The Effect of Endurance Training and Omega-3 Supplementation on Inflammatory Cytokines in Endurance Runners

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Abstract

The aim of this study was to investigate the effect of endurance training and omega-3 supplementation on inflammatory cytokines in endurance runners. To this end, a total of 30 men with an average of 26.9±2.6 years of age, 176.2±4.5 cm height, 77.0±3.49 kg weight, 24.37±1.4 kg/m² BMI and maximum oxygen uptake of 43.15±2.62 ml/kg/min among Iranian National league 5000 meters endurance runners were selected and targeted to three groups of 10 (control, placebo and supplement) based on the percent of body fat and VO2max. The experimental groups followed a 2 weeks endurance training and received an omega-3 supplementation. The exercise protocol consisted of 90 to 120 minutes of exercise at 65 % VO2max on an ergometer cycle at a frequency of 3 sessions per week. Blood samples were taken 48 hours before and after the training period and the cultures isolated immune cells, production of cytokines TNF-α and IL-1β was measured by ELISA in vitro. The findings of this research showed that endurance training significantly increases the concentration of inflammatory cytokines and that omega-3 supplements significantly affect TNF-α levels by reducing its in endurance training but had no significant effect on the IL-1β. Omega-3 supplementation have positive role in decreasing TNF-α concentration during endurance training.

Keywords: IL-1β, TNF-α, Endurance training, Omega-3 supplements.

Introduction

The elite athletes should deal with increasing and intense exercises to get the best body fitness. These types of exercises usually will lead to the release of Prostaglandin E2 (PGE2), and leukotriene B4 (LTB4) from immune cells. PGE2 and LTB4 are produced from unsaturated fatty acids metabolism exist in phospholipid membrane of immune cells specially Arachidonic acid (AA) (Shreedhar et al., 1998; Andrade et al., 2007). The release of PGE2 and LTB4 will lead to increase in production of inflammatory cytokines as IL1-β and TNF-α (Koyama et al., 2007; Lo et al., 1999; Richard et al., 2008; Zhao et al., 2004). The Increase in such inflammatory cytokines is related to some complications such as expressing adhesive molecules and blood clots formation, the response to acute inflammation phase and body’s soft tissue damage. They also have an effect on the hypothalamus increases the release of (Glucocorticoids), overtraining symptoms such as sleeping disorders, Anorexia, weight loss and depression (Nieman et al., 2003; Huffman et al., 2008; Forman et al., 1997). The major function of TNF-α is to adjust cell immunity (Grimble, 1996). IL-1β is also an inflammatory cytokines which play an important role in immune defense against infections (Dinarello, 2006).

It should be mentioned that some other studies have shown that endurance type exercise, especially if it is done in consistent regular basis, reduces the sympathetic tone and inflammatory cytokines by creating some adaptability and increases anti-inflammatory cytokines. Although some researches have shown increased inflammatory cytokines after a period of endurance training (Huffman et al., 2008; Koyama et al., 2007; Koch, 2010; Lee et al., 2011). On the other hand, some studies have been conducted which show that taking certain supplements following an activity affects the inflammatory cytokines levels (Andrade et al., 2007; Shreedhar et al., 1998; Mozaffarian & wu, 2001; Nieman et al., 2003; Roman et al., 2006; Toft et al., 2000; Walse et al., 2006). According to researches with using omega-3, its content of Eicosapentaeonic acid (EPA) and Dacosahexaenoic acid (DHA) are replaced relatively with AA in phospholipid membrane of immune cells. The decrease in AA will lead to decrease in PGE2 and LTB4 production.

As well as, consuming omega-3 will lead to increase in Prostaglandin E3 (PGE3) and leukotriene B5 (LTB5) synthesis which of less inflammatory characteristics (Kelley et al., 1999; Kelley, 2001; Mayatepek et al., 1994). Studies have shown that supplementation with omega-3 in the mixture of EPA and DHA is in some cases associated with a reduction of inflammatory
cytokines following exercise and in some cases it didn’t have a positive effect on their recovery. Walser and colleagues showed that using omega-3 supplementation after endurance exercise reduces levels of acute phase proteins (Zhao et al., 2004).

