



Comparing the Effectiveness of Nutritional Education with and without Physical Activity on Blood Lipids and Liver Aminotransferase Enzymes in Non-Alcoholic Fatty Liver Diseased Men



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ABSTRACT

Aims Fatty liver disease is a type of fat accumulation in the liver parenchyma that can be related to lifestyle. Therefore, this study compared the effectiveness of nutrition education with and without physical activity on blood lipids and liver aminotransferase enzymes in men with non-alcoholic fatty liver disease.

Materials & Methods This study is a randomized clinical trial with a control group, carried out on 80 employees working in the South Pars gas complex in Jam, Iran, suffering from fatty liver. The participants were allocated to four nutrition education groups; a physical activity group, a nutrition education group with physical activity, an education group without physical activity, and a control group (20 people each) using a random block sampling method. In all four groups, 48 hours before the intervention, 3 and 6 months after the intervention, blood lipids and liver enzymes were measured and recorded in a form. Data were analyzed using SPSS 21 software and chi-square statistics, one-way ANOVA, and repeated measures ANOVA test.

Findings Alanine aminotransferase enzyme showed a significant improvement in each of the 3 intervention groups compared to the control group at 3 and 6 months after the research intervention ($p=0.001$). Low-density lipoprotein showed a significant improvement in 6 months, which was related to the group of physical activity combined with nutrition education ($p=0.005$).

Conclusion Exercise and education interventions are more effective over a longer period of time in preventing and treating non-alcoholic fatty liver disease.

Keywords Non-Alcoholic Fatty Liver; Diet; Physical Activity; Education; Lipoprotein; Alanine Aminotransferase

CITATION LINKS

- [1] Effect of a counseling-supported treatment with the Mediterranean diet and physical ...
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- [3] Prevalence of non-alcoholic fatty liver and its related factors ...
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- [10] Non-alcoholic fatty liver disease—a global public ...
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- [26] Effect of 8 weeks of aerobic training and using chicory extractive ...
- [27] Effects of endurance and endurance–strength ...
- [28] Stage of change and motivation to ...
- [29] A Greater Improvement of intrahepatic fat contents after ...
- [30] The effects of eight weeks interval endurance training on lymphocyte ...
- [31] The effect of physical activity on serum lipids, lipoprotein, ...

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fat in the liver, with more than 5% of the liver's total weight being fat [1]. The prevalence of NAFLD worldwide ranges from 8 to 45% [2]. In Iran, the prevalence of mild, moderate, and severe fatty liver disease is estimated at 26.7%, 7.6%, and 0.5%, respectively [3]. This systemic disorder has a multifactorial and complex pathogenesis [3-5]. Decreased physical activity and unhealthy diets in Asian countries have led to a 25% increase in obesity and the prevalence of this disease [6]. The main risk factors for this disease include obesity, insulin resistance, and dyslipidemia [7]. NAFLD, coupled with the obesity epidemic, is estimated to be one of the leading causes of liver-related morbidity and mortality by 2030 [8]. NAFLD can manifest from asymptomatic to fatigue, dyspepsia, right upper quadrant pain, and hepatosplenomegaly and is associated with age, gender, ethnicity, and endocrine disorders [8] as well as metabolic disorders, cardiovascular disease [9], chronic kidney disease, sleep apnea, obesity, insulin resistance, and diabetes [10].

With an increase in hepatic triglyceride concentrations, the lipid profile in patients with NAFL is affected, characterized by a reduced HDL and an increase in LDL [11]. Elevated levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are features of NAFL disease [12]. Therapeutic interventions for fatty liver disease may include insulin sensitizers and incretins, lipid-lowering medications (such as statins), antioxidants including vitamin E, and treatment for vitamin D3 deficiency [13]. Drug therapies have not always been successful in reducing steatosis or liver inflammation or have undesirable side effects such as weight gain [1, 14]; hence, no drug or surgical method has been approved for NAFL treatment [17]. Therefore, early diagnosis and intervention and prevention of complications are proposed as optimal strategies for managing NAFL. Lifestyle modification, including dietary interventions and exercise, remains the primary treatment method for patients with this disease [15].

