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# Effect of pilates training on hepatic fat content and liver enzymes in middle-aged men with non-alcoholic fatty liver disease

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**A** Study Design

**B** Data Collection

**C** Statistical Analysis

**D** Data Interpretation

**E** Manuscript Preparation

**F** Literature Search

**G** Funds Collection

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## abstract

**Background:** The objective of this study is to investigate the effects of eight weeks' Pilates training on the hepatic fat content and liver enzymes in middle-aged men with non-alcoholic fatty liver disease (NAFLD).

**Material and methods:** Twenty middle-aged men with NAFLD were randomly divided into Pilates training (n = 10) and control (n = 10) groups. The Pilates group participated in the Pilates training program for eight weeks (three sessions per week, 60 minutes per session). The body composition, anthropometric indices, liver fat content, and serum levels of ALT, AST, and ALP were measured before and after the training period.

**Results:** After eight weeks of Pilates training, the liver fat content in the Pilates group was significantly lower than that of the control group (P = 0.001). Also, the serum levels of ALT, AST, and ALP significantly decreased in the Pilates group, compared to the controls (P < 0.05). In addition, eight weeks of Pilates training significantly reduced the patients' body weight, body mass index (BMI), fat percentage, and the waist-to-hip ratio (P < 0.05).

**Conclusions:** The results of the present study indicated that Pilates training could be effective in improving the liver fat content and reducing the serum levels of ALT, AST, and ALP in middle-aged men with NAFLD.

**Key words:** pilates, liver fat, liver enzymes, non-alcoholic fatty liver disease.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a growing health concern around the world [1, 2], with an estimated prevalence of 25–35% in Western countries and 19–32% in Asian communities [3]. NAFLD comprises a wide spectrum of liver damage, ranging from simple steatosis to cirrhosis and hepatocellular carcinoma [4]. It is also significantly associated with other comorbidities, such as cardiovascular disease, type II diabetes, and metabolic syndrome [5]. Due to lack of successful and specific treatments for NAFLD, the burden of this disease and its complications is relatively high.

NAFLD is characterized by an increased level of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as increased fat content of liver cells [6]. Although the pathogenesis of NAFLD and determinants of its severity have not been fully elucidated, evidence suggests that a sedentary lifestyle, obesity, and insulin resistance are among the most important NAFLD risk factors [7]. So far, few drug treatments have been proposed for NAFLD, including drugs increasing insulin sensitivity, drugs reducing fat content, and antioxidant drugs for fatty liver; nevertheless, because of their high cost and side effects, their continuous use is not recommended [8]. Currently, lifestyle changes, including dietary modifications and physical activity, are the most important treatment suggestions for patients with NAFLD [7, 9]. In this regard, exercise training is an important component of NAFLD treatment, as approved by the American Gastroenterological Association [10]. Evidence suggests that exercise training, especially when associated with weight loss, can improve liver function and insulin resistance [11].

Pilates training is an effective training method, which has been highlighted in recent years. Pilates, as a holistic exercise involving muscle stretching and enhancement [12], emphasizes the person's breathing rhythm and mental state and strengthens the deep muscles of the body with the least possible damage [13]. Despite some contradictory reports [14], most studies in recent years have confirmed the important role of Pilates training in improving physical fitness [15], body composition [16, 17] and insulin resistance [18]. However, limited research has been conducted on the effects of these exercises on liver enzymes and liver fat content. According to our review, only one study has been carried out by Hagner-Derengowska et al. [11] regarding the effects of Pilates training on ALT and AST enzymes in obese postmenopausal women. The results of this study showed that ALT and AST enzymes did not significantly change after 10 weeks of Pilates training in these women, despite a significant reduction in body weight and BMI [11]. Therefore, it is still unclear whether these exercises have any effects on the liver fat content and enzyme levels of ALT, AST, and ALP in middle-aged men with NAFLD. Considering the increasing prevalence of NAFLD, the widespread acceptance of Pilates training, and limited information about the effects of this type of exercise on the characteristics of NAFLD, the present study aimed at investigating the effects of eight weeks of Pilates exercise on the liver fat content and liver enzymes of middle-aged men with NAFLD.

