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Effects of aerobic and anaerobic training programs together with omega-3 supplement on interleukin-17 and CRP plasma levels in male mice

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Abstract

Background: Herein, we studied the effects of two different exercise protocols on IL-17 and CRP plasma levels along with the anti-inflammatory effects of fish oil. The purpose of the present study was to investigate the effect of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) consumption along with two different types of physical activities on IL-17 and CRP plasma levels in trained male mice.

Methods: A total of 130 adult male mice of Syrian race with the age of 2 months and the weight of 35±1 grams were selected. At the beginning, 10 mice were killed in order to determine the amounts of pre-test variables. The rest of the mice were randomly divided into 6 groups including control group (n=20), supplement (n=20), aerobic exercise (n=20), anaerobic exercise (n=20), supplement-aerobic exercise (n=20), and supplement-anaerobic exercise (n=20). Blood samples were withdrawn from the tail under intraperitoneal ketamine and xylasine anaesthesia. The anaerobic training program included 8 weeks of running on treadmill, 3 sessions per week; the aerobic training program included 8 weeks of running on treadmill, 5 sessions per week. At the end of the training program, the blood sample from each group was taken in order to measure the CRP and IL-17 levels. The analysis of variance (ANOVA) was used to determine the differences among the groups.

Results: The results showed that there was a significant difference in IL-17 and CRP plasma levels between the groups after 8 weeks ($P<0.05$).

Conclusion: Following the two different training programs, both IL-17 and CRP plasma levels increased, although these observed increases were not same for two measured variables. The results might also show that the effect of the supplement depends on the type of training.

Keywords: Omega-3, Interleukin-17, C reactive protein, Physical activity.

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Introduction

Polyunsaturated fatty acids are vital for both animals' and humans' health. Unsaturated fatty acids in fats are omega-3 and omega-6 fatty acids both of which play fundamental roles in body metabolism. Omega-3 fatty acids are classified into three groups of Alfa-linolenic Acid (ALA), Docosahexaenoic Acid (DHA), and Eicosapentaenoic Acid (EPA) (1) . It appears that omega-3 fatty acid fulfills its biological effects by changes in lipid membrane or by directly activating the substrates for metabolism. The role of omega-3 fatty acids in body is to fight with various diseases and inflammation (2). Therefore, inflammatory responses are necessary in preventing diseases and it seems that the true balance in consuming omega-3 and omega-6 fatty acids can help the issue (1).

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Different parts of immune system are involved in fighting with inflammation. Cytokines are one of the subdivisions of this system (3,4). The word "cytokine" is applied to a group of public regulatory factors including lymphokines made of lymphocytes and monokines made of monocytes (5). One of these cytokines is IL-17. IL-17 is clearly involved in inducing and mediating the inflammation. Although IL-17 is derived from a T cell, it is a pre-inflammatory cytokine, and it is assumed to have some role in progression of various inflammatory diseases, secretion of IL-16, and activating neutrophil in the tissue (6). The prognosis of inflammation and pathogenic factors in the body, on the other hand, is one of the most important and sensitive roles of immune system undertaken by some proteins. Quantity of the proteins in plasma and serum are changed due to the factors such as inflammation called acute phase proteins (APP) (7). The role of most of these proteins is to decrease the inflammatory damages in tissues (8) . The C - reactive protein (CRP) is one of the acute phase proteins produced by liver and is one of the primary indicators of inflammation in the body (9). Measuring CRP is the best way to diagnose tissue damages due to its rapid increase at the onset of the tissue damage and its rapid decrease soon after the recovery. Physical activity is one of the ways that causes inflammation (10). In the past two decades, it is said that the physical activity has deep impacts on immune system, and the metabolic changes resulting from physical activity with muscle contraction and hormonal changes can be found in some organs like liver and adipose tissue (10). In some studies on the effect of physical activity on CRP and IL-17, the intensity of training has been highly emphasized. For example, the results of a study showed that one session intense training would increase IL-17 in mice whereas one session physical activity with moderate intensity would not change IL-17 production in mice. In this study, it was also found that the intense and moderate training would cause an increase in IL-16 level in the trained mice (11). The result of the two types of intense and light training programs was also studied and the outcomes proved that after eight weeks, the level of this cytokine increased in a group with intense training but there was no alteration in another group (10). In contrast, Golzari Z. et al. found that if a combined program (including warm-up, stretching exercise, aerobic exercise, strength training and a relaxation program at the end of each session) is used for eight weeks, IL-17 level do not increase; in some people, there will be some decrease as a result of low level of training intensity (12). On the other hand, some studies has been done on CRP after physical activity (8,13,14). One of the training studies in this regard was performed by Kim HJ, et al. They studied the effect of running distance during marathon (42.195 Km) and ultra marathon (200 Km) on CRP and found that after marathon, the level of this protein does not change but after one day, it increased ¾ times and after four days, it went back to its original level. They also stated that the CRP level after ultra marathon increased 40 times and it remained in the same level up to six days after the race (14). In contrast, in another study, it was stated that the CRP level in healthy people and those with cardiovascular disease will decrease following twenty weeks of training with the intensity of 75% maximum oxygen consumption on ergometer bike but this decrease is more notable in subject patients (13).

