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Aerobic-Resistance Training with Royal Jelly Supplementation has a Synergistic Effect on Paraoxonase 1 Changes and Liver Function in Women with NAFLD

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Abstract: Introduction: Nonalcoholic fatty liver disease (NAFLD) is a clinical pathological syndrome characterized by steatosis and fat accumulation in liver parenchymal cells without a history of excessive alcohol drinking in a patient. Currently, there is no definitive treatment for NAFLD, and its prevalence increases with age, obesity, and after menopause. Among the ways to treat it, we can mention regular sports exercises and the use of natural supplements. Therefore, the aim of this research is to investigate and compare the effect of aerobic-resistance training with royal jelly supplementation on changes in paraoxonase 1, oxidized LDL, liver function and lipid profile in postmenopausal women with non-alcoholic fatty liver disease. **Methods:** This semi-experimental study on 23 women with non-alcoholic fatty liver disease with average weight (71.34 ± 11.63 kg), age (48.54 ± 3.88 years), body mass index (27.63 ± 4.20 kg/m2) who were randomly Two groups of exercise+supplement (n=12) and exercise+placebo (n=11) were divided; done Both groups performed 8-station resistance exercises (8-12 repetitions in 2-4 sets) for 8 weeks, 3 sessions per week (for 35-40 minutes, from 10-15 RPE) and then From 10-15 minutes of active rest, they performed aerobic exercises with an intensity of 40-85% of the target heart rate, in two-minute intervals with 45 seconds of active rest. Royal jelly supplement (500 mg on training days, before each training session) was consumed. Blood sampling was done before and 48 hours after the last training session. Statistical analysis was performed using variance test with repeated measures (two groups x two stages of pre-test-post-test) in SPSS software with a significance level of $p<0.05$. **Results:** The results of the statistical analysis showed that the effect of eight weeks of exercise+supplement and exercise+placebo on PON1, oxLDL, lipid profiles (HDL, LDL, TC and TG) and liver enzymes (ALT, AST) in women with fatty liver non-alcoholic, there is a significant difference ($P<0.05$). The results showed a significant increase in PON1 ($P=0.008$) and HDL ($P=0.005$) in the exercise+supplement group compared to the exercise+placebo group. But a significant decrease for oxLDL ($P=0.031$), TC ($P=0.045$), TG ($P=0.013$), LDL ($P=0.027$), ALT ($P=0.015$) and AST ($P=0.009$) was observed in the exercise+supplement group compared to the exercise+placebo group. **Conclusion:** Based on the results, it can be concluded that aerobic-resistance exercises with the addition of royal jelly can probably be an efficient and recommended strategy to minimize the harmful effects of non-alcoholic fatty liver disease by affecting the activity of liver enzymes. paraoxonase 1, LDL oxidation and lipid profile. However, in order to obtain more accurate scientific evidence, it is necessary to investigate more doses and timing of royal jelly in future studies.

Keywords: aerobic-resistance exercises; royal jelly; paraoxonase 1; oxidized LDL; liver function; lipid profile; postmenopausal women with non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinical pathological syndrome characterized by steatosis and the accumulation of fat within the parenchymal cells of the liver, occurring in the absence of a history of excessive alcohol consumption in the affected individual [1]. Currently, there is no definitive treatment available for NAFLD [2]. The prevalence of this condition increases with advancing age, obesity, and post-menopausal status, being reported to be twice as common in post-menopausal women [3]. During menopause, a deficiency of estrogen contributes to the accumulation of visceral fat, elevated triglyceride and cholesterol levels, and a reduction in energy expenditure, ultimately leading to enhanced lipogenesis, fat accumulation in the liver, and the onset of inflammation [4].

Increased oxidative stress and inflammation are pivotal contributors to the onset and progression of liver disease. One important biomarker indicative of the severity of liver dysfunction or non-alcoholic fatty liver disease is Paraoxonase, specifically the activity of the enzyme Paraoxonase-1 (PON1) [5]. The PON1 enzyme functions as an antioxidant, mitigating oxidative stress through the hydrolysis of lipoperoxides. Given the elevation of beta-oxidation of fatty acids, alongside the production of lipid peroxides and oxidative stress associated with non-alcoholic fatty liver disease, the significance of the PON1 enzyme becomes evident [6]. Furthermore, research indicates that the activity of this enzyme may play a crucial role in preventing the progression of liver damage from steatosis to advanced stages of fatty liver [7].

