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EFFECTS OF PURPOSEFUL AND OMEGA-3 EGG DIET ON BLOOD METABOLITES IN MALE AEROBIC ATHLETES

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ABSTRACT

The present study aims to investigate the effects of purposeful and Omega-3 rich egg diets on blood metabolites in male aerobic athletes. Therefore, 45 athletes were randomly put into three groups of 15 and went under a 12-week exercise program with three sessions in a week. The exercise and non-exercise groups received on supplementary omega-3 egg every other day. However, the subjects in control group did not have any interventions. Blood sampling was conducted 24 hours before exercise and supplementary diet began from all three groups. In order to ensure normality of data distribution, Kolmogorov–Smirnov, dependent T, variance analysis and Tukey's post hoc tests were utilized. The results revealed that density of triglyceride and LDL in omega-3 supplemented groups with and without exercise decreased significantly compared to the control group. Moreover, density of HDL and cholesterol did not have a significant change. BMI decreased significantly in exercise group supplemented with Omega-3 compared to the supplemented group without exercise and the control group. Moreover, aerobic capacity of the aerobic exercise group supplemented with omega-3 was significantly higher than both other groups. Total plasma antioxidant capacity density and MDA increased in both exercise and non-exercise groups supplemented with omega-3; however, the increase was not significant. Findings demonstrated that the decrease in the levels of triglyceride, cholesterol and LDL along with the increase in the level of total plasma antioxidant capacity and MDA could be used as an efficient method for preventing damages induced by oxidation stress in endurance athletes and lower the risk of cardiovascular diseases.

Keywords: *Omega-3, Enriched Egg, Blood Metabolites, Aerobic Exercise*

INTRODUCTION

Several studies have been conducted on enrichment of eggs with omega-3 unsaturated fatty acids for these eggs could be added to the diet to prevent cardiovascular diseases (Bourre, 2005). However, this enrichment is not of any benefit unless it is enhanced by antioxidant vitamins like C and E and Selenium, as a powerful protection for omega-3 derivatives like DHA. Not only do they have antioxidant properties but also they improve general health, multiply and guarantee the effects of enriched nutrients (Arazi *et al.*, 2012). Fatty acids from omega-3 family are commonly believed to be the miracle discovery in the world of nutritional sciences and have an indispensable position in it. On the other hand, the egg is a rich source of protein. Surai (2006) and Hill *et al.*, (2007) demonstrated that physical education coupled with consuming fish oil significantly decrease body fat in subjects. Defina (2011) reported that aerobic exercises with 56- 80% Vo_{2max} along with 20 to 30 minutes of strength training and eating 3 grams of omega-3 fatty acid decrease weight significantly in obese people. Omega-3 is of anti-inflammatory and hypolipidemic properties and has recently been recommended to help keep homeostasis balance in body (Maroon, 2006). These beneficial hypolipidemic effects together with weight loss reveal the size of fat cells, plasma triglyceride and fasting blood sugar (Wooten *et al.*, 2009). Shahidi *et al.*, (2015) and Tartibian *et al.*, (2011) demonstrated that long term aerobic exercises along with consuming of omega-3 supplement have synergic effects on reducing inflammation and increasing minerals bone density in postmenopausal period. Besides, studies have reported that 8 weeks of aerobic exercises together with consuming omega-3 fatty acids can decrease serum distribution of sticking molecules, cytokines and inflammation and prevent, control and decrease atherosclerosis in elderly obese people (Bolboli *et al.*,

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2014). Consuming omega-3 fatty acids decrease the level of blood triglyceride and as a result, the risk of mortality by stroke or irregular heartbeat decreases (Chan, 2009; Galli, 2009).

Researchers also investigated the effects of a marine oil and PUFA omega-3 extracted from a New Zealand oyster named *Perna Canaliculus* on inflammation index of nonathletic men. Results revealed that consuming supplements of marine oil and PUFA and compound N-3 LC (PCSO-524) extracted from New Zealand green oysters in the diet of men without exercise significantly decreased DOMS of the lower body, pain in the quadriceps, tiredness of peripheral muscles, create a protective effect against knee bending ROM and decrease isometric force induced by running down slopes (Timothy *et al.*, 2015).

