Antioxidant effect of omega-3 fatty acids on exercise-induced oxidative stress in rats

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Abstract. – OBJECTIVE: The aim of this study is to examine the effects of omega-3 supplementation on Catalase (CAT) activity, Malondialdehyde (MDA), advanced oxidation protein products (AOPP) and reduced glutathione (GSH) levels in long-term aerobic exercises in rats.

MATERIALS AND METHODS: 28 male Wistar albino rats (8 weeks old, 220-350 g body weight) were included in the study. The rats were given treadmill exercise for 20 minutes at an average speed of 15 cm/s, 5 days a week, for 8 weeks. The experiment was terminated at the end of the eighth week. Blood samples were taken. CAT, MDA, AOPP and GSH analyses were performed. SPSS v. 21 package program was used in the analysis of the data. The distribution of the data was examined with the normality homogeneity test, and it was determined that it was a normal distribution. As a result, the One-Way ANOVA test, one of the parametric tests, was used. Tukey test was used to determine the difference between groups. Significance levels were evaluated as ($p < 0.05$).

RESULTS: Statistical analysis showed a statistically significant difference between groups in CAT, MDA and GSH levels ($p < 0.05$), while there were no differences between the groups in AOPP levels ($p > 0.05$).

CONCLUSIONS: In the conclusion of the study, it was determined that omega-3 supplementation caused a decrease in MDA level, an increase in CAT activity and GSH level in rats exposed to chronic long-term exercise. Thus, it can be said that omega-3 supplementation in chronic long-term exercise will provide antioxidant protection against potential oxidative damage.

Key Words:
Antioxidant, Chronic exercise, Omega-3, Free radicals.

Introduction

Many published studies[1,2] show that regular exercise training plays an important role in the prevention of chronic diseases such as cardiovascular diseases, diabetes, cancer, hypertension, obesity, depression, and osteoporosis. However, strenuous physical exercise has also been shown[3] to cause oxidative stress by increasing the production of reactive oxygen species (ROS). In addition, ROS production is a stimulus of the post-exercise inflammatory process that occurs following exercise and accompanies muscle damage[4].

Various factors affect the oxidant/antioxidant balance in the body. It has been suggested[5,6] that exercise-induced oxidative stress and inflammatory/acute phase responses can be reduced by supplementing with antioxidants. Omega-3 fatty acids are a family of fatty acids that have shown[7,8] 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. 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 doceohexaenoic fatty acids (DHA) found in fish and fish oils[9]. Dietary ω-3 fatty acids are essential for human health and cannot be synthesized in mammalian tissues.

Despite studies[1,10,11] investigating the effects of aerobic exercise on oxidative stress and inflammatory reactions or indicating that dietary

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omega-3 fatty acids can reduce oxidative stress and inflammation, few researchers[12-15] have investigated the effects of omega-3 supplementation in addition to exercise on redox status and systemic inflammatory response. This study was designed to examine the effects of long-term aerobic exercise with omega supplementation on Catalase (CAT), Malondialdehyde (MDA), advanced oxidation protein products (AOPP), and reduced glutathione (GSH) levels in rats, and to examine the dynamic balance between oxidative challenge and antioxidant defense in the biological system, considering that omega fatty acid supplementation may have effects on exercise performance.

Materials and Methods

Experimental Design and Animals

This study was carried out in Van Yüzüncü Yıl University Experimental Animals Laboratory. Eight weeks-old (220-350 g body weight) male Wistar rats obtained from the same center were used. Rats were fed a standard diet that was regularly renewed daily in a standard laboratory environment (temperature: 22 ± 25°C, relative humidity: 55 ± 5% and 12/12 hour light/dark cycle), and their hygiene was maintained.

Power analysis was performed to generalize the results obtained for the assignment of the experimental groups and to obtain a sufficient number of samples. In order to determine the sample size to be included in this study, a power analysis was performed with reference to the study conducted by Pancar et al[16] (2022) (5% margin of error, 95% power), and the sample size resulted in at least 28 rats with 7 members in each group.

- Control group (n = 7): The rats in this group were fed with standard chow during the experiment. No additional application was made.
- Exercise group (n = 7): Animals in this group were fed a daily standard diet and performed 20 minutes of daily treadmill exercise for 8 weeks.
- Omega group (n = 7): In addition to the daily standard diet, the animals in this group were given omega-3 fatty acid component (Ocean brand) supplement (400 mg/kg/day) by gavage method for 8 weeks.
- Omega+exercise group (n = 7): In addition to the daily standard diet, the animals in this group were given omega-3 fatty acid component (Ocean brand) supplement (400 mg/kg/day) by gavage method for 8 weeks. For 8 weeks, 20 minutes of daily treadmill exercise was done[17].