Given that both lipid metabolism and oxidative stress are significant components of the pathogenesis of non-alcoholic steatohepatitis (NASH), it is not surprising that these processes are interconnected and result in the production of oxidized inflammatory lipids that adversely affect liver health. One of the most extensively studied examples of an oxidized lipoprotein associated with inflammation is oxidized low-density lipoprotein (oxLDL). OxLDL is widely regarded as a toxic compound capable of eliciting detrimental inflammatory responses in the liver and other related organs. The mediation of these inflammatory responses induced by oxLDL occurs through several fundamental mechanisms, which can ultimately lead to the progression of liver damage [8]. On the one hand, the utilization of supplements, medications, and the adherence to a healthy lifestyle can serve to prevent the progression of diseases and promote individual improvement. Recently, due to the adverse side effects associated with conventional medications, a significant number of researchers and health specialists have shifted their focus toward the application of natural supplements [9], among which royal jelly is considered one of the most significant. Royal jelly is a viscous, lemon-colored substance that is water-soluble and exhibits acidic properties. It is secreted by the hypo pharyngeal and mandibular glands of young worker honeybees and is consumed by the larvae within the hive for a limited duration and by the queen bee throughout her lifespan [10]. Royal jelly contains essential compounds such as proteins, sugars, lipids, amino acids, vitamins, and minerals. Furthermore, it possesses a diverse array of medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, anti-allergic, anti-tumor effects, and protective effects on the immune, inflammatory, and nervous systems [11].

Exercise is not only one of the foundations of a healthy lifestyle but also a critical factor influencing PON1 activity [11]. Research has demonstrated that aerobic exercise reduces the expression of pro-inflammatory markers in the liver and promotes a shift in Kupffer cells towards a more anti-inflammatory phenotype via metabolic reprogramming [12,13]. Resistance training can also induce beneficial changes in the health of visceral adipose tissue (VAT) by remodeling the extracellular matrix, decreasing the size of adipocytes, lowering inflammatory markers, and increasing muscle mass [14]. In the context of non-alcoholic fatty liver disease (NAFLD), regular moderate-intensity resistance exercise performed three times a week for at least 30 minutes per session over three consecutive months has been shown to reduce VAT, total cholesterol (TC), and glucose levels [12]. Engaging in regular physical activity can lead to decreased liver fat, increased energy expenditure, enhanced lipid oxidation, reduced abdominal fat tissue, and improved transport of fatty acids to the liver [15]. Notably, a 13% reduction in liver fat was reported in participants with non-alcoholic fatty liver after eight weeks of resistance training, occurring without any concomitant weight loss [16].

However, existing literature indicates that studies examining combined exercise interventions in individuals with NAFLD remain limited [17]. Resistance training [18] has yielded sometimes

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contradictory results. For example, Ghasemi et al. (2017)[19] investigated the effects of 16 weeks of aerobic exercise at an intensity of 65-70% of target heart rate for 45 minutes, three days a week, on the activity of the enzyme PON1, arylesterase, and lipid profile in postmenopausal women. The findings indicated that 16 weeks of aerobic exercise resulted in a significant increase in PON1 and high-density lipoprotein (HDL) activity levels, along with a significant reduction in TC and very-low-density lipoprotein (VLDL). However, no significant effects were observed in low-density lipoprotein (LDL) and triglyceride (TG) levels. Additionally, Ahmadi et al. (2016) [17] compared the effects of eight weeks of aerobic and resistance exercise on the activity of the PON1 enzyme, aryl esterase, and lipid profiles in obese women. In this study, 30 obese women were randomly assigned to three equal groups: aerobic exercise, resistance exercise, and a control group. Aerobic exercise was performed on a treadmill at an intensity of 60 to 75% of heart rate reserve, while resistance exercise was conducted at an intensity of 55 to 75% of one-repetition maximum (1RM) over eight weeks. The results indicated that aerobic exercise significantly impacted PON1 enzyme concentration in obese women; however, no significant differences were found between the two exercise modalities regarding TC, TG, and LDL levels in plasma among the obese women. Furthermore, plasma HDL levels significantly increased, yet no significant differences were observed for plasma VLDL levels. In a study conducted by Banitalebi et al. (2018) [20], 10 weeks of combined training resulted in a significant reduction in visceral fat, liver fat accumulation, and improvements in the patients' condition. Therefore, combined exercise is recommended for enhancing fatty liver conditions in women.

The utilization of natural products, particularly royal jelly, has recently garnered significant attention in the academic community [21,22]. Numerous clinical studies have investigated the antioxidant properties, improvements in lipid profiles, and anti-inflammatory effects associated with royal jelly [23]. However, despite the extensive body of research, no studies have concurrently examined the effects of combined exercise and royal jelly supplementation on these parameters in patients diagnosed with non-alcoholic fatty liver disease. Therefore, the objective of this research was to investigate the effects of an 8-week regimen of aerobic-resistance training, with and without royal jelly supplementation, on alterations in Paraoxonase 1, liver function, oxidized low-density lipoprotein (OxLDL), and lipid profiles in women suffering from non-alcoholic fatty liver disease.