Lewis *et al.*, (2015) investigated the effects of 21 days of omega-3 fatty acid supplement in mammals on improving neural and muscular aspects of performance in male athletes and compared the effect with the olive oil placebo. Findings revealed that consuming N-3 PUFA supplement improve peripheral neural muscular performance and has anonymous effect on removing pains in central neural muscular performance.

Omega-3 fatty acids, vitamins C and E, antioxidants, and selenium supplements are strongly effective on different individuals including athletes under training and therapeutic exercises to improve physical and sports performance.

Future studies should probe the effects of Omega-3 PUFA supplement on well-built bodies and should consider the protocol of specific time and severity for a stronger oxidative and an efficient response for inflammations. In spite of all the studies conducted on feeding omega-3 supplement to lab animals and migrant birds, very few have focused on its effects on humans. Lewis *et al.*, (2015) and Timothy (2013) believed that it is difficult to give any recommendations on the amount and length of consuming omega-3 PUFA supplement to athletes.

Therefore, in spite of a wide range of studies on the effects of Omega-3 on various people, very few studies have investigated the addition of this supplement to egg as a common nutrient for athletes. Furthermore, no study has investigated the effects of omega-3 supplement coupled with aerobic exercises on blood factors of athletes.

Thus, the present study aims to probe the effects of purposeful and omega-3 rich eggs on blood metabolites of athlete and non-athlete men.

MATERIALS AND METHODS

The present quasi-experimental study used data collected through fieldwork via pre and posttests. Statistical society of the study included students of physical education in Shahid Azarbaijan Shahid Madani University in the school year 2013. Sixty students volunteered to participate in this study in response to a call, 15 of whom were not qualified according to the criteria of the study. Eventually, 45 healthy male athletes with similar BMI, age, height, and weight were put into three groups randomly. The first group did not have an exercise program and enriched egg diet. The second group did not have an exercise program but received an enriched egg diet. The third group had an exercise program and received enriched egg diet.

All the participants were asked to fill out a form and declare their consent and will to participate after being synchronized. The subjects did not smoke, drink alcohol or take any medicine including various antioxidant supplements like vitamins E, C or nutrients rich with selenium or omega-3 added to their daily diet and during the last two weeks before the study commenced.

Providing and Consuming Purposeful and Omega-3 Rich Egg Supplement and Blood Sampling

The first part of the study began with enrichment of eggs with sources of omega-3, various levels of vitamins E and C and selenium. Then their biochemical properties were measured to be 800 milligrams of omega-3, 220 milligrams of vitamin E, 0.8 milligram of vitamin C and 0.1 milligram of selenium in each gram of yolk of the enriched eggs.

Later on, the eggs were given to subjects to be consumed. Blood sampling was conducted 24 hours before the exercise program began and 24 hours after the end of the final session of the 12 week exercise program with 3 sessions per week. Blood samples were taken from the right arm vessel in elbow and by a

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lab specialist. Blood samples were then centrifuged and then sent to the lab to be analyzed in -70 degrees Celsius.

According to Reference Daily Intake, nutritional guidelines for different individuals and considering their test condition each subject in the second and third groups received one egg every other day with his routine diet before exercising began.

Referring to scientific resources and consulting with the specialist committee assisting the present study, subjects' diets were examined and recorded carefully and they were strictly asked not to take any medicine like Paracetamol, methenamine, Methocarbamol and Naproxen in any form or method 72 hours before blood sampling was conducted.

Exercise Programme and Aerobic Exercise Test in Pre and Post-Tests (Exercising Protocol)

Pretest was given 24 hours before the beginning of exercises and 24 hours after the final exercise session, aerobic posttest and recording was conducted from all groups with maximum aerobic power of the subjects.

Aerobic exercise program and estimation of aerobic stamina of the subjects included a 36-session exercise program with three 60-minute sessions per each of the 12 weeks program with Cooper aerobic test. Each session included 15 minutes of warm-up with jogging, stretches and light exercises. Then the Cooper test was conducted with running two by 3000 meters in 12 minutes with half of the test time intervals between each two tests. Finally, the session concluded with 15 minutes of cooling down exercises including jogging and stretches.

