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Synergistic Effects of Omega 3 Supplementation and Exercise on Markers of Liver (ALP, AST, and ALT) and Muscle (LDH and CK) Damage in Male Karate Athletes

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ABSTRACT

Background: Enzymatic adaptations in response to liver and muscle damage with exercise and omega 3 supplementation has not been well understood. The aim of the present research was to examine the synergetic effects of exercise and omega 3 supplementation on indices of liver (ALT, AST, and ALP) and muscle (LDH and CK) damage in elite male karate athletes. **Objective:** 16 elite karate athletes with mean age of 26.07 years and mean weight of 72 kg were divided into exercise-omega 3 supplementation (EO) and exercise-placebo (EP) groups. The EO group performed karate high-intensity exercises for four weeks and three session per week. They also consumed 1000 mg of omega-3 supplements four times a day for four weeks. The EP group performed similar exercises along with consuming placebo. The tests were performed 48 hours before and after the training period after a 12-14 hour fast. Data were analyzed using dependent t-test. **Results:** After four weeks, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) significantly decreased in the EO group (p=0.005). **Conclusion:** Our findings showed that omega-3 supplementation can decrease the serum values of liver and muscle damage indicator enzymes in male elite karate athletes.

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INTRODUCTION

Today, physical education not only ensures society's health and increases body's ability to function efficiently, but it also has influenced all the aspects of human life. Physical education strengthens different body systems using exercise science, thus allowing athletes to set new records each year. To get the best results from exercise, it is important to prevent muscle soreness and damage to muscle cell membrane. Changes in serum enzymes are a good indicator for determining cell or tissue damage. Considering the metabolic and clinical role of enzymes and the effect of physical exercise on them, studying exercise-induced changes in enzymes has become more important Amir-Shaqaqi (2006).

Studies show that a bout of exercise can increase free radicals, which can lead to damages and injuries if the body's natural antioxidant defense system is weak. Consuming antioxidant supplements such as omega-3 along with exercise can prevent liver and muscle damage. However, some researchers believe antioxidant supplementation to be ineffective Azizi *et al* (2010).

Indicators of Liver Damage:

Liver enzymes are normally within liver cells, but these enzymes are released into the blood when the liver is damaged. The most critical and most widely used liver enzymes are aminotransferases such as AST and ALT. Increase in the level of these enzymes indicates liver damage.

Liver aminotransferases and diagnosis of diseases

Aspartate aminotransferase (AST) is naturally found in body tissues such as liver, heart, kidney, muscles, and brain. When any of these tissues is damaged, AST is released into the blood. For instance, serum AST levels are elevated during heart attacks or muscle injuries. Unlike AST, alanine aminotransferase (ALT) is mainly found in the liver. The normal range of values for AST is 5 to 40 units per liter of serum, and the normal range of values for ALT is 7 to 56 units per liter of serum. Elevated AST and ALT levels are critical markets of

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different liver diseases, but higher than normal levels of these enzymes does not necessarily indicate liver damage.

Exercise and indicators of muscle and liver damage:

It is well-established that sudden strokes to tissues affect the activity of enzymes in the plasma. Muscle damage is mostly associated with release of AST enzymes into the bloodstream .AST is present in both cytoplasm and mitochondria and is released into the blood after injury or death of cells Paknejad *et al*(2003). Many researchers believe that enzymes such as creatine kinase (CK) and AST and most metabolites such as lactic acid are stimulants that cause pain and injury in involved muscles; exercises with higher intensity and longer duration have a greater effect on these enzymes Nameni *et al*(2004).

Researchers have reported the relationship between accumulation of lactic acid in the muscles and reduced peak muscle tension, which is due to increase in lactic acid and subsequent hydrogen ion accumulation and decrease in pH Naimikia *et al* (2006). Regular exercise with low-to-moderate intensity decrease liver enzymes and improve symptoms Smuts *et al*(2003), González-Périz *et al*(2003).However, long-duration and high-intensity exercises can elevate liver enzymes Tartibian *et al* (2011).

High levels of serum ALT and AST has been seen immediately after activities like cycling where body weight is supported. Elevated AST ad ALT levels have also been observed after high-intensity, resistance, and eccentric exercises Clarkson *et al*(2006).

Omega-3 Fatty Acids:

Omega-3 fatty acids are unsaturated fatty acids that have a double bond between the third and fourth carbon along the carbon chain. Omega-3 fatty acids are vital for human metabolism, but they are not produced inside the body.

Omega-3 fatty acids are converted into prostaglandins 1 and 3 (PG1 and PG3). Prostaglandins have a role in blood pressure regulation, blood coagulation, nerve transfers, allergic and inflammatory reactions, renal and gastrointestinal function, tissue regeneration and repair, energy production, regulation of sex hormone, and synthesis of other hormones. Moreover, PG1 and PG3 have anti-inflammatory and anticoagulant properties Mancardi *et al*(2009).