Research Methodology

The current study employed a semi-experimental, applied design utilizing a pre-test-post-test framework. The target population comprised women in Mashhad diagnosed with stage one non-alcoholic fatty liver disease. Participants were recruited through a public announcement and subsequently confirmed by a medical professional. A total of 30 individuals were selected based on specific inclusion criteria, which included: general health status, the ability to engage in physical exercise, an age range of 45 to 55 years, a diagnosis of stage one non-alcoholic fatty liver disease, a minimum of one year post-menopause, and the absence of any dietary or exercise regimen for the preceding six months. Initially, based on previous studies, to determine the sample size from the G power software, taking into account the effect size components of 0.3, the alpha level of 0.05, and the power of the test at 0.95, the number of 24 people was considered; however, this number was increased to 30 to accommodate potential attrition. Participants were then randomly assigned to two groups: 1) a combined exercise group receiving a placebo and 2) a combined exercise group receiving royal jelly supplementation. During the exercise program, seven participants were excluded due to unforeseen circumstances, such as illness, personal issues, or irregular attendance at training sessions. Ultimately, data were collected and analyzed for 23 individuals (12 in the supplementation group and 11 in the placebo group). This study was conducted with ethics approval under the identification code IR.HSU.REC.1402.024 from Hakim Sabzevari University. To monitor the dietary intake of the participants, a 24-hour dietary recall questionnaire was administered at baseline, one week prior to the commencement of the study, and during the final week of the intervention. Following participant selection, comprehensive information regarding the exercise programs and evaluations was provided during an orientation session, and participants completed a consent form as well as the relevant questionnaires. A blood sample was collected one week prior to the initiation of the first training session and again during the post-test phase. The combined exercise program spanned eight weeks, with three sessions conducted each week. Additionally, 500 mg capsules of a uniform color were utilized for both the supplementation and placebo groups. The supplementation was administered

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in a double-blind manner, with 500 mg of royal jelly contained within the oral capsules, while similar capsules filled with starch powder were designated for the placebo group. Both the supplement and placebo were consumed by the participants one hour prior to each training session.

Exercise Protocol

The order of exercise in this study included resistance exercises with elastic bands and then aerobic exercises on a treadmill. Each exercise session commenced with a general warm-up lasting 10 minutes (Includes 5' walking, 5' specific dynamic mobility), followed by a resistance training regimen utilizing elastic bands for 35 to 40 minutes in a circuit format. After a 10 to 15-minute active rest period, aerobic exercises were conducted on a treadmill, culminating in a 5-minute cool-down (Includes stretching movements for upper and lower limbs). The resistance training program encompassed exercises targeting both upper and lower muscle groups, including elbow flexion while seated, elbow extension while standing, upper body extensors (chest and triceps), upper body flexors (latissimus dorsi and trapezius), calf raises while lying down, knee flexion while lying, hip abduction and adduction while standing, and ankle flexion and extension while seated, and time under tension/velocity for each movement were "2s eccentric, 1s isometric, 1s concentric". The load and intensity of the exercises were progressively increased throughout the intervention. The intensity for each stage was determined based on the color of the bands and the Rate of Perceived Exertion (RPE) scale [10-15], and when +1 set, when band change. This progressive overload approach ensured that participants were adequately challenged while maintaining safety in their workout regimen. Table 1 presents details on the specific resistance levels associated with different band colors and their corresponding RPE scores. 1 week of familiarization with the correct way of movements and RPE was done. The initial resistance load for all participants was established using yellow elastic bands. Every two weeks, the training program was reviewed, and the band color was adjusted based on individual variations in performance and progress. During the aerobic phase, exercises were executed in 2-minute intervals, followed by 45 seconds of active rest, until participants reached 40% of their target heart rate on the treadmill.

Over the eight-week duration, participants attained 70-85% of their predicted maximum target heart rate as calculated using the Karvonen formula. In addition to monitoring the increase in intensity through heart rate assessments, the number of repetitions progressed from 8 to 12, tailored to each participant's ability and fitness level throughout the intervention. The additional load was adjusted according to the participants' tolerance and progress, ensuring that the perceived exertion score, measured using the Borg Rating of Perceived Exertion (RPE) scale, was maintained within the range of 10 to 15 (as illustrated in Table 2). This systematic approach facilitated appropriate and safe progression for all participants involved in the study.

Table 1. Resistance training program.

