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Combined Training Attenuates Lipid Dysregulation and Liver Injury Markers in a Rat Model of Diet-Induced NAFLD

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ABSTRACT

Introduction: This study aimed to evaluate the effects of combined exercise training on dyslipidemia and liver injury biomarkers in rats with diet-induced nonalcoholic fatty liver disease (NAFLD).

Methods: Twenty male Wistar rats were allocated to three groups: normal control, NAFLD, and NAFLD plus combined training. NAFLD was induced by feeding the rats a high-fat, high-fructose diet for 15 weeks. After NAFLD was confirmed, the combined training group completed an eight-week exercise program. Serum lipid parameters and hepatic enzyme activities were measured to assess metabolic and hepatic changes. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

Results: Compared with the NAFLD group, rats that underwent combined training showed significant reductions in triglyceride (TG) and aspartate aminotransferase (AST) levels ($P<0.001$). However, combined training did not significantly alter low-density lipoprotein (LDL) or alkaline phosphatase (ALP) levels ($P\geq0.05$).

Conclusion: Combined training improved the lipid profile and reduced markers of liver injury in rats with diet-induced NAFLD, supporting its potential as a nonpharmacological strategy for attenuating NAFLD-related metabolic disturbances. Larger studies are needed to confirm these findings.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is recognized as a prevalent chronic liver disorder that encompasses a broad pathological spectrum, beginning with simple lipid accumulation in hepatocytes (steatosis) and potentially progressing to more advanced conditions, including nonalcoholic steatohepatitis (NASH), fibrotic remodeling, cirrhosis, and ultimately hepatocellular carcinoma. In recent years, the increasing prevalence of NAFLD has attracted considerable scientific attention and created an urgent need to better understand its underlying mechanisms and strengthen strategies for prevention and management. This growing concern underscores the importance of continued research on this condition (1). Previous studies have provided substantial evidence that regular physical activity can improve key pathogenic factors involved in the development and progression of several major metabolic disorders, including hypertriglyceridemia, hyperglycemia, and obesity—conditions that represent considerable public health challenges (2). Most individuals with NAFLD exhibit elevated circulating aminotransferase levels, which serve as important biochemical indicators of hepatic involvement. Among these enzymes, alanine aminotransferase (ALT) is particularly associated with hepatocellular lipid accumulation and the inflammatory responses that accompany NAFLD. This association underscores the role of ALT in the disease process and provides valuable insight into the mechanisms contributing to hepatic injury (3). Metabolic disorders are chronic in nature, and lifestyle modification alone often provides

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limited long-term success. Therefore, identifying safe and sustainable therapeutic options remains an important priority. Current interventions, including increased physical activity, dietary modification, comprehensive lifestyle programs, bariatric surgery, and pharmacotherapy, have demonstrated beneficial effects in the management of obesity (4). Physical activity is widely regarded as a central component in the prevention and management of metabolic disorders, and consistent evidence from both human and animal studies supports its effectiveness. Regular exercise has been shown to reduce body weight, body fat, and hyperglycemia. Experimental models also indicate that combining dietary modification with physical activity can reduce nutrient absorption, visceral fat accumulation, and metabolic disturbances associated with high-fat diets. Collectively, these findings reinforce the role of structured physical activity as an effective strategy for mitigating obesity and its related metabolic complications (5). Additional research has emphasized that physical activity is a primary therapeutic approach for metabolic diseases and has an important role in the management of NAFLD (6). Exercise modalities such as resistance training (RT) and high-intensity interval training (HIIT) are well established because of their beneficial effects on metabolic health and body composition. RT improves muscular strength and lean mass, whereas HIIT is strongly associated with improvements in cardiorespiratory fitness and reductions in adiposity. When combined, these training modalities may produce complementary or synergistic effects and provide individuals with obesity-related conditions with broader and more comprehensive metabolic benefits (7). The beneficial effect of physical activity on reducing hepatic fat appears to depend largely on creating an energy imbalance through increased energy expenditure relative to energy intake (8). Research further suggests that high-volume exercise may provide more substantial benefits than low-volume, high-intensity protocols.