Andrade and colleagues had studied the effect of omega-3 in fish oil supplementation on immune responses in elite swimmers to six weeks following the endurance swimming and concluded that taking this supplement reduces IL-1β by reducing PG2 (Andrade et al., 2007). However, there are some researchers such as Toft and Niemann cited studies (Nieman et al., 2003; Toft et al., 2000), have shown that omega-3 supplementation has no effect on the levels of inflammatory cytokines. Although, studies on the effect of acute exercise and adaptations to endurance training as well as the effect of supplementation with omega-3 on endurance exercise response are abundant but the results are inconsistent. To the best knowledge of the present researcher, no study has yet examined the impact of these interventions simultaneously. So the aim of this study was to investigate the effect of endurance training on inflammatory cytokines and omega-3 supplementation in endurance runners.

**Methodology**

**Subjects**

A total of 30 men with an average of 26.9 ± 2.6 years of age, 176.2±4.5 cm height, 77.03 ± 3.49 kg weight and 24.37 ± 1.4 kg/m² BMI and maximal oxygen consumption (VO₂max) of 43.15 ± 2.62 ml/kg.min, among Iran’s National League endurance runners who responded to the call for cooperation, were selected firmly based on the percentage of fats on their bodies (with a history of sport and equivalent physical activity level). After a session to familiarize the participants with the process of implementation, understanding of research procedures and filling medical information forms, physical activity level questionnaire, consents, weight and height measurement (digital scale of Glass Scale GES-07 made in United States, with an accuracy of ±0.1 kg, wall height gauge of 4440, made in Iran’s Kaveh company, with an accuracy of ±1 cm) and VO₂max (gas analyzer, GANSHORN model made in Germany) they were selected and targeted in 3 groups of 10 (control, placebo and omega-3 supplements).

To measure the percentage of body fat using a Harpenden body fat caliper gauge, subcutaneous fat content (chest, abdomen, and thigh) was determined using Jackson/Pollock formula. The research was conducted on each of the experimental groups by participating in the program of endurance training on an ergometer cycle (Monark ergometric 828E model, made in Sweden), as well as taking the required values of omega-3 supplementation and placebo group (supplementation Group with 6 grams of oil capsules daily, containing 3.6 grams of omega-3: 1200 mg EPA + 2400 mg DHA) and placebo group (6 grams placebo capsules daily containing only mineral oil) receiving on a daily basis and a blind for two weeks. To reduce the effects of the last training session on the measured factors, blood samples were collected 48 hours following the last day of the training period. The participants were prohibited from physical activity and alcohol, caffeine and food consumption, 24 hours prior to taking blood samples. All blood samples (10 ml of heparinized blood samples) in fasting from 8 to 10 AM were collected from the elbow vein in a sitting position.

It should be noted that none of the participants had any metabolic disease, and nor were smokers. Foods containing high levels of omega-3 and medicines which affect the body’s immune system were introduced not to use them during the research. They also didn’t use any vitamin C and E supplement.

**Blood analysis**

Peripheral blood mononuclear cells (PBMCs) are separated at density of 1.077 on ficoll through Density Gradient method. At first, the blood is diluted with cell culture RPMI 1640 at ratio of 1:1 and then it gradually transferred on ficoll at ratio of 1:2; the above set is centrifuged for 20 minutes and the corresponding layers of PBMCs are gathered from ficoll with a Pasteur pipette. Immediately, the vital living cells test is carried with Trypan Blue staining. After separation, the PBMCs are cultured soon in the concentration of 1million cells per well, in plates of 24 in cell culture of RPMI 1640 for 48 hours. It's containing fetal bovine serum 1%. For stimulating Cytokine production, 10 mg/ml of Phytohaemagglutinin (PHA), polyclonal stimulation of T cells is added to cell culture. After 48 hours, the Supernatant in cell culture are gathered and frozen at temperature of -70°C, so that to be prepared for ELISA test. For measuring IL-1β and TNF-α, kits of eBiosience, American company with sensitivity of respectively 0.7 and 0.92 pg/ml is used.

**Measurement VO₂max**

At least one week prior to the implementation of the protocol, the subjects were asked to attend Tehran University Laboratory of Physical Education Faculty for VO₂max estimation. VO₂max test was obtained using an ergometer cycle to a voluntarily progressive exhausting. After stretching and also 5 minutes of warm up on the cycle, the test started with 50 watts resistance and then it was increased 25 watts every two minutes and it continued until the subject's inability stopped them from going further. Meanwhile VO₂max was measured using gas analyzer (GANSHORN, Germany) in 30-seconds periods.