Week	Repetition	Sets	Active rest (stretching movements between each station)	Rest between sets	Color of the elastic band	RPE
1	8 - 12	2	60 - 90 seconds	90 seconds	Yellow	10-11
2	8 - 12	2	60 - 90 seconds	90 seconds	Yellow	12-13
3	8 - 12	3	60 - 90 seconds	90 seconds	Green	12-13
4	8 - 12	3	60 - 90 seconds	90 seconds	Green	13-14
5	8 - 12	3	60 - 90 seconds	90 seconds	Blue	13-14
6	8 - 12	4	60 - 90 seconds	90 seconds	Blue	14-15
7	8 - 12	4	60 - 90 seconds	90 seconds	Red	14-15
8	8 - 12	4	60 - 90 seconds	90 seconds	Red	14-15

Table 2. Aerobic exercise program.

Week	1	2	3	4	5	6	7	8
Target maxim	70	70	75	75	80	80	85	85

um heart rate* (percentage)								
Set and repeat	5×2	6×2	7×2	8×2	9×2	10×2	11×2	12×2
Active rest between sets (with 40% of target heart rate)	45 seconds	45 seconds	45 seconds	45 seconds	45 seconds	45 seconds	45 seconds	45 seconds

* Target heart rate =(Reserve heart rate x training intensity percentage) + Resting heart rate. Reserve heart rate= Resting heart rate - Maximum heart rate .

Blood Sampling

Blood samples were collected between 7-9 AM in the morning after 12h fasting one week prior to the initial exercise session and 48 hours following the final training session to mitigate any potential short-term effects of exercise. Pre-test standardization included no exercise, standard meals, 8h sleep. A total of 10 milliliters of blood was extracted from the antecubital vein. The samples collected in tubes containing blood anticoagulant with a concentration of 3 to 4 mg/ml ethylene amine tetraacetic acid were quickly centrifuged (at a speed of 2000 rpm for 10 minutes) and then the obtained plasma was kept in a freezer until the time of testing. They were kept at minus 80 degrees Celsius. Finally, plasma was prepared for the analysis of total cholesterol (TC), high-density lipoprotein (HDL), oxidized low-density lipoprotein (oxLDL), low-density lipoprotein (LDL), triglycerides (TG), Paraoxonase 1 (PON1), and liver enzymes (ALT and AST). The plasma samples were subsequently stored at -80°C until analysis. The levels of TC, HDL, oxLDL, LDL, and TG were quantified using an automatic chemistry analyzer in conjunction with enzymatic assay kits. The concentration of PON1 and liver enzymes was determined using enzyme-linked immunosorbent assay (ELISA) methods with specific human kits sourced from ZellBio, Germany, which possess a sensitivity range of 5 to 150 nanomoles per liter. This standardized methodology ensured accurate measurement and reliable results for the biochemical markers of interest in the study.

Statistical Analysis

Data were analyzed utilizing both descriptive and inferential statistics. To assess the normality of the data distribution, the Shapiro-Wilk test was employed. Variance homogeneity was evaluated using Levene's test. In the inferential statistics section, a repeated measures ANOVA (two groups × two stages of pre-test and post-test) along with Bonferroni's post hoc test were conducted to analyze the data. All calculations were executed using SPSS software version 26, with a significance level set at 0.05 ($P < 0.05$) and 95% confidence interval. The outcomes of the Shapiro-Wilk test and Levene's test indicated that the data distribution was normal ($P > 0.05$) and that homogeneity of variances among the groups was maintained ($P > 0.05$). The results of the repeated measures ANOVA demonstrated a significant difference between the effects of eight weeks of combined training with supplementation and combined training with placebo on changes in PON1 levels in women with non-alcoholic fatty liver disease. Specifically, the mean values of this enzyme in the exercise plus supplementation group exhibited a significant increase compared to the exercise plus placebo group (8.4% vs. 1.1%) ($P < 0.05$). Similarly, a significant difference was observed regarding oxLDL levels between the studied groups, with the mean values of this index in the exercise plus supplementation group demonstrating a significant decrease compared to the exercise plus placebo group (9.6% vs. 1.3%) ($P < 0.05$) (see Table 3 and Figure 1).

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Table 3. Results of statistical test analysis for variables PON1and oxLDL.