## MATERIAL AND METHODS

### PARTICIPANTS

In this semi-experimental study with a pretest-posttest control-group design, the statistical population consisted of middle-aged men with NAFLD from Qazvin, Iran. The sample was recruited by advertising in medical and administrative centers. The study sample included middle-aged men (31–49 years old). The main inclusion criterion was diagnosis of steatosis grade 1 or higher (> 5% triglyceride), which was confirmed via ultrasonography with an acceptable diagnostic accuracy. On the other hand, the exclusion criteria were as follows: genetic, metabolic, and endocrine diseases; alcohol consumption; cardiovascular and respiratory diseases; use of lipid-lowering drugs or dietary supplements; and lack of regular exercise in the past six months.

The sample size was determined based on previous studies, using the infinite population formula at 95% confidence level and margin of error of 5%. A total of 20 eligible candidates were selected through purposive sampling. Before the study, a full description was presented to the subjects about the study implementation and Pilates protocols. Next, the consent form, health questionnaire, and 24-hour dietary recall were completed by the participants. All principles of ethical human research were considered in this study. The subjects were aware of all aspects of the research and could withdraw from the project at any time. The participants were randomly divided into two homogeneous Pilates (n = 10) and control (n = 10) groups, based on the pretest results.

### PILATES TRAINING PROTOCOL

The Pilates protocol included 60 minutes of exercise, i.e., 10 minutes of warm-up, 40 minutes of Pilates training, and 10 minutes of cool down, three times a week for eight weeks. The intensity of Pilates training gradually increased from basic levels to advanced stretching, muscular endurance, balance, flexibility, and musculoskeletal coordination training. The exercises focused on the upper and lower muscles of the upper trunk and were performed in standing, sitting, and lying-down positions, without any need for advanced equipment. The applied exercises included the fundamental, basic and intermediate Pilates exercises (Table 1).

Table 1. Fundamentals, basic and intermediate of Pilates exercises

Fundamentals	Basic	Intermediate
Breathing	Hundred	Hundred
Imprinting	Roll Up	Roll Up
Leg Slides	Roll Down	Lunges
Spinal Bridging	Single Leg Circles	Reverse Crunch
Knee Sway	Rolling Like a Ball	Teaser
Pelvic Bowl	Basic Squat	Shoulder Bridge Single Leg
Knee Folds/Stirs	Lunges	Toe Touches
Leg Slides	Single Leg Stretch	RT.LT Side Crunch
Prone Hip Extension	Double Leg Stretch	Side plank
Cervical Nod	Swimming	Plank
Head Float	Leg Pull Front	Seal
Ribcage/Angel Arms	Saw	Corkscrew
Rotating Arms	Legs Up and Down	Single Leg Stretch
Torso Twist	Spine Stretch Forward	Double Leg Stretch
Flight		Single Straight Leg
Cat		Double Straight Leg
Bowing		

In order to comply with the overload principle, the speed and repetition of movements in each session increased compared to the previous session, starting from 10 repetitions in the first week and reaching 20–25 repetitions in the eighth week. Also, to control exercise intensity, the maximum heart rate formula (peak heart rate = 220 minus age) and a polar heart rate sensor were used. The exercises began in the first week at 50–55% of maximum heart rate and reached 75–80% of maximum heart rate in the eighth week (an increase of approximately 5% in exercise intensity per week). It should be noted that the control group did not engage in any sports activities or exercises during the study.

## **ANTHROPOMETRIC INDICES AND BODY COMPOSITION**

One week before the study, the participants visited the Sport Sciences Laboratory of Imam Khomeini International University in Qazvin to be familiarized with the study tools and methods. Some anthropometric and body composition indices were measured before the training period and 48 hours after the final Pilates training session. The participants' height was also measured using a Seca scale (Germany) with 0.01 cm accuracy. Moreover, the waist and hip circumferences were measured using a tape measure, and then, the waist-to-hip ratio was calculated. The weight and body fat percentage were also calculated based on bioelectrical impedance analysis, using a body composition analyzer (ZEUS 9.9, Korea) according to the manufacturer's guidelines.

## **HEPATIC FAT CONTENT AND LIVER ENZYMES**

The hepatic fat content and serum levels of liver enzymes were measured before the training period and 72 hours after the final Pilates training session. The liver fat content was examined using an ultrasound system (Medison SonoAce X8, Korea). Ultrasonography was performed by an expert at the Advanced Medical Imaging Center after at least five hours of fasting. Hepatic steatosis was graded from one to three: grade I (mild), a slight increase in echogenicity; grade II (moderate), a moderate increase in echogenicity; and grade III (severe), a significant increase in echogenicity [19].