(9). Hashida et al. reported that resistance training (RT)—an exercise modality that uses external loading to improve muscular strength and endurance—could improve NAFLD despite requiring relatively low energy expenditure (10). Mirghani et al. further demonstrated that strength-training sessions performed three times per week for eight weeks were associated with reductions in high-density lipoprotein (HDL) levels and increases in total testosterone levels. Resistance training has also been reported to produce favorable changes in lipid profile parameters (11). Another study indicated that resistance training reduced HDL concentrations, whereas no significant changes were observed in the other biochemical components of the lipid profile or in body mass index (BMI) (12). Morze et al. proposed that combined training (CT) may represent one of the most effective approaches for reducing obesity-related complications; however, empirical evidence regarding its influence on hepatic fat accumulation remains limited (13). Current physical activity guidelines recommend that individuals with metabolic disorders incorporate RT together with moderate- to vigorous-intensity exercise, such as HIIT, to support effective disease management. In this context, HIIT can be integrated into a combined training (CT) program. Nevertheless, the available evidence remains insufficient to determine whether combining RT and HIIT produces reductions in hepatic fat that are comparable with or greater than those achieved by either exercise modality alone.

(14) It was hypothesized that a supervised program combining resistance training and high-intensity interval training (RT+HIIT) would produce greater reductions in hepatic steatosis and greater improvements in the lipid profile and ALT, AST, and ALP levels than the control conditions by enhancing hepatic fatty acid oxidation, after accounting for baseline metabolic risk factors. Accordingly, this study investigated whether CT consisting of RT and HIIT could reduce hepatic fat and improve serum liver injury biomarkers (ALT, AST, and ALP) and lipid profile parameters more effectively than the control conditions in rats with diet-induced NAFLD over the intervention period.

Materials and Methods

Animals and Diet

Twenty-nine male Wistar rats were obtained from the Shahid Mirghani Research Institute (Golestan, Iran). The animals were housed under controlled conditions (20–24 °C; 12:12 h light/dark cycle) with ad libitum access to food and water. After one week of acclimatization, NAFLD was induced according to the protocol described by Eslami et al. (15). Twenty-nine male Wistar rats were sourced from the Shahid Mirghani Research Institute (Golestan, Iran) and maintained under standardized conditions (20–24 °C; 12:12 h light/dark cycle) with ad libitum access to food and water. NAFLD was induced by feeding the rats a high-fat, high-fructose diet (HFFD), as described previously [16, 17]. After 15 weeks of HFFD feeding, five rats were randomly selected for blood sampling to confirm successful NAFLD induction. Following confirmation, the remaining animals were allocated to three groups: (1) normal control (n = 8), (2) NAFLD (n = 8), and (3) NAFLD + combined training (CT) (n = 8). Five Wistar rats were excluded because of injury.

Training Protocol

Rats in the training group completed an eight-week combined training (CT) program with five sessions per week. Each rat performed a maximal running-speed test to determine HIIT intensity, and the mean maximal speed (37 m/min) served as the reference for subsequent HIIT sessions. HIIT intensity was set at 85–90% of each rat's mean maximal running speed, with a 1:1 work-to-rest ratio. The intensity, number of repetitions, weekly frequency, and running speed used for RT and HIIT within the CT protocol are presented in Tables 1 and 2.

The adaptation phase involved ladder-climbing sessions on a wooden ladder measuring 110 cm in height and 18 cm in width and positioned at an 80° incline. A resting platform was located at the top of the ladder, where the rats remained for 2 minutes before beginning the next repetition. During this phase, the animals learned to climb in response to tail stimulation with forceps. The adaptation protocol comprised up to six consecutive sessions over one week, after which external stimulation was no longer necessary. Training was performed in the afternoon five times per week for eight weeks, for a total of 40 sessions. Each session comprised 15 ladder-climbing repetitions. The resistance training (RT) and high-intensity interval training (HIIT) protocols are presented in Tables 1 and 2, respectively [18, 19].

Biochemical Analysis

After a 12-hour fast, the rats were anesthetized 48 hours after the final training session by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) (Merck, Germany). Blood samples were collected from the thoracic cavity and transferred to gel-separator tubes. Serum was separated by centrifugation and analyzed for liver enzyme activities and lipid profile parameters using enzymatic kits (Pars Azmoon, Tehran, Iran) and a BT 3500 autoanalyzer (Medsystem, USA).

Ethical Statement

The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The experimental protocol was approved by the institutional ethics committee (IR.SSRC.REC.1402.121). All procedures were conducted to minimize animal distress and reduce the number of animals used.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Between-group differences were assessed using one-way ANOVA followed by Tukey's post hoc test. Statistical analyses were performed using SPSS software (version 16), and $p \leq 0.05$ was considered statistically significant.