Therefore, regarding the effect of physical activity on inflammation, the purpose of this research is to study the effect and comparison of eight weeks intense aerobic or anaerobic physical training on possible changes in some systematic inflammatory markers (CRP, and IL-17). We are also looking for possible effects of omega-3 fatty acids on CRP and IL-17 levels and if yes, it relation to the type of activity.

Methods

Data

In this experimental study, 130 mice of

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Table 1. Anaerobic exercise program (speed, slope and time)

Training sessions	No. of cycles	Speed (minute/meter)	Slope (degree)	Time (second)	Rest between cycles (minute)
1-6	3	24	5	30	1
7-8	4	24	5	30	1
9-12	4	27	10	30	1
13-16	5	27	10	30	1
17-18	5	30	15	30	1
19-24	6	30	15	30	1

Table 2. Aerobic exercise program (speed, slope and time)

Days	Variable	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Monday	Speed (m/min)	10	10	12	13	17	19	22	27
	Slope (degree)	5	10	15	15	15	15	18	18
	Time (m)	15	15	60	60	60	60	60	60
Tuesday	Speed (m/min)	10	10	12	13	17	19	22	27
	Slope (degree)	5	13	15	15	15	15	18	18
	Time (m)	15	15	60	60	60	60	60	60
Wednesday	Speed (m/min)	10	12	12	13	17	19	22	27
	Slope (degree)	8	13	15	15	15	15	18	18
	Time (m)	15	15	60	60	60	60	60	60
Thursday	Speed (m/min)	10	12	12	13	17	19	22	27
	Slope (degree)	8	15	15	15	15	15	18	18
	Time (m)	15	15	60	60	60	60	60	60
Friday	Speed (m/min)	10	12	12	13	17	19	22	27
	Slope (degree)	10	15	15	15	15	15	18	18
	Time (m)	15	45	60	60	60	60	60	60

Syrian race with the age of 2 months and the weight of 35±1 grams were selected. At first, 10 mice were killed to determine pretest variables; Unconscious was induced using Ketamine (30 to 50 mg per kg body weight, intraperitoneally) mixed with Xylazine (3 to 5 mg per kg body weight, intraperitoneally) and then blood samples were taken from their tail. The rest of mice were randomly divided into six groups including control group (n=20), supplement (n=20), aerobic exercise (n=20), anaerobic exercise (n=20), supplement-aerobic (n=20), and supplement-anaerobic (n=20). The mice were kept in separate cages (6 mice per cage), with a mean temperature of 23±2 °C and a light cycle of 12 hours light and 12 hours darkness. The mice had free access to water and food packets. The physical activity started using rodents' treadmill (with 7 simultaneous lines). The aerobic training program included 8 weeks of running on treadmill, 5 sessions per week (15). In order to get familiar with the treadmill, the aerobic exercise and aerobic-supplement groups began to run on treadmill for one week, with the speed of 5 meters per minute and zero slopes for 10 minutes. After getting familiar with the treadmill, the speed increased to 7 meters per minute, the slope raised to 5 degrees and the duration to 15 minutes. On the other hand, the anaerobic training program included 8 weeks of running on treadmill, 3 sessions per week (10). The mice in the anaerobic and supplement-anaerobic groups ran on the treadmill for one week (3 sessions) with the intensity of 12 meters per minute. The training program included the speed, slope, and duration of running on treadmill (see the aerobic program in Table 1 and the anaerobic program on Table 2).

The mice in three groups of supplement, aerobic-supplement and anaerobic-supplement were fed (using gavage) 0.2 ml (0.06 ml/g of body weight) of fish oil (omega 3) including DHA and EPA for 8 weeks on a daily basis. At the end of the exercise, the blood samples from each group were taken in order to measure the

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CRP and IL-17 levels. Measurement were performed using Elisa kits, MOUSE-KIT (ID Labs inc., Hungary) for IL-17 and CRP-KIT (Neflometry, England) for CRP.