During the test, their heart beat rate was controlled continuously by a digital heart rate measuring (Polar Vantage NV, Polar Electro, Kempele, Finland). VO₂max was determined using The British association of sport and exercise sciences (BASES) institute approved criteria. The measures included respiratory exchange ratios (RER) greater than 1.15, oxygen consumption remain unchanged despite the increased rates of load and heartbeat rate closer to the maximum heart rate based on age (HRmax=220-age).
Exercise protocol

Both omega-3 supplement and placebo groups participated in endurance training program. Subjects training sessions ran in 6 exercise sessions with 1 to 2 days rest in between for 2 weeks. The first two sessions of 90 minutes duration were constant pedalling with intensity equal to 0.65 of the maximum oxygen consumption, and these periods were increased to 105 minutes in the second session and 120 minutes at the last two sessions. VO\textsubscript{max} Every session included warming up, working out and cooling down. Warm-up included stretching for 2 minutes, then pedaling the cycle unloaded. Then the exercise started with the intensity of 50 watts or until the participant reached 0.65 of his and continued with the same intensity till the end of the session. After the exercise, the session was ended in cooling-down including walking and stretching (Gibala and et al, 2006).

Statistical analysis

All data were evaluated for normality of distribution and homogeneity of variance before hypothesis testing. All statistical analysis and graphic presentation was accomplished using SPSS (v 19). Main effects of training modality (Endurance training with placebo and endurance training with Omega-3 consumption) and time (pre-exercise (baseline) and post-exercise), were assessed using Two-Way Analysis of Variance. Statistical significance was conferred at P=0.05. When main effects were detected, the Tukey test was used for post hoc comparisons.

Results

No significant differences were found between groups for age and body composition (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Placebo</th>
<th>Omega-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age</td>
<td>26±2.6</td>
<td>27±2.4</td>
<td>27.7±2.8</td>
</tr>
<tr>
<td>High</td>
<td>176.8±4.89</td>
<td>175±4.44</td>
<td>176.9±4.42</td>
</tr>
<tr>
<td>Weight</td>
<td>75.91±3.23</td>
<td>77.03±3.49</td>
<td>77.57±2.47</td>
</tr>
<tr>
<td>BMI</td>
<td>24.17±1.35</td>
<td>25.33±0.78</td>
<td>23.69±1.29</td>
</tr>
</tbody>
</table>

The results of this research demonstrated that following 2 weeks of endurance training and Omega-3 Consumption, there were significant differences in the groups for TNF-α (P=0.009), post hoc test results showed that there were significant difference between placebo and omega-3 supplementation (P=0.01). TNF-α in Placebo group increased, but in Omega-3 group its decrease respectively 12.56% versus 6.56%. Notwithstanding this after endurance training and Omega-3 Consumption, there were no significant differences in groups for IL-1β (P=0.694). IL-1β in both groups decreased, but in Omega-3 group it decreased 14.84% versus 13.16% in Placebo group(Table 2).

Table 2. Research variables in all groups before 2 weeks supplementation and 48 hr post-exercise.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre test</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β(pg/ml)</td>
<td>253.8±40.3</td>
<td>223.2±38.8</td>
</tr>
<tr>
<td>TNF-α(pg/ml)</td>
<td>7130.2±905</td>
<td>7732±898.8</td>
</tr>
<tr>
<td>VO\textsubscript{max}(ml/kg/min)</td>
<td>43±2.61</td>
<td>42.16±2.58</td>
</tr>
<tr>
<td>Fat percent</td>
<td>13.73±1.75</td>
<td>15.52±1.79</td>
</tr>
<tr>
<td>Omega-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β(pg/ml)</td>
<td>262.1±26.9</td>
<td>223.2±28.8</td>
</tr>
<tr>
<td>TNF-α(pg/ml)</td>
<td>7017±968.8</td>
<td>6556.5±938.5</td>
</tr>
<tr>
<td>VO\textsubscript{max}(ml/kg/min)</td>
<td>43±2.62</td>
<td>44.05±2.53</td>
</tr>
<tr>
<td>Fat percent</td>
<td>13.56±1.81</td>
<td>14.39±1.38</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β(pg/ml)</td>
<td>268.1±26.9</td>
<td>232.8±23.1</td>
</tr>
<tr>
<td>TNF-α(pg/ml)</td>
<td>7153±1281.8</td>
<td>8054±1280.2</td>
</tr>
<tr>
<td>VO\textsubscript{max}(ml/kg/min)</td>
<td>42.6±2.78</td>
<td>44.2±2.57</td>
</tr>
<tr>
<td>Fat percent</td>
<td>14.6±1.69</td>
<td>14.36±1.18</td>
</tr>
</tbody>
</table>