Table 1. Resistance training protocol

	Session	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**
1st week	Repetition	1	1	1	1	1	1	1	-	-
Intensity	%	0%	20%	30%	40%	30%	20%	0%	-	-
2nd week	Repetition	0	1	2	2	2	1	0	-	-
Intensity	%	0%	20%	30%	40%	30%	20%	20%	-	-
3rd week	Repetition	0	1	1	1	1	1	1	1	-
Intensity	%	20%	30%	40%	50%	40%	30%	20%	0%	-
4th week	Repetition	0	1	1	1	1	1	1	1	1
Intensity	%	0%	20%	30%	40%	50%	40%	30%	20%0	0
5th week	Repetition	0	1	1	1	2	1	1	1	1
Intensity	%	0%	20%	30%	40%	50%	40%	30%	20%	0%
6th week	Repetition	0	1	1	1	1	2	1	1	1
Intensity	%	0%	30%	40%	50%	60%	50%	40%	30%	0%
7th week	Repetition	1	1	1	2	2	1	1	-	-
Intensity	%	0%	40%	50%	60%	50%	40%	0%	-	-
8th week	Repetition	1	1	2	3	1	1	1	-	-
	%	0%	40%	50%	60%	50%	40%	0%	-	-

Table 2. High-intensity interval training protocol

Week	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**
Repetition	2	2	3	4	4	5	6	6
Min	2	2	2	2	2	2	2	2
Intensity	90%	90%	90%	90%	90%	90%	90%	90%

Results

Weekly body weight measurements for all groups throughout the eight-week intervention period are presented in Figure 1. No significant between-group differences in body weight were detected during the final four weeks of the intervention ($p > 0.05$). During the first four weeks, rats in the CT group exhibited a decline in body weight after the intervention began, whereas rats in the NAFLD group continued to gain weight relative to the normal control group. Significant between-group differences in mean body weight were observed only during weeks 1–4, when the groups showed clear differences in mean body weight ($p < 0.05$) (Figure 1). The comparative results for the serum biochemical parameters—including TG, LDL, HDL, AST, ALP, and ALT—are presented in Figure 2 and 3. One-way ANOVA identified significant between-group differences in TG concentrations ($p = 0.001$). Significant differences among the groups were also observed for LDL ($p = 0.001$) and HDL ($p = 0.008$) (Figure 2). Analysis of the hepatic enzyme profiles also revealed significant between-group differences in ALT ($p = 0.004$), as well as in AST and ALP (both $p < 0.001$). Post hoc analysis indicated that the normal control group differed significantly from the NAFLD group in TG and LDL levels ($p = 0.001$), as well as in AST ($p = 0.003$) and ALP ($p = 0.002$). Moreover, TG and AST values differed significantly between the NAFLD and NAFLD+CT groups ($p < 0.001$), whereas no significant changes were observed for the remaining biochemical parameters (Figure 3).

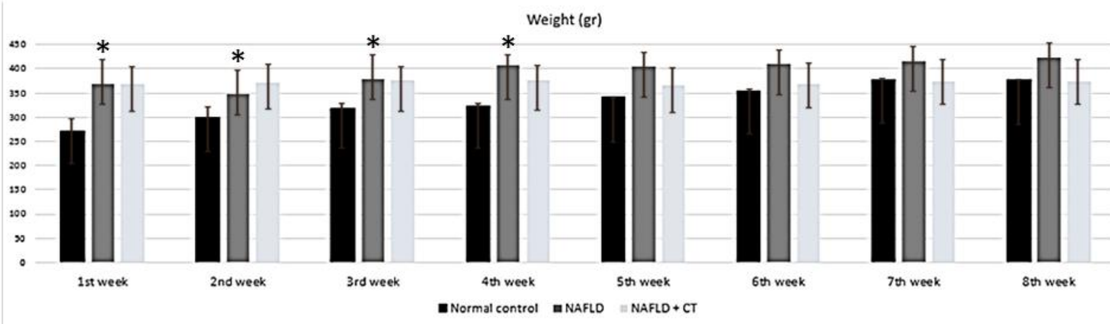

Figure 1. Comparison of body weight (g) among the groups during the eight-week intervention. *$p \leq 0.05$ compared with the normal control group.