To determine the serum levels of liver enzymes, including ALT, AST, and ALP, blood samples were collected from the antecubital vein after 8–12 hours of fasting. The collected blood sample was poured into a test tube containing an anticoagulant. Then, the blood sample was centrifuged, and the separated serum was stored at -70°C until further analysis. The levels of ALT and AST enzymes were evaluated using quantitative detection kits (Pars Azmoon, Iran), based on the kinetic VV method in an Advia 1200 system (USA) according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standards. Also, the level of ALP enzyme was determined using a quantitative diagnostic kit (Bionic), based on the photometric method according to the German Society for Biochemistry standards in an Advia 1200 system (USA).

## **STATISTICAL ANALYSIS**

After data collection, the normal distribution of data was evaluated by Shapiro-Wilk test. For descriptive statistics, mean and standard deviation were measured. Paired t-test was used to examine intra-group differences, while independent t-test was performed for inter-group differences. The significance level was set at 0.05, and all statistical analyses were performed in SPSS software version 18.0 (SPSS, Inc. Chicago, Illinois, USA).

## RESULTS

Shapiro-Wilk test was first used to examine the normal distribution of data in different groups. The results indicated that the collected data had a normal distribution, and the curve of the sample was assumed to be normal ( $P > 0.05$ ). The anthropometric characteristics and body composition of the participants before and after Pilates training are presented in Table 2. The findings showed that eight weeks of Pilates training caused a significant reduction in the body weight, body mass index (BMI), fat percentage, and waist-to-hip ratio of middle-aged men with NAFLD, while no significant changes were observed in the control group (Table 1).

Table 2. Characteristics of subjects before and after eight weeks Pilates training in the two groups <sup>a, b</sup>

Variables	Control (n = 10)				Pilates (n = 10)			
	Pretest	Posttest	Δ	P Value	Pretest	Posttest	Δ	P Value
Age, y	39.30 ±4.64	-	-	-	41.67 ±5.62	-	-	-
Height, cm	173.40 ±4.81	-	-	-	174.11 ±6.33	-	-	-
Weight, kg	82.10 ±7.44	82.50 ±7.47	0.4 ±0.84	0.16	83.21 ±6.16	80.33 ±6.28	-3.66 ±0.86*	0.000
BMI, kg/m <sup>2</sup>	27.39±3.40	27.52 ±3.41	0.13 ±0.28	0.18	27.57 ±2.54	26.56 ±2.52	-1.21 ±0.30*	0.000
Body fat, %	23.68±2.32	23.74 ±2.31	0.06 ±0.18	0.34	24.45 ±2.50	22.74 ±2.09	-1.71 ±0.61*	0.000
WHR, ratio	0.91 ±0.01	0.91 ±0.01	0.003 ±0.00	0.39	0.93 ±0.02	0.91 ±0.01	-0.03 ±0.01	0.000

<sup>a</sup> Values are presented as Mean ±SD

<sup>b</sup> Abbreviation: BMI, body mass index; WHR, waist-to-hip ratio.

\* $P < 0.01$ , significant difference between control and Pilates Training.

Also, the results showed that the liver fat content, which was evaluated via ultrasonography, significantly decreased in the Pilates group after eight weeks of training, whereas no significant change was observed in the control group (Figure 1). Moreover, there was a significant difference between the Pilates and control groups with respect to the posttest liver fat content ( $P = 0.001$ ) and the range of changes ( $P = 0.019$ ).

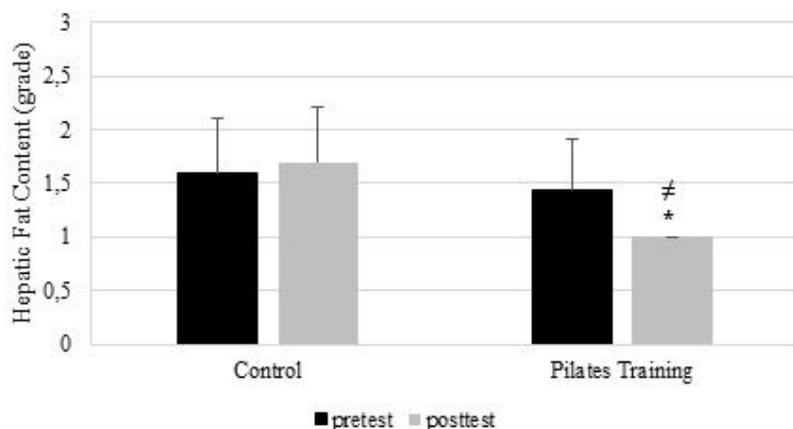


Fig. 1. Hepatic fat content in the control and Pilates groups before and after eight weeks Pilates training

\* $P < 0.05$ , significant difference between pre and post; ≠  $P < 0.01$ , significant difference between control and Pilates training groups.