Statistical Analysis

Data analysis was performed using the SPSS version16. The analysis of variance (ANOVA) was used to determine the differences among the groups; if significant, Tukey's post hoc test was used. The significance level of statistical analysis is $\alpha<0.05$.

Results

The results showed that there was a significant difference in interleukin-17 level among the groups ($p<0.05$). This research showed that the supplement consumption (without physical activity) causes a significant decrease in IL-17 level ($p<0.05$) but applying both training programs leads to a significant increase in this cytokine; this increase is more notable after doing anaerobic exercise ($p<0.01$). Furthermore, when aerobic exercise is together with supplement consumption (aerobic-supplement group) there is no difference between the results of this group and the aerobic group; but when the supplement consumption is together with anaerobic exercise, it doesn't increase and there is no significant change in IL-17 level (Table 3). On the other hand, the results of recent research showed that there is a significant difference in the levels of CRP among the groups ($p<0.05$). Comparing the average CRP before and after the exercise in the groups indicated that the supplement consumption results in a notable decrease in this protein. During the physical activity, the CRP level will increase but this increase is more notable in aerobic group ($p<0.01$) (Table 4). There is also a significant difference between supplement-aerobic group and supplement-anaerobic group and the results showed that the supplement consumption with aerobic exercise will only decrease the slope whereas the supplement consumption with anaerobic exercise makes no significant change.

Discussion

The results of current research show that there is a significant difference in IL-17 and CRP plasma levels after EPA and DHA supplement consumption along with physical activity. These dependent variables are involved in regulation of immunity and inflammatory responses(16,17). Although pre-inflammatory cytokines are necessary for immunity defense, the maximum production of these cytokines can cause the inflammation and then the injury to skeletal

Table 3. Changes in interleukin-17 (pg/ml) before and after aerobic and anaerobic exercises in groups

Groups	Before exercise (Mean±SD)	After exercise (Mean±SD)
Control group	120.98±5.67	126.00*±8.42
Supplement group	120.98±5.67	93.06*±6.25
Aerobic exercise	120.98±5.67	126.06*±9.32
Anaerobic exercise	120.98±5.67	143.96**±8.54
Supplement-aerobic exercise	120.98±5.67	129.12*±5.68
Supplement-anaerobic exercise	120.98±5.67	121.32±5.98

* Stars indicate there is a significant difference ($p<0.05$)
** Stars indicate there is a significant difference ($p<0.01$)

Table 4. Changes in CRP (u/l) before and after aerobic and anaerobic exercises in groups

Groups	Before exercise (Mean±SD)	After exercise (Mean±SD)
Control group	0.82±0.35	0.99±0.32
Supplement group	*0.82±0.35*	0.30*±0.20
Aerobic exercise	*0.82±0.35*	2.30**±0.95
Anaerobic exercise	*0.82±0.35*	1.70*±1.10
Supplement-aerobic exercise	*0.82±0.35*	1.35*±0.98
Supplement-anaerobic exercise	*0.82±0.35*	1.10±0.89

* Stars indicate there is a significant difference ($p<0.05$)
** Stars indicate there is a significant difference ($p<0.01$)

