Research Article

The Simultaneous effect of high-intensity interval training (HIIT) and omega-3 supplementation on malondialdehyde (MDA) levels in the serum of obese male rats.

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Received: 31 July2025
Accepted: 22 September2025

Abstract

Introduction: High-intensity interval training (HIIT) and omega-3 supplementation are effective strategies to reduce obesity-induced oxidative stress. Malondialdehyde (MDA) serves as a key marker of oxidative damage and helps us learn about these interventions' effectiveness. the aim of this study is to examine the effect of omega-3 fatty acids on Malondialdehyde (MDA) level in long-term aerobic exercise in the serum of obese rats.

Methods: In a semi-experimental and applied study, 40 obese male rats of 6 months with Wistar breed with a weighted average of 332.28 Grams and standard deviation of 9 after 7 days of acquaintance with the laboratory environment were randomly divided into 4 groups of 10 (control, training, supplement and training-supplement). The training-supplementation and training groups underwent aerobic training for 6 weeks and 4 sessions in each week, according to the training protocol. Supplement and training- Supplements Groups also for 6 weeks and 4 meals every week, one hour before the start of training, 400 mg of omega-3 per kilogram of body weight was gavage. 24 hours after the last training session, while fasting for 12 hours, all subjects were anesthetized with ketamine and xylazine solution and blood was taken from the posterior orbital sinus of the rats. The collected blood samples were immediately transferred to the laboratory to measure the variables. In order to analyze the data at the descriptive level, mean and standard deviation tests were used, and at the inferential level, the Shapiro-Wilk test was used to check the normality of the data, and the two-way ANOVA test was used to check the interaction effect of the groups. data analysis It was done using spss software version 26 and at a significance level($P \leq 0.05$).

Results: The statistical results of the study showed that all three variables of intensity interval training, Omega-3 supplementation, and exercise-supplementation caused a significant decrease in serum levels of the enzyme malondialdehyde. ($p < 0.05$).

Conclusion: Based on the results of the present study, it seems that the interactive effect of endurance training and omega-3 supplementation can be used to reduce oxidative damage caused by obesity in the body.

Keywords:
High Intensity Interval Training, Omega-3, oxidative stress, obese.

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1. Introduction

Obesity is a chronic disease of multifactorial origin that develops from the interaction of social, behavioral, psychological, metabolic, cellular, and molecular factors. It is the condition under which adipose tissue is increased and can be defined as an increase in body weight that results in excessive fat accumulation(1).

It is estimated that more than 115 million people in developing countries suffer from obesity-related problems. Today, the obesity epidemic has become such a global phenomenon that the World Health Organization has coined the term globesity(2). Obesity increases mortality and the prevalence of cardiovascular diseases, diabetes, and colon cancer(3). The excessive fat accumulation in adipose tissue increases reactive oxygen species (ROS) production through different mechanisms and subsequently promotes systemic oxidative stress (OS)(4). OS occurs also in other tissues, such as skeletal muscle and liver, where ectopic fat accumulation promotes ROS production(5). Oxidative damage can arise from an imbalance between increased production of reactive oxygen species (ROS) and/or reduction of antioxidant defenses(6). Malondialdehyde (MDA), the main end product of lipid oxidation caused by ROS, can cause cross-linking polymerization of proteins, nucleic acids, and other living macromolecules(7). and do great damage to the activities of mitochondrial respiratory chain complex and key enzymes in mitochondria(8). At present, a lot of evidence has confirmed that blood MDA is an important indicator of the degree of oxidative damage in vivo and in vitro(9). High levels of circulating glucose and lipids can result in an excessive supply of energy substrates to metabolic pathways, which in turn can increase the production of ROS(10). MDA has been widely used for many years as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids(11). The Strategies to lower obesity-induced OS include physical activity programs (indirectly via weight loss and directly by increasing antioxidant enzyme activities), and the antioxidant rich diets(12). The recent understanding about advantages and the effectiveness of low-volume high-intensity interval training (HIIT) can aid to overcome that obstacle.

