EFFECT OF OMEGA-3 SUPPLEMENTATION ON THE BLOOD LEVELS OF OXIDATIVE STRESS, MUSCLE DAMAGE AND INFLAMMATION MARKERS AFTER ACUTE RESISTANCE EXERCISE IN YOUNG ATHLETES

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Abstract:
The present study was conducted to assess the effect of dietary omega-3 fatty acid supplementation on the levels of oxidative stress, muscle damage, and inflammatory markers after acute resistance exercise in young athletes. In a randomized double-blind design, twenty subjects were divided into two equal groups; each subject receiving three capsules per day (3000 mg) of either omega-3 or a placebo for seven days. All subjects underwent high intensity acute resistance exercise. Venous blood samples were collected one week prior to the exercise, immediately pre-exercise, and 24 hours post exercise. Malondiadehyde (MDA), plasma total antioxidant capacity (FRAP), C-reactive protein (CRP), creatine kinase (CK), and lactate dehydrogenase (LDH) were measured in the serum. MDA, CRP and CK concentrations were significantly higher 24 hours post exercise in the placebo versus the omega-3 group (p=.005). The mean of total antioxidant capacity in both groups showed no significant differences immediately pre-exercise and 24 hours post exercise (p>.005). LDH activity was significantly higher 24 hours post exercise in both groups (p=<.05). The results of this study indicate that high intensity resistance exercise induces oxidative stress, systemic inflammation, and cellular damage indices in athletes. However, seven days of omega-3 fatty acid supplementation may ameliorate these effects.

Key words: malondiadehyde, plasma total antioxidant capacity, omega-3, resistance exercise, C-reactive protein, creatine kinase, lactate dehydrogenase, young adults, collegiate men, handball players

Introduction
Many published studies have indicated that regular exercise training is associated with prevention of chronic diseases such as cardiovascular diseases, diabetes, cancer, hypertension, obesity, depression, osteoporosis and premature death (Belviranl & Gökbel, 2006, Thirumalai, Therasa, Elumalai, & David, 2011). However, strenuous physical exercise is also shown to increase reactive oxygen species (ROS) production resulting in oxidative stress (Fatouros, et al., 2004). Furthermore, the production of ROS is part of the post-exercise inflammatory process (increases inflammatory cytokines in the circulation) that accompanies muscle damage, such as that which occurs following eccentric exercise (Clarkson & Thomson, 2000).

Despite numerous studies investigating the effects of aerobic exercise on oxidative stress and inflammatory reactions (Belviranl & Gökbel, 2006; Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005), few researchers have investigated the effects of resistance exercise on the redox status and the systemic inflammatory response. Deminice and colleagues (2010) recently reported that oxidative stress biomarkers decreased following an acute session of resistance exercise in humans. In contrast, resistance exercise has been shown to lead to increasing circulating inflammatory and cellular
damage markers (Roth, et al., 2000; Damirchi, Rahmani-Nia, & Mehrabani, 2011). Moreover, it has been suggested that heavy resistance exercise, especially that with an eccentric component, results in muscle tissue damage, which initiates an inflammatory response that eventually produces oxygen free radicals and lipid peroxidation (Gützel, Hazar, & Erbas, 2007).

It has been suggested that exercise-induced oxidative stress and inflammatory/acute phase responses can be reduced by supplementation with antioxidants. Omega-3 fatty acids are a family of fatty acids that have shown promise and favorable effects in modifying a host of disease processes involving the inflammatory and immune pathways, cardiac dysrhythmias, rheology, and lipid regulation in the general population (Ho, Maple, Bancroft, McLaren, & Belch, 1999; Friedman & Moe, 2006). By down-regulating pro-inflammatory eicosanoids, omega-3 fatty acids may reduce the risk for atherosclerotic progression (De Caterina & Zampoli, 2004).

Omega-3 fatty acids may have antioxidant effects by inhibiting lipid peroxidation (Taccone-Gallucci, et al., 2006). Recently, Tartibian, Maleki and Abbasi (2011) showed that omega-3 fatty acid supplementation attenuates the inflammatory response following an eccentric exercise in untrained men. Accordingly, consumption of omega-3 fatty acids may be beneficial in ameliorating the negative effects of ultra-structural damage and the subsequent inflammatory response following a hard resistance training session.

Therefore, this study was conducted to assess the effect of omega-3 fatty acids on the blood levels of oxidative stress, muscle damage and inflammation markers after acute resistance exercise in young adult athletes.

Methods

Study design and subjects

In a randomized double-blind placebo-controlled trial, twenty collegiate young male handball players were allocated into two equal groups — a supplement (treatment) group, and a placebo (control) group. Supplements were consumed for seven days, before which an overnight fasted blood sample was taken and anthropometric measurements were made. On the eighth day, all subjects underwent an intense resistance exercise session. Further samples were taken immediately before exercise and 24 hours following the exercise test.