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muscle, weakness and increased risk of infection (18) IL-17 cytokine is able to activate the macrophages, the fibroblasts, secretion of IL-1, IL-6, and IL-8 cytokines, the secretion of E2 prostaglandins and nitric oxide (19). Some researchers believe that IL-17 plasma levels may be a helpful biochemical marker to determine the acute inflammation presented in skeletal muscle (20,21). As you can see in table 3, anaerobic exercises caused the highest levels of IL-17. The results of current research, like some other researches, show that the intensity of the exercise is a key factor (12). Another research indicated that in an activity with high intensity, IL-17 level was increased but in a group with low intensity, there was no change (11). Lowder et al. studied the effect of four weeks physical activity on treadmill and the results showed that IL-17 had decreased. These researchers also stated that the main cause of this decrease was probably due to the low intensity of the exercise (19). In current research, it was also specified that the changes to IL-17 levels differed in physical activities with various intensities (aerobic & anaerobic) and this increase was more in anaerobic exercises (it should be noted that there is any way some increase in aerobic exercises). However, these results are inconsistent, compared with some other studies(19), and it can be due to the number of weeks. Similar to this, Duzova H.et al also stated that the production of IL-17 was increased by high intensity or long time activities but it was not increased in a group with short time physical activity (one week) with moderate intensity (11). The involved mechanism is probably due to the fact that the high intensity physical activity leads to the release of pre-inflammatory cytokines and these cytokines, in turn, will cause the production of anti-inflammatory cytokines such as IL-2, IL-6, and IL-10 (6). It appears that the consecutive production of these pre-inflammatory and anti-inflammatory cytokines will cause IL-17 to be produced by blood peripheral leukocytes and skeletal muscle (17). The results of supplement consumption along with aerobic and anaerobic exercises should also be taken into consideration. The results of current research show that the supplement consumption in aerobic group didn't have any effect on IL-17 levels (129.06 pg/ml in aerobic group and 129.12 pg/ml in aerobic-supplement group); but omega-3 consumption along with anaerobic physical activity prevents the increase in this cytokine, and there is no change in IL-17 level as compared with the values before the exercise (after the exercise completion, 143.96 pg/ml in anaerobic group and 129.12 pg/ml in aerobic-supplement group). The reason for the differences in supplement consumption can be related to the different IL-17 levels as a result of aerobic and anaerobic physical activities. It is foreseen that the IL-17 level should be reached to the extent so that the effect of omega 3 supplement consumption can be observed. . Therefore, a threshold for IL-17 production for the effect of supplement consumption can be mentioned (meaning that IL-17 level doesn't reach the desired threshold to make the supplement effective as a result of aerobic exercise). With regard to the results of above studies, it can be said that the intensity or duration of the physical activity is a key factor in increased production of interleukin-17, but the supplement consumption can change the result. Thus, it was specified that the supplement consumption, the supplement consumption together with aerobic exercises and supplement consumption along with anaerobic exercises respectively will cause a decrease in IL-17 (as compared with control group), will have no effect on IL-17 (as compared with aerobic group), and will prevent the IL-17 increase (as compared with anaerobic group).

The CRP normal range in adults is about 0.8-5 ml per liter blood, but it can

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be increased up to 1000 times as a result of infection or physical activity because the amount of secretion of APPs depends directly or indirectly on factors such as the production of cytokines and T cells (8) . The gathered information shows that the CRP plays an important role in inflammation (7). This acute phase reactant can be found in human arteriosclerosis plaque together with LDL (8). CRP stimulates the expression of plaque in endothelial cells and enhances the adhesion of the plaque to these cells. This information shows that CRP is not merely an inflammation marker but a risk factor in turn (9) . Some researchers believe that the amount of production of this protein depends on the duration, intensity, type of exercise and the distance travelled by an individual (9) . For example, in a study, researchers found that the level of this protein doesn't change soon after marathons; however, there is a remarkable increase after ultra-marathons (14). The results of current research indicate that the CRP level in aerobic exercise group has increased more than the anaerobic exercise group (2.3 and 1.7 mg/L respectively). It was also specified that the amount of this protein would increase more in exercises with more distance travelled. Table 4 shows that the supplement group has the least amount in CRP and this indicates the effect of omega-3 consumption on this protein. In conformity with the result of current research, another study proved that omega-3 consumption for 8 weeks by 6 women and 6 men led to a CRP decrease (2). In another study, the researchers stated that consuming 6 grams of EPA daily for 8 weeks would cause a decrease in the CRP level from 11 mg/L to 8 mg/L (probably due to a decrease in IL-6). However, in contrast, the researchers in a study found that consuming 4 grams of fish oil daily doesn't increase the CRP level in fat individuals. Short time supplement consumption (one week) has been mentioned as a reason for this finding (9). Therefore, time is a key factor for supplement to be effective (9). On the other hand, this study showed that when the supplement is used, the level of CRP increases in supplement-aerobic group less than the aerobic group (without supplement consumption); for instance, the CRP level increases about 180% as a result of aerobic exercise and 64% when it goes with supplement consumption. Comparison of these four groups (aerobic, anaerobic, supplement-aerobic and supplement-anaerobic) reveals that supplement consumption has affected the CRP level (incremental changes with a CRP milder slope in supplement-aerobic group as compared with aerobic group and insignificant increase in supplement-anaerobic group). Why supplement consumption cannot prevent its increase in aerobic exercises (contrary to anaerobic exercises)? It appears that the distance travelled and high level of this protein followed by aerobic exercise (without supplement consumption) is a reason. It should also be noted that the supplement consumption led to a decrease in the the slope of CRP increase**.**

Conclusion

Finally, it can be said that EPA and DHA supplement consumption decreases the IL-17 plasma level. The results of this study shows that the amount of cytokine depends more on the intensity of exercise but the CRP level depends more on the distance travelled. Besides, the effect of omega-3 consumption on these two variables depends on the type of exercise.

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