This study was approved by Van Yüzüncü Yıl University Animal Experiments and Local Ethics Committee (2021/02-12) and was supported by Van YYU Scientific Research Projects Coordination Unit as project numbered TYL-2021-9497. All experiments were performed in accordance with internationally recognized standard Ethical Guidelines for Laboratory Animal Use and Care (EEC Directive 86/609/EEC of 24 November 1986) as described in European community guidelines.

Treadmill Workout Programs

The rats in the Exercise and omega+exercise groups were exercised on a special treadmill (MAY-TME 0804, Commat Ltd., Ankara, Turkey) 5 days a week for eight weeks. In order to ensure adaptation, before the exercise protocol, running exercise was applied at 5 m/min speed on the treadmill for 5-10 minutes daily for 2 weeks. At the end of the 2-week adaptation period, the rats run at a speed of 15 m/min for 20 minutes on the exercise days. Exercise practices were carried out continuously between 08:00 and 10:00[17].

Biochemical Analysis

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 and centrifuged at 4,000 rpm for 5 minutes to separate the serum fraction. The serums were transferred into pre-assigned and numbered Eppendorf tubes. It was stored at -80°C until analysis. CAT, MDA, AOPP, and GSH analyses were performed using Andy Gene ELISA kits (AndyGene Biotechnology Co., Ltd., Fengtai District, Beijing, China) according to the manufacturer's protocol.

Statistical Analysis

SPSS v. 21 package program (IBM Corp., Armonk, NY, USA) was used in the analysis of the data. The distribution of the data was examined with the normality-homogeneity test and it was determined that the variances showed normal distribution. Descriptive statistics for the featured features are expressed as Mean and Standard

Table I. CAT, MDA, AOPP and GSH levels in all groups.

	Control	Exercise	Omega	Exercise+omega	*p*-value
CAT (U/L)	328.46 ± 9.67^{a}	361.3 ± 15.1^{b}	$334.98 \pm 23.14^{a,b}$	$349.81 \pm 13.35^{a,b}$	0.031*
MDA (mmol/L)	0.64 ± 0.01^{a}	0.71 ± 0.33^{b}	0.64 ± 0.03^{a}	0.64 ± 0.03^{a}	0.001*
AOPP (mmol/L)	37.69 ± 1.84^{a}	39.39 ± 1.74^{a}	37.28 ± 1.5^{a}	38.97 ± 2.68^{a}	0.177
GSH (mmol/L)	$1.59 \pm 0.25^{a,b}$	1.98 ± 0.18^{c}	1.51 ± 0.17^{a}	$1.89 \pm 0.17^{b,c}$	0.000*

*$p < 0.05$. Catalase (CAT), Malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPP), Glutathione (GSH). A statistically significant difference was found between groups with different letters in the same line.

Deviation. One-Way ANOVA analysis was performed for the statistical analysis of all parameters. Tukey test was used to compare different groups. Significance levels were evaluated as ($p < 0.05$).

Results

The results obtained in the study and the statistical analyses of these results are presented in Table I.

While there was a statistically significant difference between the groups in CAT, MDA and GSH levels ($p < 0.05$), there was no difference between the AOPP levels ($p > 0.05$). The averages of the variables are given in Figure 1.

Discussion

In our study, it was aimed to determine the degree of oxidant damage caused by aerobic exercises in rats 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[18,19]. For this purpose, MDA and AOPP levels, which are indicators of oxidative stress, CAT enzyme activity, which indicates the defense response against oxidative stress, and GSH levels were measured.

When the results were examined, MDA levels were statistically significantly different in the exercise group compared to all other groups ($p < 0.05$). MDA is an indicator of lipid peroxidation and is one of the final degradation products with numerous harmful effects on biological systems. Trained swimmers have been found[20] to have higher MDA levels compared to control subjects. Many studies[21-23] have reported that acute exercise increases plasma MDA levels. In contrast, there are studies[23,24] that reported higher plasma MDA levels in untrained subjects compared to trained subjects and did not observe any changes