Farzanegi *et al*. (2012) examined the effect of endurance training and curcumin supplementation on the liver indices of male rats exposed to lead poisoning. The results showed that eight weeks of lead injection significantly increased AST and ALP activity in the experimental group compared to the control group. Endurance training together with curcumin supplementation significantly decreased AST activity. Also ALP activity decreased non-significantly as a result of endurance training and curcumin supplementation. The researchers concluded that exercise and curcumin supplementation may inhibit liver damage due to lead poisoning Farzanegi *et al*. (2012).

Alizadeh *et al*. (2011) examined the changes in plasma interleukin-17 (IL-17), C-reactive protein (CRP), and creatine kinase (CK) in male rats after eight weeks of aerobic training and omega-3 supplementation. At the end of the training, CK levels decreased in the experimental groups compared to the control group.

Azizi *et al*. (2010) studied the effect of antioxidant supplements on oxidative stress and muscle damage following a period of high-intensity training. They reported a non-significant decrease in CK and AST levels in the supplementation group Azizi *et al*. (2010) .

Nobahar *et al*. (2012) examined the effect of incremental exhaustive exercise on indicators of muscle damage in active girls. Subjects performed incremental exercises every day for one week. Blood samples were collected 24 hours before and after the first, fourth, and seventh days. The findings showed a significant increase in AST levels after the first, fourth, and seventh days of training. CK values increased on the fourth and seventh days. LDH significantly increased on the seventh day and after the 24-hour recovery Nobahar *et al*. (2012).

Padalino *et al*. (2007) compared the effects of training and overtraining on certain indicators in trotters. The results showed significant higher AST, ALP, and LDH levels in the overtraining group compared to the training group Padalino *et al*. (2007)

Sinclair AJ *et al*. (2011) evaluated the indicators of liver damage (AST, ALT, and ALP) following exercise. The results suggested a significant increase in AST levels immediately after running. AST increased 24 hours after exercise, while in none of the subjects did ALT and ALP levels exceed the maximum values Sinclair AJ *et al*. (2011).

Parker *et al*. (2011) did a research on the effects of omega-3 polyunsaturated fatty acids on people with non-alcoholic fatty liver disease. They found that ALT and AST decreased as a result of omega-3 consumption Parker *et al*. (2011).

Al-Attar (2010) studied the effects of plant oils supplementation on diabetic rats. He examined 120 Wistar rats in 12 groups. The results showed that diabetic rats supplemented with normal diet experienced reduced body weight gain rates, reduced liver/body weight ratio, and increased kidney and spleen/body weight ratios. Serum glucose, creatinine, urea, uric acid, triglycerides, cholesterol, LDL-C, VLDL-C, and the activities of

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PAM, CK, GPT, GOT, and ALP were significantly elevated. Supplementation of the tested oils in diabetic rats showed remarkable lowering effects of physiological abnormalities changes. Further, between-group comparisons showed that soybean oil treatment is more effective to improve the physiological parameters which are abnormally altered due to diabetes mellitus Al-Attar (2010).

Methodology:

A single-blind, quasi-experimental, controlled, pretest-posttest design was used to examine the synergetic effect of omega-3 supplementation and exercise on certain indicators of liver (ALP, AST, and ALT) and muscle (LDH and CK) damage in elite male karate athletes. The population consisted of the karate athletes of Tabriz City within the 20-30 age range. Given the nature and purpose of the research, 20 subjects were non-randomly selected from 40 volunteers, and on the blood collection day the number of subjects decreased to 9 subjects in the experimental group (exercise and omega-3) and the control group (exercise and placebo) because some of the subjects did not follow the 12-14 hour fast before the tests. Measurement of plasma values and biochemical analysis of ALP, ALT, and AST values were done using ELISA test kits and according to the instructions of the manufacturing company. Dependent t-test was used to examine within-group changes. All the analyses were done in SPSS at the 0.05 significance level.

Results:

The results of dependent t-test for examining the difference between pre- and post-exercise AST levels in the experimental group are provided in Table 1.

Table 1: Results of dependent t-test for examining AST levels in the experimental group

Experimental Group	Mean	SD	Mean Difference	df	Observed t-value	Critical t-value	p-value
Pre-exercise AST	20.32	4.56	5.82	8	4.089	2.30	0.005*
Post-exercise AST	14.5	2.34					

* Significant at $p < 0.05$

The results indicate that the observed t-value is greater than the critical t-value (t = 2.30) and thus there is a significant difference between pre- and post-exercise AST levels of the subjects in the experimental group ($p < 0.05$).

The results of dependent t-test for examining the difference between pre- and post-exercise ALT levels in the experimental group are provided in Table 2.

Table 2: Results of dependent t-test for examining ALT levels in the experimental group

Experimental Group	Mean	SD	Mean Difference	df	Observed t-value	Critical t-value	p-value
Pre-exercise ALT	10.13	1.56	3.10	8	9.892	2.30	0.000*
Post-exercise ALT	7.03	1.04					

* Significant at $p < 0.05$

The data in Table 2 indicate that the observed t-value is greater than the critical t-value (t = 2.30) and thus there is a significant difference between pre- and post-exercise ALT levels of the subjects in the experimental group ($p < 0.05$).