Regarding the level of liver enzymes, the results showed that all three enzymes, including AST, ALT, and ALP, significantly reduced in the Pilates group after eight weeks of training, while no significant changes were observed in the control group (Table 3). Also, there was a significant difference between the Pilates and control groups regarding the posttest serum levels of AST, ALT, and ALP and the range of enzyme changes (Table 3).

Table 3. Liver enzyme changes before and after eight weeks Pilates training in the two groups<sup>a, b</sup>

Variables	Control (n = 10)			Pilates (n = 10)		
	Pretest	Posttest	Δ	Pretest	Posttest	Δ
ALT (U/L)	31.30 ±5.90	31.70 ±6.78	0.40 ±1.26	28.88 ±3.98	24.11 ±2.61 <sup>*,†</sup>	-4.77 ±1.78 <sup>†</sup>
AST (U/L)	23.80 ±3.01	24.20 ±2.89	0.40 ±0.84	24.1 ±3.87	20.77 ±4.11 <sup>*,†</sup>	-3.21 ±2.1 <sup>†</sup>
ALP (U/L)	157.55 ±53.41	157.77 ±69.60	0.22 ±2.27	154.63 ±26.82	145.44 ±45.30 <sup>*,†</sup>	-10.33 ±6.04 <sup>†</sup>

<sup>a</sup> Values are presented as Mean ± SD.

<sup>b</sup> Abbreviations: ALT, alanine amino transferase; AST, aspartate amino transferase; ALP, alkaline phosphatase.

\* P<0.01, significant difference between pre and post; †P<0.05, significant difference between control and Pilates Training.

## DISCUSSION

Considering the importance of sports activities in the treatment of patients with NAFLD, it is essential to identify the most effective exercises for these patients. The present study was conducted to investigate the effect of eight weeks of Pilates training on the liver fat content and liver enzymes of middle-aged men with NAFLD.

The results indicated that eight weeks of Pilates training significantly reduced the liver fat content (decreased liver steatosis grade) in middle-aged men with NAFLD. The grade of liver steatosis decreased in 31% of subjects in the Pilates group. The reduction of liver fat content was associated with a significant decrease in the serum levels of ALT, AST, and ALP following eight weeks of training. The serum levels of ALT, AST, and ALP enzymes in the Pilates group showed 17%, 14%, and 7% reductions, respectively. In the present study, the Pilates exercises often involved flexibility, balance, and muscular endurance activities, with 10–25 repetitions at a relative intensity of 55–75% of the maximum heart rate.

Although limited research has been conducted on the effects of Pilates training in patients with NAFLD, the majority of previous studies incorporating aerobic or resistance training have indicated the positive effects of this training on reducing the liver fat content and liver enzymes. In this regard, Houghton et al. showed that 12 weeks of combined exercise training reduced the liver fat content, as well as ALT and AST levels in patients with NAFLD [16]. Also, Hallsworth et al. reported that 12 weeks of cycling significantly reduced the fat mass and ALT and AST levels in patients with NAFLD [20]. Moreover, Haus et al. reported that even short-term exercises could improve the liver fat content in NAFLD patients, thereby reducing the risk of disease progression [21]. Although the exact mechanism contributing to the increased liver fat content and liver enzymes after exercise training remains unclear, evidence suggests that aerobic and muscular endurance training can improve NAFLD through activation of lipolysis, regulation of UCP-1 and PPAR $\gamma$ , and changes in adipocytokines [22]. Guo et al. in a review study argued that aerobic training could be effective in improving NAFLD by stimulating lipid metabolism, along with inhibition and modulation of oxidative stress, inflammation, and liver apoptosis [23].