Discussion and Conclusion

The results of this study showed that during incremental endurance training, Omega-3 Consumption for two weeks lead to decreases in TNF-α, but in placebo group its increased. Also after consumption Omega-3 there were no significant differences between experimental groups for IL-1β. These results demonstrated that despite increased levels of omega-3 intake to 3600 mg per
day and significant increase in their plasma EPA and DHA, no group differences in IL-1β outcomes relative to placebo were measured after a 14-day period of intensified exercise. Although these results in one way match with the results of some previous researches (Bloomer et al., 2006) but in another, they are in contrast with some other researches that shows adaptations to endurance training or using omega-3 reduces inflammatory cytokines (Andrade et al., 2007; Nieman et al., 2003; Toft et al., 2000). Different studies suggested several mechanisms regarding to the effect of unsaturated fatty acids on inflammatory factors. With disturbing the ratio of omega-3 to omega-6 in dietary pattern, the amount of AA of omega-6 in immune cells phospholipid membrane will increase.

The result of unsaturated fatty acids metabolism in immune cells phospholipid membrane (specially AA), are PGE2 and LB4, respectively, which produced from enzyme pathways of Cyclooxygenase (COX1, COX2) and Lipooxygenases (5-LOX) with many inflammatory effects as inflammatory cytokines production. Consuming omega-3 complements make this ratio to be balanced and consequently decrease AA on the immune cells membrane surface, then AA is replaced by EPA and DHA. Decrease in AA leads in PGE2 and LTB4 production and finally decrease in inflammatory cytokines concentration in body. On the other hand, EPA and DHA are placed as a substrate on the enzyme pathways of COX1, COX, 5-LOX and results in PGE3 and LTB5 production. It seems they are of less inflammatory characteristics comparing to PGE2 and LTB4 (Richard and et al, 2008; Fritsche, 2006). The late studies discover a new group of three hydroxy Eicosapentaenoic acid intermediates called ResolvinEs series (RvE1, RvE2) that are derived from Eicosapentaenoic acid in enzyme pathways of 5-LOX and COX2. These regulators could lead to the strong anti-inflammatory activity among neutrophils, macrophages, dendrite cells and T-cells. In addition, the other derived intermediates from DHA referred to as Resolvin D (RvD1, RvD2) a produced from the same enzyme pathways and are of anti-inflammatory activities (Serhan et al., 2008). According to the above explanation, it's interesting that most unsaturated fatty acid effects on the production of inflammatory intermediate molecule don't restricted to bringing change in Eicosanoids production. But this inflammatory intermediates exercise their effects along with gene encoding change. It's suggested that omega-3 unsaturated fatty acids most probably effect cytokines gene expression with activity change in Nuclear Factors kappa-B (NF-κB) or peroxisome proliferator-activated receptors (PPAR-γ). NF-κB is activated by a signaling cascade. This cascade is stimulated and initiated with an extra-cellular inflammation that leads to phosphorylation inhibitory subunit (IκB) of NF-κB, and subsequently transmission of NF-κB toward nucleus will be possible (kumar, 2004; Perkins, 2007; Sigal, 2006). It appears to be EPA is associated with decrease in IκB phosphorylation that is possibly in turn resulted from decrease in the activity of Mitogen activated protein kinase (Perkins, 2007) and will lead to decrease of NF-κB activity in human Monocytes culture (Novak et al., 2003; Walser et al., 2006; Lo et al., 1999). These observations also suggest omega-3 unsaturated fatty acids with hindering transcription factor activation have a direct effect on inflammatory gene expression. It also seems PPAR-γ, second transcription factor could have anti-inflammatory behaviors. PPAR-γ not only regulates inflammatory gene expression directly, but it contributes in NF-κB activity (Vandenburgh and et al, 1993). It is so believed that PPAR-γ could attach to different fatty acids as omega-3 unsaturated fatty acids and then activated (Kilewer et al., 1997; Forman et al., 1997). These acids more probably will lead to increase in PPAR-γ activity that has anti-inflammatory effects.

These research findings indicates the positive role of omega-3 supplementation in decreasing TNF-α concentration in endurance training. However, It appears to be changes in Cytokines response after Omega-3 Consumption are associated with anti-oxidation body's system Potential and exercise intensity.

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