None of the subjects had ingested omega-3, or any other dietary supplements or drugs during six months preceding the study. In addition, none reported a past history of kidney, heart and liver disease, diabetes or any physical damage. All subjects were experienced in resistance training, as circuit training was a part of their pre-competition regime. Experimental procedures and potential risks were explained and informed consent was obtained from all the subjects. All were asked to abstain from intense physical activity for 48–72 hours before the measurements. The study design and experimental procedures were approved by the Regional Research Ethics Committee of Islamic Azad University, and conducted in the laboratory conditions (temperature 22–25 °C; humidity 50–55%).

Anthropometric measurements

Subjects’ height (nearest 0.1 cm) and weight (nearest 0.1 kg) were measured using a stadiometer and digital scale, and body mass index (BMI) (body weight [kg]/height [m²]) was calculated. Body fat percentage was predicted from the skinfold measurements taken on the right side of the body using specialized calipers (Baseline Economy ‘Slim-Guide’) at the triceps, abdominal, and super-iliac sites after 10 hours of fasting. Body fat percentage was then calculated by using the formula described by Brozek et al. (1963) once estimations of the body density had been made from the regression equation of Jackson and Pollock (1978).

Supplementation protocol

The subjects in the supplemented group were given omega-3 fatty acid (each capsule contained 1000 mg of omega-3 fatty acid, product Pty Ltd, Brookvale, Australia), containing 180 mg eicosapentaenoic acid, 120 mg docosahexaenoic acid in a base of natural vitamin E, gelatin glycerol, and purified water. The supplement was taken three times per day at regular intervals (breakfast, lunch and dinner) for seven days. The control group received a placebo of the same form and size of capsule as the omega-3 capsules and instructed to ingest these at the same times. During supplementation, all the subjects completed a validated food intake questionnaire and made a 24-hour food record to determine if both groups had similar diets. All subjects reported adherence to the experimental protocol and complete ingestion of the supplement.

One repetition maximum (1RM)

The predicted maximum strength as one repetition maximum (1RM) for the upper and lower extremities was estimated according to the National Strength and Conditioning Association guidelines and calculated using the Brzycki (1995) equation ([1 RM=Weight / (1.0278 - (0.0278 × Number of repetitions)]). After a warm-up period, all the subjects lifted submaximal loads until exhaustion (7–10 RM). The appropriate load was based on each subject’s weights used for circuit training in the pre-competition season (Brzycki, 1995; Eston & Evans, 2009).


**Exercise protocol**

Prior to the intervention, all participants underwent a familiarization and correct handling procedure session of the training equipment that would be utilized during the study. The subjects performed a gentle warm-up before the start of the exercise test. They then performed a resistance exercise session which consisted of three leg exercises including leg press, leg extension, and leg curls at 120% of the participants’ predetermined predicted 1RM for each exercise. The participants completed 40 repetitions (4 sets x 10, with 3 minutes rest between sets) of each exercise. The resistance exercise protocol utilized in this study has been reported in previous studies and is sufficient to provide significant eccentric work to the muscle groups involved (Cooke, Rybalka, Stathis, Cribb, & Hayes, 2010).

**Blood collection and analysis procedures**

Blood samples were collected one week prior to supplementation for baseline analysis of oxidative stress, cellular damage and inflammatory markers. The blood collection was performed using vacuum tubes, one containing an anticoagulant and the other without it. The collected blood samples were centrifuged at 4 c, at a speed of 2000 rpm for 10 minutes, so that the determination in the serum as well as in the plasma was performed. S/P was aliquot in 1 cc micro tubes and was stored at -20 degrees until use. Further samples were taken immediately before exercise, and 24 hours following the exercise test. Blood (5 ml) was extracted from the subject’s antecubital vein and collected into a specialized blood tube (5 ml; made by SUHA Co.). Plasma levels of C-reactive protein (CRP) were measured by a highly sensitive enzyme linked immunosorbent assay (ELISA) technique as described previously (Wong, et al., 2007). Afterwards, the serum was separated by a centrifuge (SAHAND Co.) and serum CK and LDH activities, as cellular damage indices, determined by commercial kits (Sigma Chemical Co.) with automatic analyzers (RA-1000; American TECHNICOM Co.). The total antioxidant capacity of the plasma was evaluated by applying the FRAP assay (ferric reducing antioxidant power or ferric reducing ability of plasma) according to the method of Benzie and Strain (1996). The method is based on the reduction of ferric (Fe³⁺) to ferrous (Fe²⁺) ion at low pH. This causes a formation of blue colored ferrous tripyridyltriazine (Fe²⁺·TPTZ) complex, which absorbs at 593 nm. Moreover, plasma malondialdehyde (MDA) concentrations, as an oxidative stress indicator, spectrophotometrically were assayed by measurement of thiobarbituric acid reactive substances (TBARS) assay according to the procedure of Uchiyama and Mihara (1978).