Gauging Devices and Methods

The present study used a TINA Bioelectrical impedance analysis device made in Japan to measure subjects' body composition. Their height was measured by SECA height measuring system with a precision of 0.1 centimeters made in Iran. LDL, CHOL, HDL and TG were analyzed using a kit made by Pars Azmoon Co. in Iran through enzymatic methods. In order to measure the serum Malondialdehyde as an index of lipid peroxidation, Thiobarbituric acid test and spectrophotometer were utilized. Furthermore, total plasma antioxidant capacity was also measured using a spectrophotometer.

$$VO_{2Max} = (\text{Distance run in meters} - 504.9) / 44.73$$

Statistical Method

The data collected were analyzed through descriptive and inferential statistics. Normality of data distribution was evaluated by Kolmogorov–Smirnov test. Intergroup differences were measured by dependent t test and the intragroup differences were measured using one-way variance test. In case the difference was significant, Tukey range test was also utilized. Statistical analysis was conducted with SPSS₂₁ computer application with the level of significance (P<0.05).

RESULTS AND DISCUSSION

Results

Descriptive properties of subjects are represented in table 1 in the form of mean ± standard deviation. Table 2 represents the results for the effects of enriched eggs with omega-3 coupled with aerobic exercises on LDL, HDL, triglyceride, cholesterol, Malondialdehyde and total plasma antioxidant capacity. Data from table 1 show that the differences in BMI, body fat percentage, age, height and weight of the subjects were not significant; therefore, they were homogenous.

Table 1: Descriptive Properties of the Subjects

Variables	Age	Height	Weight	Bmi	Fat %
Exercising + Supplement	20.05± 2.3	179.5± 0.5	74.05± 5.3	21.45± 2.42	17.14± 2.2
No Exercising + Supplement	19.62± 2.55	179.15± 1.3	73.05± 6.27	22.05± 1.5	18.1± 1.81
Control Group	20.45± 2.34	180.2± 1.25	75.05± 4.54	22.3± 1.2	18.29± 1.68
Level of Significance	0.84	0.88	0.6	0.14	0.23

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Variables	Exercising + Supplement			No Exercising + Supplement			Control Group		
	Pretest	Posttest	P	Pretest	Posttest	P	Pretest	Posttest	P
LDL	89.05± 1.36	75.5± 1.52	0.04	86.05± 1.33	79.45± 1.66	0.5	88.1± 1.6	87.77± 1.45	0.71
HDL	49.12± 1.62	47.6± 1.3	0.09	47.2± 1.55	45.5± 1.3	0.11	48.62± 1.1	49.01± 0.03	0.32
CHOL	178.2± 7.6	171.5± 9.4	0.12	180.2± 7.7	182.5± 9.3	0.2	179.62± 9.7	185.5± 25.2	0.76
TG	102.45± 14.4	84.45± 15.65	0.01	105.45± 14.02	83.23± 16.33	0.03	102.25± 14.21	103.2± 14.8	0.9
MDA (µmol/l)	0.64± 0.3	0.62± 0.8	0.09	0.66± 0.5	0.34± 0.21	0.05	0.59± 29	0.61± 0.65	0.11
TAC (µmol/l)	4.35± 1.3	4.31± 1.5	0.62	4.22± 1.4	3.82± 1.74	0.08	4.87± 1.42	4.9± 1.25	0.83
BMI	24.12± 2.62	22.66± 2.11	0.04	24.332± 3.11	24.52± 2.92	0.21	25.12± 2.72	25.3± 2.41	0.36
Aerobic Capacity	47.2± 7.6	55.5± 7.4	0.03	48.2± 7.7	47.5± 6.3	0.71	48.62± 6.1	48.5± 5.2	0.8

Findings revealed that the density of triglyceride and LDL decreased significantly ($P \leq 0.05$) 24 hours after aerobic exercises in both groups with supplements and with and without exercising compared to the control group. The level of Malondialdehyde decreased in both groups with supplement and with and without exercising; yet the decrease is significant only in the group without exercising ($P \leq 0.05$). Moreover, the study failed to observe any significant change in the levels of plasma cholesterol and HDL in any of all three groups ($P \leq 0.05$). However, the level of cholesterol decreased noticeably in the group of omega-3 supplement with exercising and it had increased slightly in the other two groups. Findings of the study also revealed that BMI decreased significantly 24 hours after aerobic exercises in the group receiving omega-3 supplement and doing aerobic exercises compared to control group and the group receiving supplement and not doing exercises ($P \leq 0.05$). Moreover, aerobic capacity of the group receiving supplements and doing aerobic exercises had increased significantly.