Variable	time effect			Time × group interaction effect		
	F value	P value	Effect size (n^2_p)	F value	P value	Effect size (n^2_p)
PON1 (ng/mL)	14/24	0/001	0/404	8/71	0/008	0/293
oxLDL (ng/L)	3/26	0/085	0/134	5/34	0/031	0/203

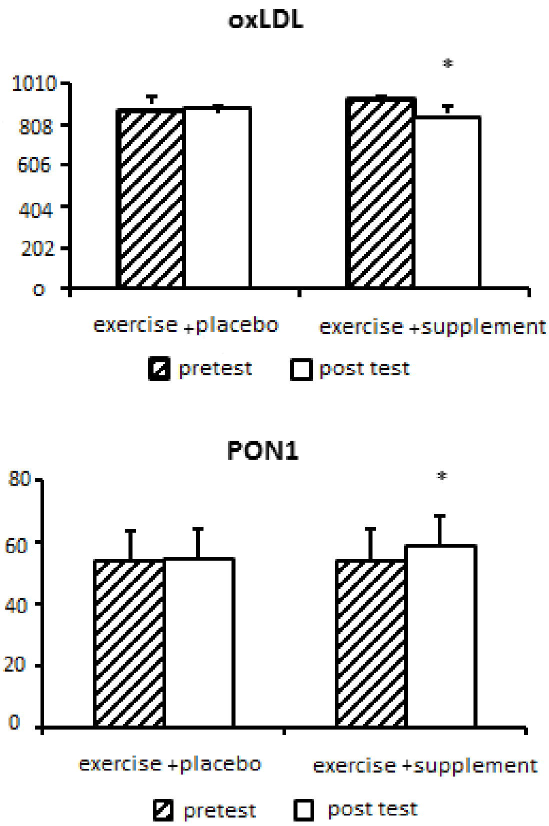

Figure 1. Changes of PON1 and oxLDL (means ± SD) in two groups following eight weeks of intervention. *indicates P>0.05.

The results of the statistical analysis indicated a significant difference between the effects of eight weeks of combined training with supplementation and combined training with a placebo on the lipid profile (HDL, LDL, TC, and TG) in women diagnosed with non-alcoholic fatty liver disease (P > 0.05). The mean values for LDL, TC, and TG in the exercise and supplementation group exhibited a significant decrease compared to those in the exercise and placebo group (LDL: 22.5% vs. 1%; TC: 13.6% vs. 2%; TG: 23.3% vs. 1.1%). Conversely, the mean HDL values in the exercise and supplementation group demonstrated a significant increase relative to the exercise and placebo group (19.6% vs. 1.6%) (See Table 4 and Figure 2).

Table 4. The results of repeated measures analysis of variance for lipid profile.

Variable			time effect	Time × group interaction effect		
	F value	P value	Effect size (n^2_p)	F value	P value	Effect size (n^2_p)
HDL (mg/dL)	14/06	0/001	0/401	10/01	0/005	0/323
LDL (mg/dL)	5/91	0/024	0/220	5/69	0/027	0/213
TC (mg/dL)	9/95	0/005	0/321	4/54	0/045	0/178
TG (mg/dL)	8/70	0/008	0/293	7/33	0/013	0/259

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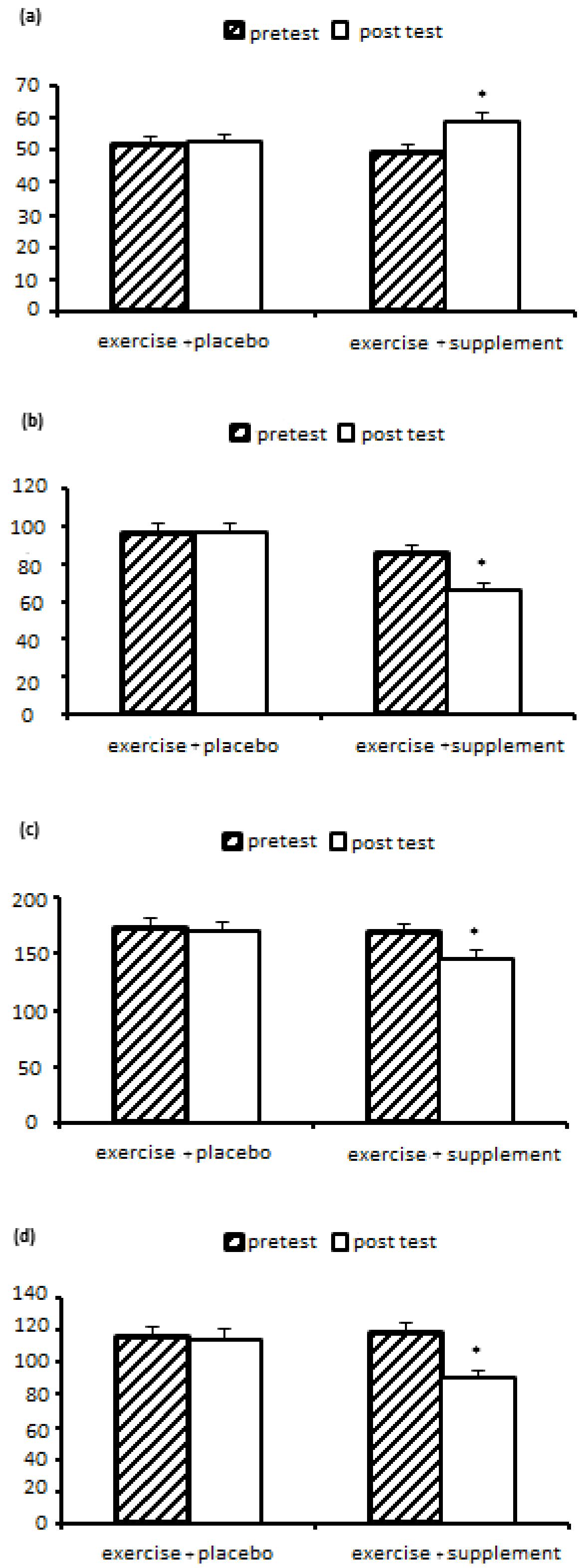

