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The Effect of Resveratrol with Interval Training on Oxidative Stress Biomarkers and Liver Enzymes in Men with Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Introduction: As resveratrol and exercise may influence oxidative stress processes in non-alcoholic fatty liver disease (NAFLD), the present study aimed to investigate the effects of interval training combined with resveratrol supplementation on liver enzymes, total antioxidant capacity (TAC), and malondialdehyde (MDA) in individuals with NAFLD.

Methods: Forty-eight participants were randomly assigned to four groups, with 12 participants in each group: 1) resveratrol, 2) training, 3) training plus resveratrol, and 4) placebo. The exercise intervention was performed for eight weeks, three sessions per week. Participants in the resveratrol and training plus resveratrol groups received one 400 mg resveratrol capsule daily for eight weeks. The placebo group received one 400 mg starch capsule daily for the same duration. Liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as TAC and MDA levels, were assessed at pre-test and post-test. Two-way ANOVA and paired-samples t-tests were used for statistical analysis, with the significance level set at $P \leq 0.05$.

Results: Resveratrol supplementation significantly increased TAC ($P = 0.002$) and decreased ALT levels ($P = 0.01$). In addition, training significantly reduced ALT levels ($P = 0.001$).

Conclusion: The findings suggest that resveratrol supplementation and interval training alone may improve some NAFLD-related outcomes by improving liver enzyme status. However, their combination did not appear to exert synergistic effects.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic diseases (1). Its prevalence has been reported to range from 5% to 20% in healthy individuals (2) and exceeds 69% in the overweight population (3). NAFLD is projected to become the leading indication for liver transplantation worldwide by 2030 (4). Oxidative stress and insulin resistance are key risk factors for this disease, and their management is considered fundamental to common therapeutic approaches for NAFLD (5). Insulin resistance can enhance the hepatic uptake of free fatty acids (FFAs) and increase lipolysis, leading to elevated triglyceride synthesis and accumulation (6). Excess delivery of lipids to mitochondria increases the production of reactive oxygen species (ROS), promoting oxidative stress and lipid peroxidation. Therefore, oxidative stress plays a central role in the pathogenesis of NAFLD (7,8). Consumption of antioxidant substances has been shown to be effective in preventing and managing complications related to oxidative stress (9). Resveratrol, or 3,4',5-

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trihydroxystilbene, which is found in red wine, berries, and grapes, is known to exhibit multiple biological activities (10). Its potent antioxidant activity has been demonstrated in various animal studies (11–13). Resveratrol has been reported to increase the activity of antioxidant enzymes (11,14), reduce lipid peroxidation products (13,15,16), and elevate the levels of endogenous antioxidant agents (Oyenihi, 2016). These characteristics make resveratrol a promising candidate for managing NAFLD, partly through modulation of liver enzyme activity. Clinical studies have reported that resveratrol can effectively reduce serum liver enzyme levels (17,18). However, some studies have shown no significant effect of resveratrol on improving liver enzymes (19,20). For instance, Asghari et al. (2018) reported that resveratrol did not enhance oxidative/antioxidative status in individuals with NAFLD (20).

Regular physical activity has been reported as one of the most important factors in protecting various tissues against both acute and chronic diseases. Exercise exerts beneficial effects on aging, lipid peroxidation, inflammation, and apoptosis (21). Park et al. (2016) demonstrated that regular exercise can prevent the generation of free radicals and contribute to the reduction of reactive oxygen species (ROS), inflammation, ischemia, and oxidative stress (22).

Recent studies have shown that aerobic exercise can reduce serum biomarkers of liver injury, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in patients with NAFLD. For instance, Shamsoddini et al. (2015) reported that moderate-intensity treadmill exercise significantly reduced mean AST and ALT levels following an aerobic exercise program in individuals with NAFLD (23). Exercise is an affordable intervention with therapeutic and preventive benefits and can reduce cardiovascular risk factors associated with NAFLD, such as hypertension and diabetes (23). Although previous studies have examined the protective effects of resveratrol (17–20) and exercise training (23–25) separately, evidence regarding the combined effects of resveratrol supplementation and exercise training in patients with NAFLD remains inconclusive. Therefore, the present study aimed to investigate the effects of resveratrol supplementation combined with interval training on oxidative stress markers and liver enzymes in individuals with NAFLD.

Material and Methods

In this study, 48 men diagnosed with NAFLD were recruited. The study protocol was approved by the Ethics Committee of Islamic Azad University of Sanandaj, with the Clinical Trials registration code IRCT20240604062009N1.