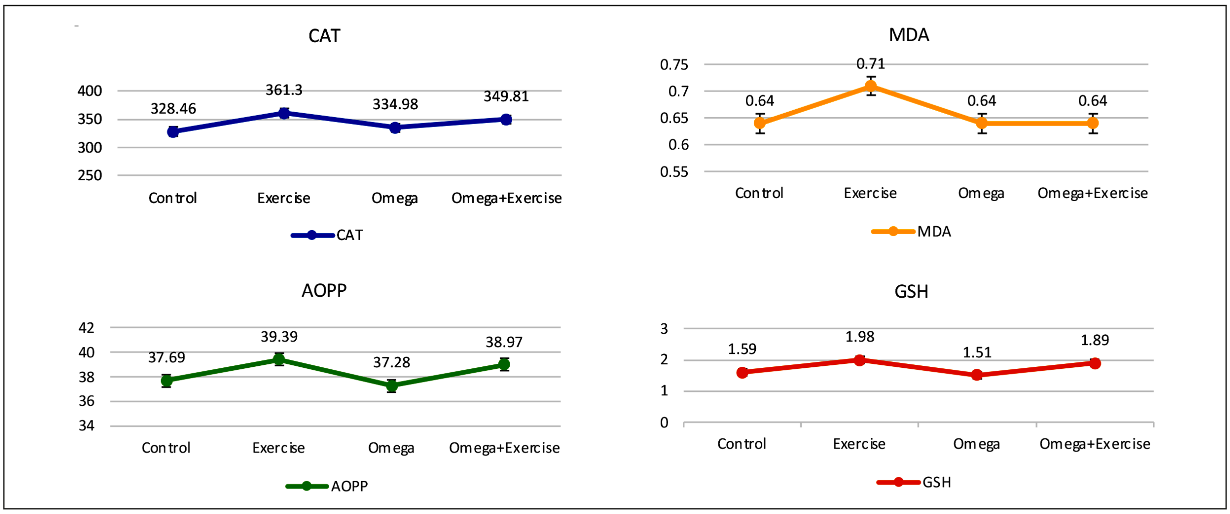

Figure 1. Average values of Catalase (CAT), Malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPP) and Glutathione (GSH).

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 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[25]. In contrast to the findings of increased oxidative stress in response to exercise in sedentary subjects, a few studies[25] using trained individuals have reported minimal increases in exercise-induced oxidative stress when exercise intensities are of medium duration and intensity (70% VO_{2max}). 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 eicosapentanoic acid (EPA) and docosahexanoic 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, Rmax, CDmax, 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]. It can be thought that the main reason for the difference in our findings may be due to the increase in anaerobic exercise-specific free radical production, which may be mediated by various pathways in addition to, for example, electron leakage that occurs during aerobic exercise. In addition, the large increases in lactic acid, acidosis, catecholamines and post-exercise inflammation, characteristic of supramaximal exercise, are other factors that can increase free radical production.

In our study, when the CAT and GSH levels were examined (Table I), it was determined that there was a statistically significant difference ($p < 0.05$) between the exercise and control groups, and when the AOPP levels were examined, there was no statistical difference between the exercise and control groups ($p > 0.05$). Poprzecki et al[29] showed that a daily dose of 1.3 g of n-3 LCPUFA for 6 weeks increased CAT activity in response to high-intensity endurance exercise without any change in oxidative parameters. Available results suggest that supplementation of ω-3 fatty acids will provide benefits during aerobic exercise. There is substantial evidence[30-32] 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, increased significantly in the exercise group compared to the control group ($p < 0.05$). According to this result, it can be said that exercise application causes oxidative stress in rats. Our results are also compatible with the literature[21-23]. 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[33-34]. Based on these results, it can be said that omega-3 fatty acid supplementation is effective in preventing exercise-induced oxidative stress in rats. CAT activity and GSH amount, which are endogenous responses to oxidative stress, seem to increase in response to oxidative stress in the exercise group. This increase is statistically significant ($p < 0.05$). In the exercise+omega group, CAT activity and GSH amount decreased due to the decrease in oxidative stress with omega-3 application. AOPP level, another oxidative stress marker, increased in exercised rats compared to the control group, but this increase was not statistically significant ($p > 0.05$). This situation may be caused by the duration of exercise. More meaningful results will be obtained with longer-term studies.

Conclusions

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[28]. 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, it can be said that omega-3 supplementation can provide antioxidant protection against potential oxidative damage, as it causes a decrease in MDA level, an increase in CAT activity, and GSH levels in rats exposed to chronic long-term exercise. For the antioxidant properties of omega-3 fatty acids supported by our findings, there may be various mechanisms, including anti-inflammatory effects, stimulation of antioxidant enzymes and inhibition of phospholipase A2. Moreover, the association of ω-3 fatty acids in membrane lipids and lipoproteins makes the double bonds in the cell membrane less available for free radical attack[33,34]. Therefore, omega-3 fatty acids can be mentioned as enhancing factors in antioxidant defense against ROS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

We would like to thank all the people who took part in our study as well as the reviewers, and editors for their opinions and suggestions.

Ethics Approval

The study was approved by Van Yüzüncü Yıl University Animal Experiments and Local Ethics Committee (2021/02-12) and was supported by Van YYU Scientific Research Projects Coordination Unit as project numbered TYL-2021-9497. All experiments were performed in accordance with internationally recognized standard Ethical Guidelines for Laboratory Animal Use and Care (EEC Directive 86/609/EEC of 24 November 1986) as described in European community guidelines.

Informed Consent

Not applicable.

Authors' Contribution

Mücahit Sarikaya, Mehdi Aslan, Nuri Mert Embiyaoğlu and Beyza Öğe conceived the study, collected the data and supervised the data collection. Salih Çibuk analyzed the data. Mücahit Sarikaya and Salih Çibuk performed the statistical analysis. Nuri Mert Embiyaoğlu and Beyza Öğe wrote the draft. All the authors read and approved the final manuscript.

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