Treatment focuses on lifestyle modifications such as physical activity and healthy eating habits because body weight and metabolic risk factors associated with metabolic syndrome are controlled [2]. Exercise helps reduce visceral fat mass, increase lipolysis, decrease the release of pro-inflammatory cytokines, and create an anti-inflammatory environment, all of which control NAFL [16]. While exercise has advantages in improving insulin sensitivity and reducing body mass index, dietary interventions may be more effective in improving aminotransferase levels [7]. Excessive food consumption and diets rich in fats or carbohydrates can reduce insulin sensitivity

and lead to non-alcoholic fatty liver disease by increasing serum-free fatty acid levels [17]. Some dietary patterns rich in whole grains, legumes, fruits, vegetables, and dairy products play a fundamental role in this effectiveness. Whole grains have a lower glycemic index and higher fiber content. Calcium content in dairy products contributes to reducing visceral fat. Increased consumption of fruits and vegetables is associated with reduced risk of hypertension, type 2 diabetes, and increased satiety, aiding in weight loss. Conversely, consuming high-energy foods such as high-fat or refined carbohydrate foods can lead to excess calorie intake, resulting in obesity, overweight, and chronic diseases, including fatty liver disease [18].

In Kastagoni *et al.*, exercise alone or combined with dietary intervention improved serum levels of liver enzymes and fat [19]. Gelli *et al.* demonstrated that a Mediterranean diet and a more active lifestyle could be a safe therapeutic approach for reducing the severity of NAFL and related diseases [1]. In Hosseini Kakhk *et al.*, eight weeks of combined aerobic-resistance training and nutrition alone did not significantly affect serum aminotransferases [20]. Shamsaldini *et al.* also found in a study that aerobic and resistance exercises alone could reduce the levels of liver enzymes in patients with non-alcoholic fatty liver disease [12].

Given the significant role of lifestyle interventions in NAFL and the conflicting results of the aforementioned studies, there is a need for more precise evidence regarding the effects of exercise and diet on this disease. Therefore, the present study aimed to compare the effectiveness of six-month nutrition education interventions with and without physical activity on blood lipids and liver aminotransferase enzymes in men with non-alcoholic fatty liver disease.

Materials and Methods

This study is a pre-post clinical trial with a control group (IRCT120180425039409) conducted from December 2018 to July 2019 in 105 employees working in the South Pars Gas Complex in Jam, Iran, diagnosed with non-alcoholic fatty liver disease. The sample size was calculated using tables based on the Cohen formula, considering an effect size of 0.4 and a test power of 80%. For the analysis of variance of four groups, the required sample size was determined to be 16 individuals per group. In addition, considering the sample size in most similar studies [21, 22] as 17 individuals and accounting for a 20% dropout rate, 20 individuals per group were selected, totaling 80 overall. The participants were selected using non-probability convenience sampling. The total population of men with fatty liver was 105, of which 14 were unwilling to participate in the research, and 11 did not meet the criteria. Inclusion criteria were being male, at least 20 years old, and confirming a

diagnosis of non-alcoholic fatty liver disease in the sonography report by a specialist physician (grade 2 non-alcoholic fatty liver disease). Exclusion criteria were receiving nutritional education intervention and regular exercise program in the past year, alcohol and drug consumption, metabolic diseases, endocrine disorders, liver diseases such as viral hepatitis B and C, hypothyroidism, and undergoing surgery for weight loss due to reasons other than the intervention.

They were allocated to the four groups using the block randomization method with equal sizes:

Nutrition Education Group (NE)

Patients received education on healthy dietary patterns and nutrition for non-alcoholic fatty liver disease (NAFLD) by a nutritionist in the researcher's presence. The education method involved group discussions and lectures, and educational materials were provided to the participants as booklets. Additionally, participants were visited by a nutritionist every two weeks (a total of 12 sessions). During each visit, the nutritionist emphasized the patients' adherence to the educational points. The educational booklet included guidance on preventive and therapeutic nutrition for NAFLD, such as recognizing and recommending adherence to a healthy dietary pyramid, daily food group consumption patterns, recommended dietary regimens for reducing calorie intake, and dietary restrictions and limitations.

Physical Activity Group (PA)

Intervention in this group was conducted through practical demonstration and group lectures, showing the skills and types of physical activity. The researcher accompanied the participants in each session to ensure proper exercise performance. An exercise physiology specialist trained all participants, and they were under the researcher's control for six months. The endurance exercise program was based on the recommendations of the American College of Sports Medicine. According to this protocol, aerobic exercise (brisk walking) was performed for 25 weeks, three sessions per week, and each session lasted for 40 minutes (5 minutes for warm-up and 5 minutes for cool-down), resulting in 12 weeks of readiness and preparation. In this way, from the first week of brisk walking exercise on flat ground and in a designated path determined by the exercise physiology specialist and the researcher in Jam County, each session started with 10 minutes of brisk walking at 50% of maximum heart rate and three minutes were added to the duration each week until the end of the twelfth week. From the end of the twelfth week to the twenty-fifth week, the brisk walking program continued steadily (50 minutes). The individual monitored the desired heart rate (exercise intensity) during the 40-minute aerobic exercise during the exercise session. Considering the participants' age and non-athlete status, the exercise

intensity of 50 to 75% of the maximum heart rate was calculated individually for each person according to the formula (maximum heart rate = $\text{age} - 220$)^[23].