Participants

From April to November 2023, 126 men with NAFLD were screened at the internal medicine clinic of Kowsar Hospital in Sanandaj, Iran. Ultimately, 48 patients with a BMI of 25–35 kg/m^2 met the inclusion criteria, which included age between 45 and 60 years and a diagnosis of NAFLD for at least six months before the start of the study. Liver ultrasonography was used to diagnose NAFLD. A single radiologist performed all transabdominal ultrasound assessments of hepatic steatosis at both baseline and the end of the study. The inclusion criteria also included non-smoking status, no alcohol consumption, no use of medications such as lipid-lowering drugs, oral antidiabetic agents, psychotropic and antidepressant medications, hormonal drugs, corticosteroids, or hepatotoxic drugs, and no use of nutritional supplements during the previous three months.

Study Design

All investigators, participants, and the statistician were blinded to group assignments. At baseline, a comprehensive questionnaire was administered to each participant. The 48 men with NAFLD were randomly assigned to four homogenized groups (n = 12 each): resveratrol (RS; age 50.5 ± 3.72 years; BMI 31.4 ± 0.94 kg/m^2), interval training (IT; age 53.0 ± 4.24 years; BMI 31.6 ± 0.68 kg/m^2), resveratrol plus interval training (RSIT; age 51.5 ± 3.55 years; BMI 31.3 ± 0.75 kg/m^2), and placebo (PL; age 50.0 ± 3.31 years; BMI 31.6 ± 0.62 kg/m^2). Participants in the RS and RSIT groups received one capsule per day containing 400 mg of pure trans-resveratrol for eight weeks. The PL group received one placebo capsule daily for the same duration, with each placebo capsule containing 400 mg of maltodextrin.

The training protocol for participants in the IT and RSIT groups consisted of 10 repetitions of 60-second running bouts, performed three times per week according to the overload principle. Training intensity was progressively increased across the eight-week intervention as follows: 40–50% of maximum heart rate during weeks 1–2, 50–60% during weeks 3–4, 60–75% during weeks 5–6, and 75–90% during weeks 7–8. The overload principle was applied by progressively increasing exercise intensity based on maximum heart rate. At the end of each training session, mean heart rate was measured using a Polar heart rate monitor (26,27).

Blood Sampling and Laboratory Assays

At the beginning and end of the study, anthropometric parameters were recorded for all participants. Body weight was measured with participants wearing minimal clothing and no shoes. Height was measured barefoot to the nearest 0.1 cm using a measuring tape. BMI was calculated as body weight in kilograms divided by height in meters squared. Dietary intake was assessed using 24-hour dietary recalls on three non-consecutive days, including two weekdays and one weekend day, at the beginning, middle, and end of the study. The mean energy and macronutrient intakes over these three days were analyzed using Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA, USA). Usual physical activity levels were determined using the short, validated version of the International Physical Activity Questionnaire (IPAQ) at baseline and at the end of the study (28).

Blood samples (10 mL) were collected at baseline and 48 hours after the last training session following an 8-hour overnight fast. AST and ALT levels were measured using enzymatic colorimetric assays with Pars Azmoon kits (Pars Azmoon Inc., Iran) and an automated analyzer (Alcyon 300, USA). Malondialdehyde (MDA) was measured using the thiobarbituric acid reactive substances (TBARS) method, and total antioxidant capacity (TAC) was measured spectrophotometrically using a Randox kit (Randox Laboratories Ltd., UK).

Statistical Analysis

The Kolmogorov–Smirnov test, paired-samples t-test, and two-way ANOVA were used for statistical analysis, with the significance level set at $P \leq 0.05$.

Results

Demographic characteristics of the participants are presented in Table 1. Means and standard deviations of the measured variables are presented in Table 2. The results of paired-samples t-tests showed that serum levels of ALT, AST, and MDA significantly decreased in the RS, IT, and RSIT groups at post-test compared to pre-test ($P \leq 0.05$). In contrast, TAC levels significantly increased at post-test compared to pre-test ($P \leq 0.05$). No significant changes were observed in ALT, AST, MDA, or TAC between pre-test and post-test in the PL group (Table 2).