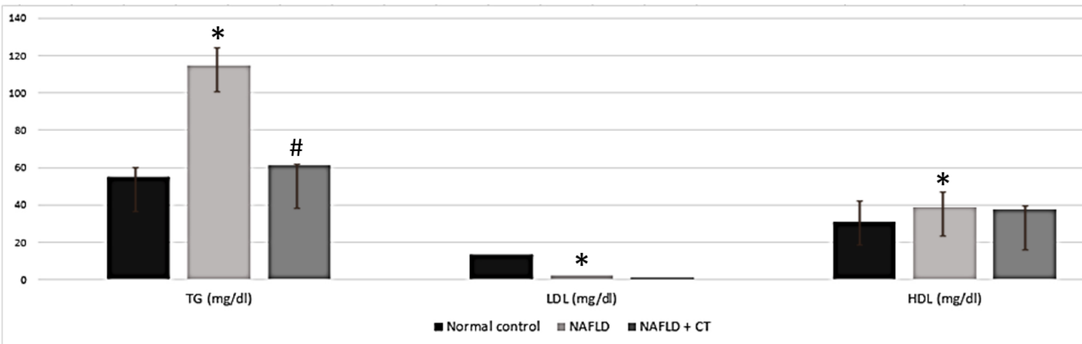

Figure 2. Lipid profiles among the study groups. *$p \leq 0.05$ compared with the normal control group; #$p \leq 0.05$ compared with the NAFLD group.

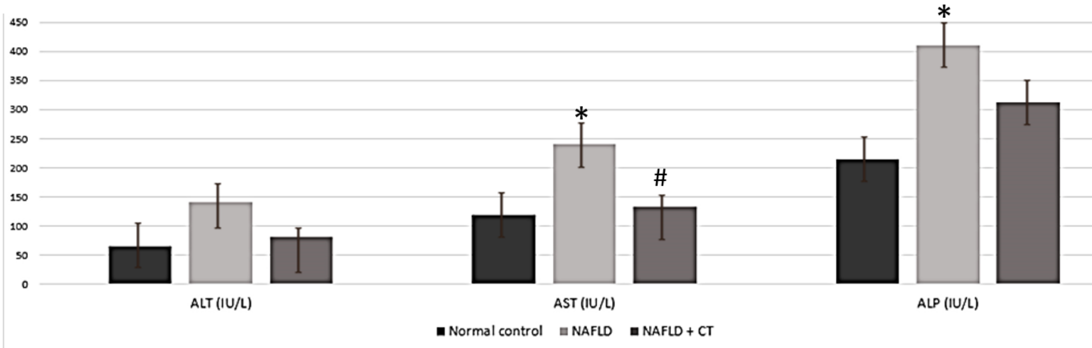

Figure 3. Comparison of hepatic enzyme levels among the study groups. *$p \leq 0.05$ compared with the normal control group; #$p \leq 0.05$ compared with the NAFLD group.