The Literatures have stated HIIT would significantly decrease subcutaneous fat, especially abdominal fat and the body fat percentage (BFP) increase the amount of VO2 Max and even improve insulin sensitivity(11). In this regard, Fattahi et al (2018) have stated exercise could remarkably decrease MDA level within the blood sample. The recent understanding about advantages and the effectiveness of low-volume high-intensity interval training (HIIT) can aid to overcome that obstacle. Literatures have stated HIIT would significantly decrease subcutaneous fat, especially abdominal fat and the body fat percentage (BFP) increase the amount of VO2 Max and even improve insulin sensitivity(11).

A growing body of evidence suggests that a chronic inflammatory state is associated with many diseases. Low-level inflammation also appears to be involved in the onset and development of PCOS. Diet, as a modifiable lifestyle factor, may play an important role in regulating chronic inflammation(13).

Omega-3 polyunsaturated fatty acids have been shown to increase athletic performance, and reduce oxidative stress, muscle damage, and inflammatory markers in athletes(14). Various factors affect the oxidant/antioxidant balance in the body. Omega-3 fatty acids are a family of fatty acids that have shown promising and positive effects in modifying a range of disease processes in the general population, including inflammatory and immune pathways, cardiac arrhythmias, other cardiovascular diseases, and lipid regulation(15). For this reason, in recent years, the scientific community has shown great interest in omega-3 (ω-3) polyunsaturated fatty acids, especially eicosapentaenoic fatty acids (EPA) and Docosahexaenoic fatty acids (DHA) found in fish and fish oils(16). Dietary ω-3 fatty acids are essential for human health and cannot be synthesized in mammalian tissues. study showed that omega-3 supplementation reduced plasma malondialdehyde(17). A study investigating the administration of n-3 PUFAs has not been shown a decrease in Malondialdehyde (MDA) levels after anaerobic exercise(18). Despite studies investigating the effects of aerobic exercise on oxidative stress and inflammatory reactions or indicating that dietary omega-3 fatty acids can reduce oxidative stress and inflammation(16) few researchers have investigated the effects of omega-3 supplementation in addition to exercise on redox status and systemic inflammatory response. The innovation of the present study is the simultaneous use of a supplement and an training program. Based on this, the study was designed to investigate the acute effect of Omega-3 on MDA after anaerobic exercise in obese rats.

2. Materials and Methods

This research is applied and semi-experimental, which was conducted with a post-test design. The statistical sample of this research included 40 6-month-old male rats with an average weight of 332.28 Grams and standard deviation of 9grams, which were placed in four groups of 10: endurance training, OMEGA-3 supplement, training supplement and control group. The inclusion criteria in the present study included healthy rats weighing more than 319 grams, and the exclusion criteria included disease in the rats during the study and lack of regular training. Rats were kept in the animal laboratory under light-controlled conditions, 12 hours of light and 12 hours of darkness (starting light at 6 in the morning and turning off at 6 in the evening), temperature 22 ± 3 centigrade and humidity about 45%, and kept for 1 week with new environment and activity on the treadmill.

In order to get familiar with the treadmill, first the rats of the experimental group worked on the treadmill for 1 week (5 sessions), for 10 to 15 minutes in each session at a speed of 6 to 10 meters per minute with a zero-degree slope, to learn about the treadmill and the running pattern. Learn about it. Then, to accurately determine the training intensity, the maximum running speed test was performed using a treadmill in an indirect way.

The training and training-omega3 groups participated in eight weeks HIIT (five weekly sessions) which involved running on a 10 lines animal treadmill with treadmill slope of 0°, intensity of 80% VO2 Max (after three min warm up with speed of five (m/min)) and spans sessions of 10 to 20 min (Table slop of 0°, and span of 5 min. The treadmill orientation training sessions had gradually increased to speed of 10 (m/min), spans sessions of 15 min, at the ending of the orientation period. Though, the treadmill slope remained 0° (14).

Table 1. Protocol of HIIT

Exercise Step HIIT / Exercise Element	Warm up	Main step (3 intervals)	Cool down
Span (min)	3 min	10 to 20 min	5 min
Intensity (% VO2 Max)	50 to 60%	80% averagely (10% weekly increase)	50 to 60%

*Slop of the treadmill remains 0°, during the entire steps/stages of the training

*From four 2 min sets at the 1st weeks to eight 2 min sets at the 8th week, and with 1 min interval rest span between each two successive sets

The OMEGA-3 and OMEGA-3 training groups received 300 mg of OMEGA-3 per kilogram of body weight by gavage for eight weeks and four times a week, one hour before the training program. OMEGA-3 was obtained from Sigma, Germany and was used after dilution in DMSO (dimethyl sulfoxide) solvent with a concentration of 15% (19).