Figure 2. Changes in (a) HDL, (b) LDL), (c) TC and (d) TG (means ± SD) in two groups following eight weeks of intervention. * indicates P>0.05.

The results of the statistical analysis indicated a significant difference in the effects of eight weeks of combined training with supplementation versus combined training with placebo on AST and ALT

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levels in women diagnosed with non-alcoholic fatty liver disease (P < 0.05). Specifically, the mean values of these enzymes in the exercise plus supplementation group demonstrated a notable decrease compared to the exercise plus placebo group (AST: 30.9% vs. 1%; ALT: 26.8% vs. 1%) (See Table 5, Figure 3).

Table 5. The results of repeated measures analysis of variance for AST and ALT.

Variable	time effect			Time × group interaction effect		
	F value	P value	Effect size (n^2_p)	F value	P value	Effect size (n^2_p)
AST (Units per liter)	9/25	0/006	0/306	8/315	0/009	0/284
ALT (Units per liter)	7/69	0/011	0/268	6/98	0/015	0/249

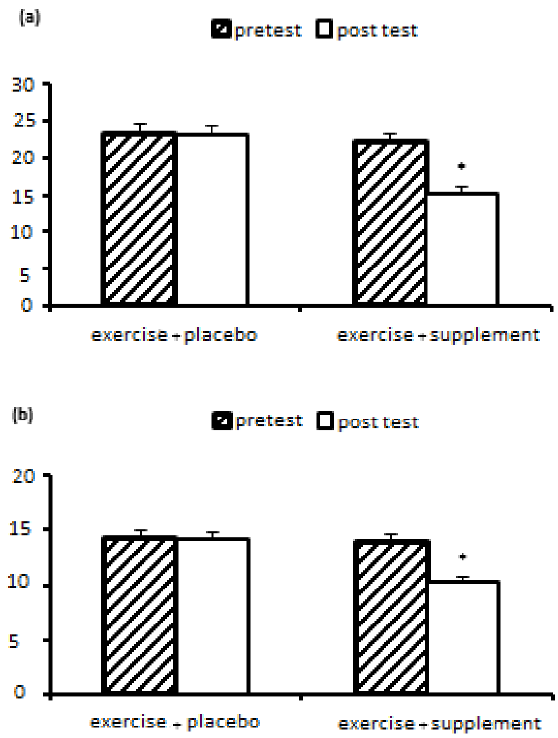

Figure 3. Changes of (a) AST and (b) ALT (means ± SD) in two groups following eight weeks of intervention. *indicates P>0.05.

It is important to note that the mean values of none of the variables associated with energy intake exhibited a statistically significant difference between the exercise plus supplement group and the training plus placebo group. Consequently, the nutritional status of the subjects did not influence the results obtained in this study.

