ursula Schwab. Positive Effects of Exercise Intervention without Weight Loss and Dietary Changes in NAFLD-Related Clinical Parameters: A Systematic Review and Meta-Analysis. Nutrients 2021, 13, 3135. https://doi.org/10.3390/nu13093135.
20. Jae Ho Park, Nam-Kyoo Lim and Hyun-Young Park. Protective Effect of Leisure-Time Physical Activity and Resistance Training on Nonalcoholic Fatty Liver Disease: A Nationwide Cross-Sectional Study. Public Health 2022, 19, 2350. https://doi.org/10.3390/ijerph19042350
21. Tannaz Eslamparast, Hossein Poustchi, Farhad Zamani, Maryam Sharafkhah, Reza Malekzadeh, and Azita Hekmatdoost. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr 2014;99:535–42
22. 22. 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), 288.
23. 23. Abdel Monem, S.M. Probiotic Therapy in Patients with Nonalcoholic Steatohepatitis in Zagazig University Hospitals. Euroasian J. Hepatogastroenterol. 2017, 7, 101–106. [CrossRef] [PubMed].
24. 24. Nabavi, Raf Raf, Soumi, Homayoni Rad, Aziz, Asghari Jafarabadi, & Mohammad. (2016). The effects of probiotic yogurt consumption on metabolic factors in people with non-alcoholic fatty liver disease. Scienti fic Journal of Kurdistan University of Medical Sciences, 20(6), 12-25.
25. 25. Wu Y, Wang B, Tang L, Zhou Y, Wang Q, Gong L, Ni J, Li W. Probiotic Bacillus Alleviates Oxidative Stress-Induced Liver Injury by Modulating Gut-Liver Axis in a Rat Model. Antioxidants (Basel). 2022 Jan 31;11(2):291. doi: 10.3390/antiox11020291. PMID: 35204173; PMCID: PMC8868294.

26. Saeed Shahmohammadi, Farshad Ghazalian, Hossein Shirvani, Hossein Abednatanzi. The effect of probiotic consumption and intense intermittent exercise on the level of liver enzymes in male rats with steatosis. Journal of Nurse and Physician within War/Winter 2023/ Tenth Year. ISSN: 2645-3940 DOI: 10.29252/npwjm.10.37.6.