The results of dependent t-test for examining the difference between pre- and post-exercise ALP levels in the experimental group are provided in Table 3.

Table 3: Results of dependent t-test for examining ALP levels in the experimental group

Experimental Group	Mean	SD	Mean Difference	df	Observed t-value	Critical t-value	p-value
Pre-exercise ALP	214	22.8	22.80	8	7.263	2.30	0.000*
Post-exercise ALP	191.2	16.4					

* Significant at $p < 0.05$

The data indicate that the observed t-value is greater than the critical t-value (t = 2.30) and thus there is a significant difference between pre- and post-exercise ALP levels of the subjects in the experimental group ($p < 0.05$).

Discussion and Conclusion:

High-intensity exercises, especially those with eccentric contractions, are followed by increased serum values of enzymes such as CK, ALT, AST, and LDH as well as skeletal muscle damage. Moreover, AST, ALT, and ALP are normally found in liver cells, but when the liver is damaged, AST and ALT catalyze the transfer of amino acids from aspartate and alanine to ketoglutaric acid to form oxaloacetate and pyruvate. These two enzymes are naturally found in different tissues such as the liver, heart, muscles, and brain, and they are released into the bloodstream upon damage to any of these tissues. For instance, the serum concentration of AST and ALT increases during heart attacks and muscle damage. Many studies have shown that high-intensity exercise can cause damage to skeletal muscles and can increase the markers of liver and muscle damage.

In the present research, four weeks of special karate training along with daily omega-3 supplementation led to significant decrease in AST by 28.6 percent, ALT by 30.6 percent, and ALP by 10.6 percent. Apparently, omega-3 supplements stabilize liver cell membrane through an unknown mechanism, thus preventing the release of these enzymes Alizadeh *et al* (2011). Consistent with the present research, decrease in liver enzyme levels with animal-based omega-3 or antioxidant supplementation has been reported in several studies. For instance, Cavas and Tarhan (2004) found that antioxidant supplements, including omega-3, play an effective role in reducing AST and liver damage .Also Santos *et al*. (2004) showed the beneficial effects of omega-3 in reducing serum AST, ALT, and ALP levels in rats Santos *et al*. (2004). Capanni *et al* (2006) showed similar effects of fatty acid supplementation AST, ALT, and ALP levels Capanni *et al*. (2006). Moreover, some studies have reported reduced ALT and AST levels after several weeks of omega-3 supplementation in patient with non-alcoholic fatty liver disease Santos *et al* (2004). Plant-based omega-3 supplementation has also led to reduced AST, ALP, and ALT levels in rats Al-Attar (2010).

In the present research, four weeks of karate training without supplementation led to non-significant decrease in AST (11.6%), ALT (5.6%), and ALP (2.1%) levels. Bashiri *et al*. (2010) found that resistance training for two months leads to non-significant increase in serum ALT and AST levels in non-athlete students . Many studies have shown that the value of these enzymes increase immediately after high-intensity exercises and competitions Chuang *et al*(1996).Therefore, non-significant decrease in ALT and AST suggests a special form of adaptation, and if exercise stress is followed by proper recovery, it will prevent more damage to the liver and muscles Bashiri *et al*(2010). One study examined the effects of a four-week short and middle distance training on AST, ALT, and lactate levels in nursing college students. The results showed significantly higher AST and ALT levels at the end of the first week compared to the values obtained at the end of the fourth week Chuang *et al* (1996). This findings supports the results of the present research, indicating that decrease in liver damage enzymes is affected by the duration of exercise. Also the experience of the subjects and whether they are athletes or not may affect the changes in ALT and AST levels. The response of AST to high-intensity exercise has been shown to be higher in Olympics rowers compared to untrained subjects Koutedakis *et al* (1993). Prolonged and endurance exercises have a greater effect on ALT and AST, because continuing these exercises require more aerobic energy generation. AST and ALT are two of the enzymes involved in hepatic metabolism, and since the liver is more intensely involved in prolonged exercises, liver membrane is more likely to be vulnerable to these activities Bashiri *et al* (2010). If training is designed in a way that a major part of energy is generated anaerobically, these enzymes are less produced and the possibility of liver damage decreases Parker *et al* (2012). Based on the theory of enzyme leakage through the cytoplasmic membrane, AST and ALT may leak into the bloodstream. Therefore, the type and intensity of exercise has a critical effect and the resulting adaptations to stabilize liver cell membrane can decrease AST and ALT release into the blood . In the present research, decrease in serum ALP levels can be regarded as a favorable adaptation in karate athletes. Farzanegi *et al*. (2012) observed a similar protective effect of exercise on AST and ALP levels in animal samples Farzanegi *et al* (2012).

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