After the experimental applications (week 8), 90 mg/kg ketamine i.p. was administered to all rats. After the animal's thorax was shaved, cleaned with alcohol and opened with a vertical incision from the midline, the heart was directly cannulated, and a blood sample was taken. The special measuring kit of Zelbio, Germany was used and the preparation of the solutions was done according to the company's instructions. Firstly, the blood samples were coagulated at room temperature for 5-10 minutes. Samples were centrifuged at 2000-3000 rpm for 5 minutes to separate the supernatant. The serums were transferred into pre-assigned and numbered Eppendorf tubes. It was stored at -20°C until analysis.

For the purpose of statistical analysis at the level of descriptive statistics of the mean and standard deviation and at the level of inferential statistics after checking the normality of the data using the Shapiro-Wilk test, for the purpose of statistical analysis of the data and comparison between the groups, two-way ANOVA test and Tukey's post hoc test was used at a significance level of less than 0.05. All statistical calculations were performed using SPSS version 26 statistical software.

3. Results

The present study was conducted with the aim of investigating the effect of a course of aerobic training and omega-3 supplementation on the serum malondialdehyde (MDA)of obese male rats. The descriptive results of the research showed that the highest average weight in the supplementary training group and the lowest average weight belonged to the control group (Table 2).

Table 2: Average and standard deviation of weight in research groups

Groups	Weighted Average
Training – Omega-3	336.25±7.4
Omega-3	332.5±9.5
Training	334.12±9.1
Control	326.25±8.4

According to the results of the Shapiro-Wilk test and the proof of the normal distribution of the data, the two-way analysis of variance test was used to investigate the interactive effect of the research variables The results of the present study showed that all three variables of intensity interval training(HIIT), omega-3 supplementation, and training+supplementation improved the malondialdehyde (MDA) index, or in other words, the use of intensity interval training(HIIT) and omega-3 supplementation significantly reduced serum malondialdehyde (MDA) levels in obese rats compared to the control group ($p \leq 0.05$).

On the other hand, intensity interval training (HIIT) and omega-3 supplementation separately also had a significant effect on reducing malondialdehyde (MDA) levels. ($p \geq 0.05$) (Table3).

In general, the results of the present study indicated a significant interactive effect of intensity interval training (HIIT) and omega-3 supplementation on malondialdehyde (MDA) levels in obese rats, or in other words, the simultaneous use of intensity interval training (HIIT) and omega-3 supplementation had a synergistic effect on improving the malondialdehyde (MDA) index in obese rats.

Table 3: Comparison of malondialdehyde of the experimental groups with the control group

Groups	MDA	
	Average	p
Training	167.38±9.12	0.050
Omega-3	155.11±7.24	0.010
Training- Omega-3	141.74±11.01	0.008
Control	184.25±4.80	

The key strengths of the present study were the simultaneous examination of two complementary variables, namely practice and measurement accuracy and validity. Its weaknesses include the lack of scientific resources in this field.

4. Discussion

In our study, it was aimed to determine the degree of oxidant damage caused by obesity in obese rat and to determine how this damage was affected by omega-3 supplementation. We chose to use a moderate-duration aerobic exercise protocol to represent a type and duration of exercise commonly used by many researchers. For this purpose, MDA which is an indicator of oxidative stress was measured. When the results were examined, MDA levels were statistically significantly different in the training group, omega-3, and omega-3+exercise compared to control group ($p < 0.05$). MDA is an indicator of lipid peroxidation and is one of the final degradation products with numerous harmful effects on biological systems(20).