Table 1. The demographic characteristic of subjects

Variable	RS group (n=12)	IT group (n=12)	RSIT group (n=12)	PL group (n=12)
Age	52.5±3.72	51.5±3.55	53.0±4.24	51.8±3.31
Weight (kg)	95.7±3.57	95.8±6.69	95.5±6.82	97.0±6.94
BMI (kg/m²)	31.4±0.94	31.3±0.75	31.6±0.68	31.6±0.62

Data are expressed as mean ± standard deviation or n (percentage).
RS = resveratrol, IT = interval training, RSIT = resveratrol+interval training, PL= Placebo

Table 2. The results of paired- sample t- test

Variable		Groups			
		RS (n=12)	IT (n=12)	RSIT (n=12)	PL (n=12)
AST	Before	43.7±3.22	43.6±2.55	42.6±3.14	44.6±2.74
	After	41.6±2.49	41.1±3.68	40.7±2.95	44.4±3.05
	P-value	**0.01**	**0.001**	**0.02**	**0.63**
ALT	Before	60.9±2.5	61.3±3.11	61.5±3.34	61.3±2.01
	After	59.1±2.02	58.9±2.74	58.0±2.84	61.0±2.39
	P-value	**0.01**	**0.001**	**0.001**	**0.55**
MDA	Before	2.46±0.38	2.49±0.61	2.56±0.6	2.53±0.46
	After	2.20±0.48	2.22±0.49	2.03±0.5	2.49±0.38
	P-value	**0.008**	**0.23**	**0.006**	**0.54**
TAC	Before	1.37±0.19	1.38±0.22	1.41±0.18	1.39±0.28
	After	1.78±0.34	1.60±0.32	1.81±0.37	1.38±0.28
	P-value	**0.001**	**0.001**	**0.009**	**0.98**

Data are expressed as mean ± standard deviation
RS = resveratrol, IT = interval training, RSIT = resveratrol+interval training, PL= Placebo, AST = aspartate aminotransferase, ALT = alanine transaminase, MDA = Malondialdehyde, TAC = total antioxidant capacity.

The results of two-way ANOVA indicated that both resveratrol ($P = 0.01$, effect size = 0.12) and training ($P = 0.001$, effect size = 0.31) had significant effects on reducing ALT levels, whereas the combined effect of resveratrol plus training was not significant ($P = 0.85$, effect size

= 0.001). Neither resveratrol (P = 0.33, effect size = 0.02) nor training (P = 0.10, effect size = 0.06) had a significant effect on AST levels; similarly, the combination of resveratrol and training was not significant (P = 0.06, effect size = 0.07). For MDA, neither resveratrol (P = 0.09, effect size = 0.06) nor training (P = 0.09, effect size = 0.06) had a significant effect, and the combination also showed no significant effect (P = 0.88, effect size = 0.001). Finally, resveratrol significantly increased TAC levels (P = 0.002, effect size = 0.19), while training (P = 0.25, effect size = 0.03) and the combination of resveratrol and training (P = 0.20, effect size = 0.03) had no significant effect (Table 3).

Table 3. Results of 2-way ANOVA test to review the effects of training and resveratrol on research variables

Variable	Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
ALT	Resveratrol	16.33	1	16.33	6.35	0.01	0.12
	Interval training	52.08	1	52.08	20.25	0.001	0.31
	Resveratrol+interval training	0.08	1	0.08	0.03	0.85	0.001
AST	Resveratrol	4.68	1	4.68	0.96	0.33	0.02
	Interval training	13.02	1	13.02	2.68	0.10	0.05
	Resveratrol+interval training	17.52	1	17.52	3.61	0.06	0.07
MDA	Resveratrol	0.72	1	0.72	2.99	0.09	0.06
	Interval training	0.72	1	0.72	2.99	0.09	0.06
	Resveratrol+interval training	0.005	1	0.005	0.02	0.88	0.001
TAC	Resveratrol	1.05	1	1.05	10.92	0.002	0.19
	Interval training	0.13	1	0.13	1.35	0.25	0.03
	Resveratrol+interval training	0.15	1	0.15	1.65	0.20	0.03

Discussion

In this study, resveratrol significantly decreased ALT levels and increased TAC, while training significantly decreased ALT levels. However, no synergistic effects were observed. In a study by Wang et al. (2023) on NAFLD rats, resveratrol gavage at a dose of 40 mg/kg/day combined with aerobic exercise for six weeks reduced ALT and AST levels (29). In many previous studies, the effects of these factors have been investigated as separate interventions. Faghihzadeh et al. (2014) reported that consumption of 500 mg/day resveratrol for 12 weeks improved ALT and AST levels in patients with NAFLD (17). Similarly, Chen et al. (2015) showed that supplementation with resveratrol at 2 × 150 mg/day for three months reduced ALT and AST levels in patients with NAFLD (18). In contrast, Heebøll et al. (2016) reported that a daily dose of 1.5 g resveratrol for six months did not change liver lipid content or ALT and AST levels (19). Asghari et al. (2018) also reported that resveratrol supplementation at 600 mg/day did not significantly affect serum liver enzyme levels in individuals with NAFLD (20). These contradictory findings may be attributed to differences in the dosage and duration of resveratrol administration, the grade of hepatic steatosis among participants, and patients' baseline metabolic profiles. In the present study, participants had elevated baseline serum liver enzyme levels, which may have contributed to the notable changes observed in these variables (23,30,31).