Nutrition Education and Physical Activity (NE/PA)

Participants received counseling on nutrition and interventions for physical activity. Thus, individuals in this group received both types of interventions from groups 1 and 2.

Control Group (Cont)

During the study period, participants did not receive counseling on nutrition and physical activity.

Since lipid, lipoprotein, and liver aminotransferase enzyme tests are conducted annually for employees of Pars Jonoubi Gas Complex in Iran, the medical records of all employees were reviewed, and individuals with abnormalities in these tests were selected. Then, a radiology specialist performed ultrasound imaging to diagnose non-alcoholic fatty liver disease (grade 2). Demographic and paraclinical data of the participants were collected in a data collection form. Research on human subjects was conducted according to the Helsinki Declaration. Participants were informed about the research objectives, and written informed consent was obtained from them.

They were also assured that their personal information would remain confidential and not be disclosed. At the end of the study, educational materials were provided to the participants in the control group. No expenses were imposed on the participants.

After a fast of 12 to 14 hours, 10cc of venous blood was drawn, and after serum separation, blood lipid profiles and aminotransferase enzyme levels were measured. Blood lipids (HDL and LDL) were measured using the Immunoinhibition method, total cholesterol, triglycerides, and fasting glucose were measured using the enzymatic-colorimetric method, and AST and ALT enzymes were measured using the IFCC method enzymatic-colorimetrically using the kit (Pars Azmoon; Iran) and the 902 autoanalyzer (Hitachi; Japan) at a wavelength of 340nm and a temperature of 37°C. These measurements were taken 48 hours before the intervention and 3 and 6 months after the intervention. Before commencing the experiment, the device's accuracy was evaluated using the TrueLab N control (Pars Azmoon; Iran). All tests were performed by a skilled technician.

The data were analyzed using SPSS 21 software, employing descriptive and inferential statistics. Before conducting the main analyses, the distribution of scores for outcome variables was assessed using the Kolmogorov-Smirnov test. Given the normal distribution of the outcome variables, the results of repeated measures ANOVA tests were reported, considering a significance level of less than 0.05. It is worth mentioning that the data analysis was conducted blindly.

Findings

No significant differences were found in demographics between the study groups. The mean age was 47.4 ± 7.4 years, 97.5% were married, and 58.8% had educational levels higher than a bachelor's degree. 58.8% were in administrative positions, with the majority having fixed day shifts. A significant difference was only observed in low-density lipoprotein, and no significant differences were observed in total cholesterol and high-density lipoprotein (Table 1). A post hoc test for pairwise comparisons indicates that these differences were

related to the physical activity group coupled with nutritional counseling and were temporally related 6 months after the intervention.

There was no statistically significant difference between groups over time for aspartate aminotransferase. However, a significant difference was observed in alanine aminotransferase among the study groups (Table 2). Post hoc analysis for pairwise comparisons showed that this difference was attributed to all three intervention groups compared to the control group at both measurement times (3 and 6 months) after the interventions.

Table 1. Comparison of the mean of serum levels of total cholesterol and blood lipoproteins between Physical Activity (PA), Nutrition Education (NE), combined (PA/NE), and Control (Cont) groups over measurement times (repeated measures ANOVA)