**Statistical analyses**

Before statistical comparison, all data sets were tested for normal distribution by a Kolmogorov-Smirnov test. Statistical analyses of the differences in physiological characteristics of the subjects in the two groups at the beginning of the research were performed using paired t-test. Data were expressed as mean ± SD and analyzed by using two (omega-3 and placebo groups) x 3 (times of measurement) repeated measures analysis of variance (ANOVA), and Bonferroni post-hoc tests were applied to locate the source of significant differences using the SPSS statistical software package (SPSS, version 16.0 for Windows, SPSS Inc., Chicago, IL, USA). Significance was set at p<.05.

**Results**

Physiological characteristics of the subjects at the beginning of the research are presented in Table 1. There were no differences between the groups at the beginning of the research for age, body weight, height, BMI and body fat percentage.

**Table 1. Physical characteristics of the omega-3 and placebo groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Omega-3 (n=10)</th>
<th>Placebo (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.24 ± 1.87</td>
<td>21.55 ± 2.34</td>
<td>.195</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.30 ± 6.94</td>
<td>72.40 ± 6.67</td>
<td>.125</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.30 ± 1.05</td>
<td>179.60 ± 2.1</td>
<td>.368</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.44 ± 2.3</td>
<td>23.50 ± 1.67</td>
<td>.219</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.39 ± 1.75</td>
<td>17.19 ± 2.81</td>
<td>.148</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation

The plasma MDA and FRAP concentrations before the resistance exercise did not differ between the supplement and placebo groups, but the pattern of change in the MDA, as a measure of oxidative stress, was significantly higher 24 hours post exercise in the placebo versus omega-3 (p=.011) group and exhibited interaction (p=.007) and time (p=.001) effects (Figure 1). However, concentrations of FRAP as a measure of plasma antioxidant potential were unaffected by the exercise in both groups (p=.119) (Figure 2).

Interestingly, C-reactive protein (CRP) was significantly increased 24 hours post exercise in the placebo group (p=.001) but not in the omega-3 group (p=.248) (Figure 3), indicating that omega-3 supplementation can prevent the increased systemic inflammation produced by acute resistance exercise.

Concentrations of lactate dehydrogenase (LDH) increased significantly after the exercise in both groups (p=.024 and p=.000, respectively), but there were no differences in the pattern of change between the groups (group effect, p=.264;
interaction effect, p=.100) (Figure 4). Creatine kinase (CK) concentration increased significantly only in the placebo group after 24 hours post exercise (p=.007), but was unchanged by exercise in the omega-3 group (p=.582) and indicated that supplementation with omega-3 could prevent the increased systemic inflammation produced by acute resistance exercise in athletes (Figure 5).

**Discussion and conclusions**

Strenuous exercise has previously been shown to induce muscle damage and oxidative stress, even in athletes. However, consumption of products rich in antioxidants may potentially ameliorate this effect. Therefore, the aim of this study was to investigate the effects of omega-3 fatty acid supplementation on the circulating markers of oxidative stress, muscle damage, and circulating inflammatory markers after acute resistance exercise in young athletes.

The most important finding of this study is that short-term (one week) omega-3 supplementation before an acute bout of strenuous resistance exercise can reduce post-exercise oxidative stress by attenuating increases in plasma MDA levels. Few researchers have assessed oxidative stress after acute resistance exercise, though reports of increased MDA in both sedentary (Güzel, et al., 2007)
and trained males (McBride, Kraemer, Triplett-Mcbride, & Sebasianelli, 1998) are available. It has been proposed that ischemia-reperfusion at the site of the active muscles is probably the mechanism for the production of reactive oxygen species and oxidative stress during high-force resistance exercise (McBride, et al., 1998; McBride, 1999). Others (McAnulty, et al., 2005) concluded that resistance exercise has no effects on oxidative stress markers (F2-isoprostanes) in trained subjects. The differences in the exercise intensity and/or training status may account for this discrepancy.

Akin to our results, though in diabetic patients, Kesavulu et al. (2002) observed that supplementation with omega-3 fatty acids is associated with decreased blood MDA concentration, and others (Poprzecki, 2003) have shown that omega-3 fatty acid supplementation may result in improved antioxidant status in male subjects following acute exercise in male subjects.

The potential mechanism by which omega-3 fatty acids may reduce oxidative stress could be related to their tight packing in complex membrane lipids and lipoproteins making the double bonds less available for free-radical damage (Mori, et al., 1999). Another potential mechanism is through increased production of catalase levels, a peroxisome-based antioxidant enzyme (Masters, 1996).