It has been suggested that the beneficial effects of exercise on NAFLD may be related to several mechanisms, including modulation of mitochondria-mediated apoptosis, attenuation of inflammation and NAFLD-induced oxidative stress, activation of PPAR-γ, enhancement of hepatic autophagy, increased fatty acid β-oxidation, and reduced intrahepatic fat content (32). Resveratrol has been reported to exert antioxidant and anti-inflammatory effects by scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation (20,33). Reducing oxidative stress and free radical production in the liver may improve clinical manifestations and liver enzyme levels in NAFLD. Oxidative stress can damage hepatocytes, thereby promoting inflammation and fibrosis. Therefore, modulation of oxidative stress may improve liver function, potentially attenuate liver injury, and contribute to the normalization of liver enzyme levels (34).

In this study, resveratrol supplementation and interval training reduced ALT levels. Given that the decreases in ALT within the RS and IT groups were significant, it is possible that a longer intervention period or a higher dose of resveratrol may be required to induce more pronounced changes in liver enzymes between the RS and IT groups compared to the PL group. NAFLD is associated with increased ROS

production, lipid peroxidation, and impaired antioxidant defenses (35). Total antioxidant capacity (TAC) provides an integrated measure reflecting overall antioxidant status rather than the sum of individual antioxidants (36). Malondialdehyde (MDA), a biomarker derived from the peroxidation of polyunsaturated fatty acids, is widely used to assess oxidative stress in biological samples. Elevated plasma concentrations of MDA have been reported in patients with NAFLD (37). Both MDA and TAC are valuable indicators for evaluating oxidative stress in the body (38). A clinical trial by Hadavi et al. (2018) on women with metabolic syndrome indicated that eight weeks of aerobic training combined with resveratrol supplementation at 400 mg/day increased TAC levels and attenuated oxidative stress (39). Similarly, another study reported that 500 mg of resveratrol for 30 days improved TAC levels (40). However, Chachay et al. (2014) reported that 3000 mg/day of resveratrol in subjects with NAFLD did not alter serum TAC levels or antioxidant enzyme activity (41).

Both resveratrol and regular exercise have been demonstrated to exert anti-inflammatory and antioxidant effects, likely enhancing the antioxidant defense system and providing anti-inflammatory benefits. Exercise has been reported to reduce pro-inflammatory cytokines, such as IL-1β and TNF-α, as well as reactive oxygen species (ROS) production by enhancing antioxidant activity (42). It has been proposed that exercise initially activates the mitogen-activated protein kinase (MAPK) pathway, which subsequently triggers the NF-κB pathway, ultimately increasing the expression of antioxidant enzymes (43). In the present study, no significant changes in MDA levels were observed among the four groups at the end of the trial. The study had several strengths, including a high compliance rate and a randomized, double-blind, placebo-controlled design, which minimized potential bias and confounding. Liver ultrasonography was employed as the diagnostic criterion for NAFLD. Ultrasonography is a preferred non-invasive, cost-effective, and accessible method for screening fatty liver in both clinical trials and population-based studies and is considered a primary diagnostic tool. However, this study had some limitations, including the short intervention duration of eight weeks and administration of a fixed dose of resveratrol. Consequently, the study could not evaluate the effects of varying doses of resveratrol or assess gene expression levels of the variables under investigation.

Conclusion

It appears that resveratrol and training alone can improve NAFLD-related outcomes by enhancing liver enzyme profiles; however, no synergistic effects were observed.

Declarations

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Conflict of Interest

The authors declare no conflict of interest

Author Contributions

F.N. was responsible for implementing the experimental protocol, providing financial support for the study, and preparing the original draft of the manuscript. Z.E. and Kh.M. supervised the study and monitored the implementation of the research protocol. F.N., Z.E., and Kh.M. jointly contributed to the revision, editing, and final approval of the manuscript.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Islamic Azad University of Sanandaj, with the Clinical Trials registration code IRCT20240604062009N1.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of Artificial Intelligence (AI)

The authors declare that no artificial intelligence (AI) technologies or AI-assisted tools were used in the design, writing, editing, or preparation of this manuscript.

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