Discussion

The results of the present research indicate a significant difference in PON1 levels among the subjects following the interventions (combined exercise and supplementation). The mean PON1 level in the exercise + supplement group was significantly higher than that in the exercise + placebo group, consistent with the findings of Fatolahi et al. (2017)[24], who observed six weeks of endurance and speed training in rats; Otocka-Kmiecik et al. (2021)[25], who conducted three repeated sessions of intense exercise in healthy men; Bacchetti et al. (2023)[26], who examined seven weeks of resistance training in overweight/obese individuals; and Ghasemi et al. (2017)[19], who reported on sixteen weeks of aerobic training in postmenopausal women. In contrast, these findings are inconsistent with those of Gharakhanlou et al. (2007)[27], who studied eight weeks of vigorous and moderate aerobic exercise in inactive healthy men, and Nalcakan et al. (2016)[28], who investigated aerobic exercise in trained women. The primary antioxidant enzyme carried by HDL particles is PON1 [29]. Empirical evidence suggests that HDLs protect against LDL oxidation, thereby preventing the production of pro-inflammatory oxidized lipids, primarily lipid hydroperoxides and short-chain oxidized phospholipids [30]. Given that PON1 activity is typically reduced in chronic liver diseases, including NAFLD, it has been demonstrated that this reduction is associated with changes in HDL particles, the expression of peroxisome proliferator-activated receptor PPAR δ, and the regulation of monocyte chemoattractant protein-1 (MCP-1) transformation. Consequently, low levels of PON1 may be regarded as a marker of lipid peroxidation and a potential alternative indicator of increased oxidative stress and fibrosis in patients with NAFLD [31]. In the present study, HDL levels increased, and oxLDL levels decreased; therefore, the current findings regarding the increase in PON1 levels are somewhat justifiable. Considering that the administration of royal jelly can mitigate oxidative stress in liver and kidney tissues, which is associated with a reduction in MDA production and an increase in the concentration of cellular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) [32], the further increase in the levels of these enzymes in the exercise + supplement group is thus accounted for.

Another significant finding was the impact of combined exercise and royal jelly supplementation on changes in LDL oxidation among women with non-alcoholic fatty liver disease. This observation aligns with the results of Rezvani et al. (2017) [18], who conducted an 8-week resistance training program with postmenopausal women, Park et al. (2015) [33], who studied 12 weeks of aerobic, resistance, and traditional Korean dance training in obese older women, and Ghorbanian et al. (2021) [34], who examined 8 weeks of rope skipping combined with purslane supplementation in overweight and obese girls. Conversely, these findings do not correspond with those of Sadeghi et al. (2019) [35], who investigated the effects of eight weeks of aerobic exercise on total cholesterol (TC) and oxidized LDL (oxLDL) levels, as well as cardiovascular risk factors in obese and overweight women, and reported no significant changes in oxLDL following the exercise regimen. The discrepancies between the present study's outcomes and those of other investigations may be attributed to factors such as the intensity, duration, and type of exercise, as well as participant characteristics. Numerous antioxidants have been shown to inhibit lipid oxidation. The principal protein in royal jelly (RJMP) exhibits several biological functions, including antibacterial, antioxidant, anticancer activities, and immune modulation. However, there is a scarcity of studies focusing on the effects of royal jelly protein hydrolysates. Some research indicates that royal jelly is rich in bioactive compounds, including 10-HDA, flavonoids (quercetin, naringenin, and galangin), phenolic acids (chlorogenic acid, caffeic acid, and ferulic acid), and essential amino acids, and it also possesses a significant inhibitory effect on oxidative DNA damage and LDL oxidation [36]. According to the researchers' reviews, no study to date has examined the effect of royal jelly on LDL oxidation levels. Nevertheless, based on the results of the present study, it was observed that the average values of this index in the exercise plus supplement group exhibited a significant reduction compared to the placebo group. Therefore, it can be concluded that, in addition to the potential effects of exercise, royal jelly supplementation likely contributes to a reduction in LDL oxidation.

One of the most significant findings of the present study was the observed changes in the levels of low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG), which decreased, alongside an increase in high-density lipoprotein (HDL) in women with non-alcoholic fatty liver disease following 8 weeks of intervention. These results are consistent with the findings reported by Cho et al. (2014) [37], Hojjati et al. (2015) [38], Petelin et al. (2019) [39], and Barani et al. (2014) [40]; however, they diverged from the conclusions drawn by Gharakhanlou et al. (2007) [27]. Some studies indicate that engagement in physical activities, particularly aerobic exercises, and the incorporation of nutritional strategies for weight management can serve as effective interventions for improving lipid profiles. For instance, Cho et al. (2014) [37] investigated the effects of long-term combined exercise on brain-derived neurotrophic factor (BDNF) levels and fat oxidation rates in middle-aged women. Their findings revealed that participants in the aerobic exercise group exhibited significant increases in VO2max and HDL-C after 24 weeks, as well as notable enhancements in muscular strength and significant reductions in TG and TC in both resistance and aerobic exercise groups. In

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contrast, Gharakhanlou et al. (2007) [27] focused on the impact of aerobic exercise on Paraoxonase 1 (PON1) activity, arylesterase (ARE) activity, and serum lipoprotein profiles, reporting no significant changes in LDL-C levels or TC concentrations following aerobic interventions. The discrepancies in these findings may be attributed to variations in sample sizes, gender representation, the statistical populations studied, and the effects of the nutritional supplements utilized in the current investigation.