Our data show that CK and LDH values, as myocellular damage indices, were increased as a result of acute resistance exercise in both groups. However, we observed significant effects of omega-3 administration on CK values; it seems that omega-3 supplementation can attenuate the increase of CK values normally seen following resistance exercise. In agreement with these findings Tartibian et al. (2011) indicated that men who ingested omega-3 (N-3) fatty acids demonstrated a significant trend toward reduction in the plasma concentration of LDH and CK immediately, 24, and 48 hours after a resistance exercise program, when compared with a placebo group. In contrast with these results, Poprzecki (2003) reported that omega-3 supplementation did not significantly influence CK activity after one hour ergocycle effort with a workload of 60% in male subjects, but this may be due to the relatively low muscular tensions required and concentric-only contractions in cycling exercise. There are also differences in the omega-3 dose and length of supplementation with this and the current study.

There is a substantial evidence that acute strenuous exercise can significantly increase pro-inflammatory markers in the circulatory pathway (Damirchi, et al., 2011; Petersen & Pedersen, 2005). Post-exercise inflammation in athletes may be caused by mechanical stress, local ischemia, and/or free radical generation in the active skeletal muscle (Ghiasvand, et al., 2010). However, in accordance with the results of this study, recently it has been shown that omega-3 supplementation significantly ameliorates inflammatory markers after eccentric exercise in untrained men (Tartibian, et al., 2011). Likewise, Jouris, McDanie and Weiss (2011) declared that one-week supplementation with omega-3 fatty acid may attenuate the inflammatory response (via measurement of soreness ratings and swelling – arm circumference) to eccentric strength exercise in healthy adult men and women. Although the mechanisms responsible for this improvement are not clear, it has been suggested that anti-inflammatory properties of omega-3 fatty acid and the mechanisms by which omega-3 decreases CRP may be through the inactivation of TLR4 (toll-like receptor 4) and NF-κB (NF-κB) and IL-6/IL-1 expression (Adkins & Kelley, 2010). Another possibility is thought that the omega-3 fatty acids (such as eicosapentaenoic acid and docosahexaenoic acid) decrease the amounts of arachidonic acid available as a substrate for eicosanoid synthesis and also inhibit the metabolism of arachidonic acid (Calder, 2006).

Taken together, our data led us to conclude that a single session of strenuous resistance exercise induces oxidative stress production and increase of CRP and cellular damage indices in young athletes. However, short-term (one week) omega-3 fatty acid supplementation may ameliorate the rise in these markers after acute resistance exercise and can therefore be an effective means of minimizing exercise-induced oxidative damage and systemic inflammation in the post-exercise period.

Declaration
The authors have no conflict of interest to declare in the generation of this research and publication of this manuscript.
References


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Istraživanje je provedeno radi utvrđivanja učinaka suplementacije omega 3 masnim kiselinama na razinu oksidativnog stresa, mišićnog oštećenja i upalnih biljega kao akutnog odgovora na trening s opterećenjem u mladih sportaša. U okviru dvostrukok slijepog dizajna eksperimenta sa slučajnim odašiljem dvadeset je ispitanika bilo podijeljeno u dvije jednake grupe u kojima je svaki ispitanik uzimao dnevno tri kapsule (3000 mg) omega 3 masnih kiselin ili, u kontrolnoj grupi, placebo kapsule tijekom sedam dana. Svi ispitanici su podvrgnuti visokointenzivnom treningu s opterećenjem. Venski krvni uzorci bili su prikupljeni tjedan dana prije treninga, neposredno prije treninga te 24 sata nakon vježbanja. Mjerene su serumske vrijednosti malondialdehida (MDA), antioksidacijskog kapaciteta u plazmi (FRAP), C reaktivnog proteina (CRP), kreatin kinaze (CK) i laktat dehidrogenaze (LDH). Koncentracije MDA, CRP i CK bile su statistički značajno veće 24 sata nakon vježbanja u placebo grupi nego li u grupi koja je uzimala omega 3 masne kiseline (p=0,005). Prosječne vrijednosti ukupnog antioksidacijskog kapaciteta u oba grupe nisu pokazale značajne razlike između mjerenja neposredno prije vježbanja i 24 sata nakon vježbanja (p>0,005). Aktivnost LDH bila je statistički značajno viša 24 sata nakon vježbanja u obje grupe (p<0,05). Rezultati ovog istraživanja pokazuju da visokointenzivne vježbe s opterećenjem izazivaju oksidativni stres, sistematske upalne procese i povećavaju razinu pokazatelja staničnih oštećenja u sportaša. Ipak, sedam dana suplementacije omega 3 masnim kiselinama može smanjiti navedene učinke treninga.

Ključne riječi: melondialdehid, ukupni plazmički antioksidativni kapacitet, omega-3, vježba s opterećenjem, C-reaktivni protein, kreatin kinaza, laktat dehidrogenaza, mlade odrasle osobe, studenti, rukometari