Parameter	PA	NE	PA/NE	Cont	p-Value
Low-density lipoprotein (mg/dL)					
Before intervention	126.7 \pm 27.6	119.7 \pm 32.2	127.7 \pm 25.9	122.0 \pm 29.8	0.7
3 months later	104.6 \pm 29.8	107.5 \pm 30.4	101.3 \pm 35.5	117.6 \pm 29.9	0.4
6 months later	103.0 \pm 28.3	104.1 \pm 27.8	88.0 \pm 26.7	113.1 \pm 32.2	0.005
High-density lipoprotein (mg/dL)					
Before intervention	40.0 \pm 7.9	41.3 \pm 9.2	42.1 \pm 7.8	41.8 \pm 7.1	0.8
3 months later	42.1 \pm 8.6	42.2 \pm 6.8	43.7 \pm 9.1	43.6 \pm 7.9	0.6
6 months later	43.5 \pm 6.8	48.2 \pm 38.9	45.3 \pm 8.2	41.4 \pm 6.9	0.8
Total cholesterol (mg/dL)					
Before intervention	203.5 \pm 33.8	194.5 \pm 36.6	193.8 \pm 38.3	191.1 \pm 52.3	0.7
3 months later	183.4 \pm 31.9	186.7 \pm 31.8	185.5 \pm 37.0	196.3 \pm 30.4	0.6
6 months later	183.6 \pm 29.1	180.0 \pm 30.7	177.1 \pm 31.7	187.0 \pm 26.3	0.7

Table 2. Comparison of the mean of serum levels of liver aminotransferase enzymes between Physical Activity (PA), Nutrition Education (NE), combined (PA/NE), and Control (Cont) groups over measurement times (repeated measures ANOVA)

Parameter	PA	NE	PA/NE	Cont	p-Value
Alanine Aminotransferase (IU/L)					
Before intervention	39.6 \pm 19.4	44.1 \pm 18.5	48.1 \pm 30.7	52.3 \pm 30.7	0.3
3 months later	40.1 \pm 18.1	37.5 \pm 14.6	33.7 \pm 14.5	57.2 \pm 21.4	0.001
6 months later	36.1 \pm 19.6	32.9 \pm 15.4	29.5 \pm 19.8	57.9 \pm 17.3	0.001
Aspartate Aminotransferase (IU/L)					
Before intervention	24.1 \pm 8.9	27.9 \pm 9.2	33.7 \pm 14.7	30.4 \pm 10.6	0.06
3 months later	24.7 \pm 8.1	26.4 \pm 7.9	24.1 \pm 7.9	30.0 \pm 9.1	0.1
6 months later	28.6 \pm 12.2	27.5 \pm 9.6	23.3 \pm 8.8	31.7 \pm 8.5	0.09

Discussion

There was no significant improvement in the liver enzyme AST, but serum ALT levels in all three intervention groups—physical activity, nutrition education, and combined physical activity with nutrition education—showed significant improvement compared to the control group at 3 and 6 months after intervention.

Regarding the significant reduction in AST and the lack of significant difference in ALT, the findings of this study in contrast with the studies by Behzadi Moghadam [24] and Hallsworth [25], where only AST showed a significant reduction after 8 weeks of exercise, while ALT remained unchanged. In the study by Hassani *et al.*, serum levels of AST and ALT significantly decreased after 8 weeks of exercise in patients with fatty liver disease [26]. In the study by Gelli *et al.*, after 8 weeks of aerobic exercise and using milk thistle extract supplement, all three liver enzymes significantly decreased during treatment in women with fatty liver. Significant improvements were observed in AST, ALT, GGT, HDL, serum glucose, LDL/HDL, TG/HDL, and NAFLD liver fat scores

between the start and end of treatment [1]. The difference in the results of the current study and the aforementioned study could be due to the addition of milk thistle intervention. Additionally, contrary to the current study, Shamseddini *et al.* concluded that aerobic and resistance exercises can reduce liver enzyme levels in patients with non-alcoholic fatty liver disease [12]. However, Skrypnik *et al.* reported that combined strength and endurance training had a greater positive effect on improving liver fat and reducing ALT and AST enzymes than endurance training alone [27]. In the current study, only ALT in the physical activity group showed a significant improvement compared to the control group, while AST did not show a significant improvement. Considering the results of the Skrypnik study and since the exercise in this study was aerobic, it may be inferred that endurance exercise alone is ineffective for improving liver enzymes, and combined strength and endurance exercises should be used. Centis *et al.* reported that patients with non-alcoholic fatty liver disease have little readiness for physical activity change, with 50% categorized as pre-contemplative

or contemplative, meaning resistant to increasing exercise [28]. One of the possible reasons for the lack of improvement in AST in patients under each intervention in this study was that although ultrasound confirmed grade 2 fatty liver in intervention groups, the patient's liver enzymes were within normal range, and we should not expect a further reduction. Furthermore, the researcher did not completely control the participants' nutrition. However, the reduction in AST in physical activity combined with the nutrition education group is evident, which, although not statistically significant, is clinically valuable.