Many studies(21) have reported that acute exercise increases plasma MDA levels. In contrast, there are studies(22) that reported higher plasma MDA levels in untrained subjects compared to trained subjects and did not observe any changes in MDA levels after a 12-week training program. When the literature was reviewed, exercise was shown to cause an increase in MDA levels, which is not consistent with our findings, but this has not always been the case, and inconsistencies in response are thought to be related to the subjects' training status(23). In contrast to the findings of increased oxidative stress in response to exercise in sedentary subjects, a few studies(24) using trained individuals have reported minimal increases in exercise-induced oxidative stress when exercise intensities are of medium duration and intensity (70% VO2max). Endurance training lowers blood lipids and plasma LDL. Therefore, part of the decrease in MDA levels may be due to a decrease in fatty acid availability. However, other factors such as improved antioxidant status and a general reduction in free radical production can be considered(8). Reducing free radical production in the body (strengthening the antioxidant system) and increasing the activity of antioxidant enzymes, thereby achieving a balance between the body's oxidative-oxidative systems (oxidants versus antioxidants), which significantly prevents lipid peroxidation(25). In terms of omega supplementation, it is thought to have positive effects on oxidative stress in some patient groups. For example, in a study(26) conducted to determine the effects on inflammation and oxidative stress in patients with type 2 diabetes,it is seen that 1 g of EPA or DHA can prevent increases in serum MDA. Moderate supplementation of ω-3 fatty acids reduced MDA levels in patients with cardiac symptom X(27). Another study examined the effects of 6 weeks of eicosatetraenoic acid (EPA) and Docosahexaenoic acid (DHA) supplementation on rest and exercise-induced lipid peroxidation and antioxidant status in judoka. They observed significantly greater increases in NO and oxidative stress in the n-3 long-chain-PUFA (LCPUFA) group with exercise (MDA, R max, CD max, and NO) compared to placebo. No major interaction effects were found for retinol and α-tocopherol. These results indicate that n-3 LCPUFA supplementation significantly increases oxidative stress at rest and after judo training(28).

In our study, when the MDA level was examined (Table 3), it was determined that there was a statistically significant difference ($p<0.05$) between the training, omega-3, training+ omega-3and control groups. Available results suggest that supplementation of ω-3 fatty acids will provide benefits during aerobic exercise. There is substantial evidence(29) that athletes supplemented with ω-3 fatty acids have increased blood flow to working muscles and reduced red cell deformation. This can increase oxygen delivery to working muscles. When the results of the study were evaluated, it was seen that the amount of MDA, an oxidative stress marker, decreased significantly in the exercise group compared to the control group ($p < 0.05$). It is seen that supplementation of ω-3 fatty acids with exercise reduces the MDA level and brings it closer to the control group. It is thought that this situation may be due to the double bond structure of ω-3 fatty acids. It is thought that the double bonds in the structure of omega-3 fatty acids reduce oxidative stress by binding free oxygen radicals(18). The mechanism of these lowering effects is related to the active participation of PPARs. Fatty acids are classically recognized as energy substrates, but they may also act as endogenous ligands for PPARs and regulate the expression of genes encoding key proteins in the control of fatty acid and fuel uptake and body composition and lipoprotein formation.

They play a role in the very low density of triglyceride carriers in the liver . Although the exact mechanism by which omega-3 fatty acids improve lipid levels is not fully understood, omega-3 fatty acids may reduce triglyceride synthesis and increase hepatic fatty acid beta-oxidation(30).

Based on these results, it can be said that omega-3 fatty acid supplementation is effective in preventing exercise-induced oxidative stress in rats.

5. Conclusion

Oxidative stress is a state of disturbed balance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) on one hand and antioxidant defenses on the other. Prolonged aerobic exercise combined with omega supplementation seems to be beneficial in maintaining the dynamic balance between oxidative challenge and antioxidant defense in the biological system. Future studies are needed to confirm that the same findings can be applied to different exercise intensity ranges. In conclusion, can be said that omega-3 supplementation can provide antioxidant protection against potential oxidative damage, as it causes a decrease in MDA level in obese rats exposed to chronic long-term exercise.

Acknowledgements

We thank everyone who helped us conduct this research.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: Z.N , A.F.B ; Methodology: Z.N , A.F.B ; Software: Z.N , A.F.B ; Validation: Z.N , A.F.B ; Formal analysis: Z.N , A.F.B ; Investigation: Z.N , A.F.B ; Resources: S Z.N , A.F.B ; Data curation: Z.N , A.F.B ; Writing - original draft: Z.N , A.F.B ; Writing - review & editing: Z.N , A.F.B ; Visualization: Z.N , A.F.B ; Supervision: Z.N , A.F.B ; Project administration: Z.N , A.F.B ; Funding acquisition: Z.N , A.F.B .

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