Discussion

Nonalcoholic fatty liver disease (NAFLD) is a prevalent chronic hepatic disorder that frequently co-occurs with cardiovascular disease, metabolic syndrome, type 2 diabetes, and obesity. Current therapeutic recommendations prioritize lifestyle-based interventions, particularly reducing caloric intake and increasing physical activity. In the present study, combined exercise training in rats with NAFLD resulted in clear improvements in lipid-related measures, particularly reductions in TG and LDL, together with favorable changes in liver enzyme profiles. Consistent with these findings, Nowrozpour et al. reported that combined training in older women with NAFLD led to reductions in TG and AST, whereas ALT remained largely unchanged (20). Keshavarzi et al. investigated the effects of a 14-day HIIT regimen in men with NAFLD. In addition to reporting notable improvements in lipid parameters over this relatively short period, their findings raise several points that warrant consideration. First, the rapid changes in lipid markers suggest that HIIT may induce early metabolic adaptations that do not depend solely on meaningful reductions in body weight. These responses may be explained by enhanced skeletal muscle insulin sensitivity, increased skeletal muscle fatty acid oxidation, and upregulated lipoprotein lipase activity. Second, the brief duration of the intervention raises questions regarding the durability of the observed benefits. Transient changes in circulating lipid levels may not persist without continued training, and longer follow-up periods are therefore required to evaluate sustained reductions in cardiometabolic risk. Third, the inclusion of only male participants limits the generalizability of the results; given possible sex-related differences in lipid metabolism and responsiveness to exercise, the findings should be replicated in female and mixed-sex cohorts. Fourth, because body composition, insulin sensitivity, and liver fat assessed through imaging were not measured concurrently, the proposed mechanistic explanations remain indirect. Finally, although the findings support HIIT as a time-efficient approach for improving dyslipidemia in NAFLD, future trials should include longer interventions, standardized HIIT protocols, and integrated metabolic and hepatic outcomes to determine their clinical relevance and establish causality (21). Barani et al. reported no changes in aminotransferase levels after eight weeks of RT or CT in women with NAFLD, although RT reduced ALP. The absence of changes in ALT and AST may reflect limited hepatic effects, the timing or sensitivity of enzyme measurements, or an insufficient intervention dose. Future trials should apply standardized protocols, include imaging or composite hepatic endpoints, control for body weight and dietary intake, and use longer follow-up periods to clarify the inconsistent responses of liver enzymes (22). Long-term interdisciplinary interventions that integrate aerobic and resistance training have been shown to produce greater improvements in non-invasive biomarkers of NAFLD in pediatric populations, particularly biomarkers associated with a higher risk of disease progression. From a mechanistic perspective, combined training may exert these effects by improving whole-body insulin sensitivity, increasing fatty acid oxidation, and producing favorable changes in body composition. Together, these adaptations may contribute to reduced hepatic lipid deposition and attenuation of downstream inflammatory signaling (23). Kakhkand et al. examined the effects of an eight-week program combining aerobic and resistance exercise with dietary modification in individuals diagnosed with NAFLD. Contrary to the findings of the present study, their results indicated that combined training did not produce significant changes in the biochemical markers associated with NAFLD (24). Several studies have emphasized the beneficial role of aerobic exercise in the management of NAFLD. Sargeant et al., in a systematic review and meta-analysis, concluded that exercise reduces intrahepatic triglyceride content and improves hepatic insulin sensitivity. Their analysis supports the inclusion of aerobic exercise as a recommended component of NAFLD management. In accordance with these observations, Myers et al. reported a strong association between habitual physical activity and improved metabolic health, including a substantially lower likelihood of developing NAFLD. Shamsoddini et al. reported that resistance training and aerobic training were similarly effective in reducing hepatic fat accumulation and circulating liver enzyme levels among individuals with NAFLD. Nevertheless, it is important to note that aerobic exercise may provide distinct benefits in NAFLD that occur independently of changes in body weight (25). HIIT has been shown to provide greater benefits than moderate-intensity continuous training in attenuating obesity-related disturbances. In particular, evidence from studies involving obese rats maintained on a high-fat diet indicates that HIIT may be more effective in reducing key biomarkers associated with obesity (26). The findings of the present study are consistent with those reported by Pekkala et al., who observed similar patterns of metabolic and physiological responses (27). Bacchi et al. demonstrated that resistance training and aerobic training produced comparable reductions in hepatic fat content among individuals with type 2 diabetes who also had NAFLD (28). Although both aerobic exercise and resistance exercise have demonstrated benefits in NAFLD, the current evidence supporting combined training remains limited. Consequently, it is still uncertain whether CT produces reductions in hepatic fat that are comparable with—or potentially greater than—those achieved by either training modality alone (29).

Conclusion

Exercise—particularly CT—appears to be a promising nonpharmacological strategy for the management of NAFLD. Integrating structured physical activity into treatment programs may reduce hepatic fat accumulation, improve lipid parameters, and lower liver enzyme levels. These outcomes underscore the important role of regular exercise in both the prevention and management of NAFLD. Further research in human populations is required to confirm observations obtained from animal models and to strengthen the evidence supporting the effectiveness of CT in NAFLD.

Declarations

Funding

None.

Conflict of Interest

The authors declare that they have no conflicts of interest concerning the publication of this article.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Mania Roozbayani], [Saleh Rahmati] and [Lida Moradi]. The first draft of the manuscript was written by [Seyed Ali Mirghani] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Considerations

All protocols in this study were approved by the Committee on the Ethics of Animal Experiments of Fuwai Hospital, Peking Union Medical College and the Beijing Council on Animal Care, Beijing, China (IACUC permit number: FW2010-101523), in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no.85-23, revised 1996).

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Declaration of Artificial Intelligence (AI)

No artificial intelligence tools were used in the preparation of this manuscript.

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