Royal jelly protein has been recognized as a notable factor exhibiting hypocholesterolemic properties. Royal jelly contains bile acid-binding proteins that confer a hypocholesterolemic effect. Although the influence of royal jelly on human lipoprotein metabolism has been established, the underlying mechanisms remain poorly understood. Some research suggests that as a functional dietary supplement, royal jelly may reduce total blood cholesterol levels and, when used in a long-term regimen, improve LDL and HDL levels [41]. In this context, Petelin et al. (2019) [39] conducted a study to ascertain the effects of royal jelly administration on lipid profiles, satiety, inflammation, and antioxidant capacity in overweight adults, discovering that royal jelly supplementation led to a significant reduction in TC compared to a placebo.

Based on the results of the current study, it can be inferred that combined exercise in conjunction with royal jelly supplementation may positively influence lipid profile levels in postmenopausal women and may represent a viable therapeutic approach for alleviating non-alcoholic fatty liver disease in affected individuals. Another noteworthy outcome of this research was the changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels observed in the exercise plus supplement group, which were significantly greater than those in the exercise plus placebo group. This finding aligns with the results of Kanbur et al. (2009) [42], Bahari et al. (2023) [43], and Valizadeh et al. (2011) [44], but contrasts with the findings of Barani et al. (2014) [40]. In addition to medical and pharmaceutical interventions for treating non-alcoholic fatty liver disease, physical activity and the use of traditional medicines with natural origins possessing anti-lipid and antioxidative properties may serve as beneficial complementary treatments, particularly in the realm of prevention. The advantages of aerobic exercise for individuals with non-alcoholic fatty liver disease have been illustrated in numerous studies [44,45]. For instance, Valizadeh et al. (2011) [44] explored the effects of eight weeks of targeted aerobic training on enzyme levels (AST, ALT) in men aged 20 to 45 years with fatty liver, reporting that the training resulted in reduced AST and ALT levels in the experimental group. Additionally, Xiong et al. (2021) [45] conducted a systematic review and meta-analysis assessing the impacts of various exercise modalities on eight indices in patients diagnosed with non-alcoholic fatty liver disease, revealing that aerobic exercises significantly improved indices including TG, TC, LDL, HDL, ALT, AST, and body mass index (BMI). Resistance training was shown to markedly decrease AST levels, while high-intensity interval training significantly improved ALT levels in this patient population. Conversely, Barani et al. (2014) [40] examined the effects of resistance and combined training on serum liver enzyme levels and fitness indices in women with non-alcoholic fatty liver disease, reporting that the level of alkaline phosphatase (ALP) significantly decreased only in the resistance training group, while no significant changes were observed in AST and ALT levels in the combined and control groups.

Studies indicate that exercise and physical activity, in conjunction with the consumption of appropriate dietary sources, effectively enhance fat metabolism, reduce blood lipids, and subsequently decrease hepatic fat accumulation [46,47]. The protective effects of royal jelly can be attributed to its antioxidant properties, which elevate the levels of hepatic antioxidant enzymes and contribute to a reduction in hepatic steatosis and liver damage [48,49]. For instance, Kanbur et al. (2009) [42] investigated the effects of royal jelly on liver damage induced by paracetamol (PAR) in a murine model. The study demonstrated that PAR significantly elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and malondialdehyde (MDA) compared to the control group. Conversely, significant alterations in the biochemical parameters were observed in the group that received long-term royal jelly supplementation, indicating that royal jelly restored the aforementioned parameters to their normal levels. Bahari et al. (2023) [43] conducted a systematic investigation examining the effects of royal jelly on biochemical alterations in serum and oxidative stress status in the liver and pancreas of streptozotocin-induced diabetic male Wistar rats. They found that streptozotocin-induced diabetic rats exhibited a marked increase in serum levels of AST, ALT, ALP, and fasting blood sugar (FBS), whereas treatment with royal jelly normalized these parameters. According to the results of the aforementioned studies, the group receiving exercise and royal jelly supplementation exhibited lower levels of AST and ALT compared to the exercise plus placebo group. Based on this evidence, it can be concluded that royal jelly may serve as a completely natural adjunct to physical activity, aiding in the reduction of liver enzymes and ultimately representing an effective strategy for mitigating the detrimental effects of non-alcoholic fatty liver disease.

Conclusions

The results of this study demonstrated significant positive changes in the exercise + supplement group compared to the exercise + placebo group across all dependent variables. Consequently, it can be concluded that the combination of exercise and royal jelly supplementation may constitute an effective and recommended strategy for mitigating the detrimental effects of non-alcoholic fatty liver disease, particularly through its influence on liver enzyme activity, paraoxonase 1 levels, LDL oxidation, and lipid profile.

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