Limited studies have examined the effect of Pilates training on the grade of liver steatosis and liver enzymes, and the mechanism of the effect of Pilates exercise on NAFLD remains unclear. However, these exercises may reduce the liver fat content and serum levels of ALT, AST, and ALP in patients with NAFLD by improving insulin resistance, body composition, and physical fitness. In this regard, Church et al. reported that there is a direct relationship between the plasma ALT level of patients with NAFLD and their BMI and abdominal circumference [24]. In the present study, Pilates training was associated with

a significant reduction in patients' weight, BMI, fat percentage, and waist-to-hip ratio. Therefore, reduction in the liver fat content and enzyme levels in this study can be attributed to the improvement of these indices, which is often related to improved insulin resistance. Also, the emphasis on muscular endurance training with frequent repetitions (55–75% of maximum heart rate) in the present study increased the oxidation of fatty acids in the adipose tissues, muscle cells, and liver cells [25]. Since the emphasis of Pilates exercises is to strengthen the deep muscles of the body by improving mental concentration and breathing rhythm, our results indicating the improvement of liver indices in NAFLD patients can be related to the activation of GLUT4 and AMPK in the muscles [22]. However, contrary to our results, Hagner-Derengowska et al. reported that 10 weeks of Pilates training had no significant effects on the serum levels of ALT and AST enzymes in obese and overweight women. Nevertheless, body weight, BMI, and serum triglyceride level decreased significantly after 10 weeks of Pilates training [11]. Some causes of discrepancy between the present results and the findings reported by Hagner-Derengowska et al. may be age and gender differences, as well as the intensity of Pilates training. The participants in the study by Hagner-Derengowska et al. were elderly women with an average age of 60.9 years, who were mostly in the postmenopausal period, while in the present study, the participants included middle-aged men with an average age of 40 years. Evidence suggests that the prevalence of unhealthy lifestyle behaviors (such as sedentary lifestyle) is higher at older age. In fact, the highest prevalence of NAFLD has been reported in the age group of 50–65 years [26]. Also, the prevalence of fatty liver is somewhat higher among postmenopausal women due to the lower serum levels of estrogen [26]. It seems that age and gender influence the effectiveness of Pilates training on liver enzymes and fat content, which is contradictory with the results of the study by Hagner-Derengowska et al. In the present study, the number of exercise activities was high in each Pilates session (with 10–25 repetitions), and training continued for eight weeks. To conform to the overload principle, the intensity of training gradually increased every week. The training intensity in the final week reached about 75–80% of the maximum heart rate. On the other hand, in the study by Hagner-Derengowska et al. [11], the intensity of overload in Pilates training was unknown and the energy expenditure of every Pilates session was reported to be 400 kcal, which is suggestive of mild- to moderate-intensity training. Therefore, their intensity and duration of Pilates training did not have any significant effects on the levels of ALT and AST enzymes in elderly women. According to the study by Hagner-Derengowska et al. [11], both ALT and AST enzymes in the Pilates group diminished insignificantly. Of course, this should be taken into account that Keating et al. [27] showed in a meta-analysis that exercise training has significant benefits for patients with fatty liver, without causing any changes in the serum level of ALT enzyme. The benefits of exercise training were confirmed, while the body weight of patients with NAFLD was unchanged or minimally changed [27].

The present study has certain limitations, which need to be addressed. First, considering the main inclusion criteria of our study, the number of examined patients and the sample size were limited; therefore, further research with a larger sample size is needed to increase the external validity of our findings. Second, in our study, ultrasonography was used rather than liver biopsy, which is the gold standard for measuring fatty liver [28], based on the guidelines of the American Association for the Study of Liver Disease (AASLD). Although the diagnostic accuracy of ultrasound is about 93% when liver steatosis is greater than 33%, its sensitivity reduces if liver steatosis is below 30% [29, 30]. It should be noted that liver biopsy is an invasive and costly technique, and most patients

are reluctant to undergo biopsy due to the possible side effects. Third, although the subjects were advised not to change their normal diet (in some cases, we tried to control their diet using a 24-hour dietary recall), it was not possible to control and maintain their diet during the study; therefore, consumption of some foods, such as coffee, tea, soy, and vitamin A and E supplements, might have affected our results.

## CONCLUSIONS

Based on the results of the present study, Pilates training can be effective in improving the liver fat content and reducing the serum levels of ALT, AST, and ALP in middle-aged men with NAFLD; therefore, Pilates training can be used as a therapeutic approach for these patients. However, further research is necessary to confirm our findings.

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