The findings of this study indicate that HDL and total cholesterol did not show significant improvement after the interventions. However, LDL showed significant improvement after 6 months of intervention, which was significant in the physical activity combined with the nutritional education group. Consistent with the present study, Hosseini *et al.* found that combining nutrition and physical exercise significantly affects lipids. In Hosseini's study, the nutrition and exercise group observed a significant reduction in total cholesterol, triglycerides, and LDL [20]. However, Monserrat-Mesquida *et al.* also demonstrated that the Mediterranean diet group combined with exercise had a greater weight loss and reduction in triglycerides compared to the control group [29]. In the present study, after the other interventions of physical activity and nutritional education, improvements in LDL, HDL, and total cholesterol were observed after each of the three interventions. However, these differences were not statistically significant, which can still be clinically relevant. Ghorbanian *et al.* found that fasting blood sugar, HDL, and LDL levels did not show significant differences between the two groups after 8 weeks of training. Still, total cholesterol and triglycerides were significantly lower in the exercise training group compared to the control group [30]. Although some results of this study were similar to the present study, there are differences regarding total cholesterol, triglycerides, and LDL. Since the intensity and volume of exercises significantly impact ApoA-1, HDL, and LDL, this difference can be justified, as the intensity and volume of exercises in the present study were higher and supervised by the researcher, resulting in a significant decrease in LDL. This is supported by the study conducted by Shearman *et al.*, where they demonstrated that using a low-intensity endurance exercise protocol (± 50 to 60% of maximum heart rate) on adult men showed negligible changes in ApoA-1, HDL, and LDL after six weeks. Still, significant changes in both factors were observed after fourteen weeks [31].

Furthermore, in this study, LDL did not show improvement after 3 months. Still, it showed significant improvement after 6 months, indicating that the longer the interventions, the greater the

possibility of improvement in lipoproteins. According to the results of the present study, no significant difference was observed in other blood lipids after the intervention. Lack of complete control over adherence to the diet and even the intensity of exercise may be among the possible reasons for the lack of effect of exercise and nutritional education and their combination on these variables.

In the examination of the timing, in the group receiving physical activity combined with nutritional counseling, there was a significant reduction in low-density lipoprotein (\pm LDL) in the sixth month after the start of the interventions. In contrast, the changes in the third month were not significant. However, ALT showed a significant reduction at 3 and 6 months after the intervention, although this reduction was greater after 6 months. The longer the exercise and nutritional intervention duration, the greater the improvement in LDL and AST. Therefore, maintaining a healthy lifestyle throughout life may contribute to preventing and treating fatty liver.

One strength point of this study was the researcher's control over participants' physical activity due to their collaboration with them. However, the present study also had some limitations. Due to the inability to directly control the participants' nutrition by the researcher, especially the use of self-service and workplace cafeterias, participants did not have the full opportunity to adhere to the nutritional interventions. Another limitation was the lack of initial alignment between ultrasound findings and liver enzymes. Since ultrasound is the gold standard for diagnosing fatty liver, patients were enrolled in the study based on confirmed fatty liver in their ultrasound. It seems that the number of patients studied for this research was low, and there would have been more significant changes in the study's parameters with a larger sample size.

Due to the limitations of the aforementioned study, it is recommended that further research be conducted with a larger sample size in a diverse population. This population should include individuals diagnosed with fatty liver on ultrasound and liver enzyme abnormalities. Therefore, based on the results of the current study, it is suggested that healthcare professionals and health team members implement this protocol, which includes a healthy diet pattern along with regular brisk walking, in educational and preventive programs for individuals predisposed to obesity and fatty liver, as well as patients diagnosed with fatty liver.

Conclusion

Lifestyle improvements, including physical activity and adherence to a balanced diet, can reduce liver enzymes ALT and LDL in patients with non-alcoholic fatty liver disease. When used in combination, especially for a duration of 6 months or more, the interventions will be more effective.

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Authors' Contribution: Jamshidi H±First Author), Main Researcher/Methodologist/Introduction Writer/Discussion Writer±30%); Malekzadeh JM±Second Author), Assistant Researcher/Methodologist/Statistical Analyst±10%); Afrasiabifar A±Third Author), Assistant Researcher/Methodologist/Statistical Analyst/Discussion Writer±10%); Alamdari AK±Forth Author), Main Researcher/Methodologist/Introduction Writer/Discussion Writer±30%); Zamani Habibabad H±Fifth Author) Assistant Researcher/Introduction Writer/Discussion Writer±10%); Hosseini N±Sixth Author), Assistant Researcher/Methodologist/Introduction Writer/Discussion Writer±10%)

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