Discussion

Findings of the present study revealed that the density of TRIG and LDL decreased significantly 24 hours after aerobic exercises in both groups receiving omega-3 supplement and with or without aerobic exercises compared to the control group. Besides, the level of HDL and cholesterol slightly increased in the control group and the group receiving omega-3 supplement without aerobic exercises; however, this level decreased slightly in the group with exercises and omega-3 enriched eggs. According to this finding, it could be inferred that this supplement has had effects on athletes. This could be generalized to consuming omega-3 enriched eggs coupled with aerobic exercises. Several studies have clearly shown that extra physical activity reduces cardiovascular risk factors like LDL, cholesterol and triglyceride and increase HDL slightly (Radar, 2008). The level of triglyceride in athletes and individuals with physical activities is usually lower than people in control groups and inert individuals (Gomez, 2008; Destine *et al.*, 2001). This finding concurs with findings of Hill *et al.*, (2007), Chan (2009), Galli (2009) and Defina (2011). Hill *et al.*, (2007) investigated 65 obese individuals and concluded that physical activities together with fish oil consumption significantly decreases fat percentage of the subjects. Defina (2011) reported that aerobic exercises with 56 to 80% VO_{2Max} and 20 to 30 minutes of strength training along with consuming 3 grams of omega-3 fatty acid results in significant weight loss in obese individuals. The

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reason for this concordance may be the excessive need of about 10 to 15 times in aerobic exercises for oxygen compared to the need for oxygen in resting time. The excessive need for oxygen in cells increase electron transmission chain in cells and release more ROS in red blood cells and muscles. This will probably increase oxidative stress (Benjamin *et al.*, 2013). Besides, oxidative activities of omega-3 lipid depend greatly on active participation of PPARs. Although fatty acids are considered to be Substrate of energy production, they could probably act as indigenous ligands for PPARs and genetic expression for key protein codes for controlling absorption of fatty acids and metabolism in the body along with lipoproteins with low density used for carrying triglyceride in liver (Bays *et al.*, 2008). Considering the fact that the precise mechanism of duplicating of omega-3 in improving level of fat is not yet fully discovered yet, omega-3 can still reduce triglyceride synthesis and increase beta-oxidation of fatty acids in liver (Santos-Silva *et al.*, 2012). Nevertheless, findings of this study contradict with findings of Lee *et al.*, (2005). The reason for this contradiction on lipids and blood profiles in various studies may be the dosage of fish oil or type and severity of physical activity along with different diets, exercising protocols or condition of subjects in these studies. Results from this study revealed that BMI decreased significantly 24 hours after aerobic exercises in the group consuming omega-3 enriched eggs and doing physical activities compared to the control group and the group consuming enriched eggs and not doing any exercises. Furthermore, aerobic capacity of the subjects in the group with exercises and omega-3 enriched eggs increases significantly. This finding concurs with findings of Defina (2011) and Lewis *et al.*, (2015). considering the clear advantages of omega-3 fatty acid on cardiovascular system and lipid metabolism, it could be said that its supplements could enhance benefits of physical activities and improve weight loss after burning body fat. One of the probable mechanisms for these advantages of omega-3 supplements may be the increase in lipolysis and beta oxidation. It is especially claimed that omega-3 fatty acids preferably act as metabolic fuel and as a result, lipid oxidation enzymes are adjusted to increase and genetic expression of Lipogenic are adjusted to decrease.

The increase in HDL-C after aerobic exercises raises the activity of lipoprotein lipase and this increases catabolism of lipoproteins. Thus, the level of LDL-C decreases with performance of aerobic exercises and this in turn lowers the risk of cardiovascular diseases (Asif, 2014). On the other hand, decent exercise programs decrease the level of HDL-C as an antiatherogenic factor and this induces a decrease in cardiovascular disease risk and body fat mass (Mohammad-Poor *et al.*, 2014). Besides, it is now known that while doing aerobic exercises, endocrine glands increase secretion of epinephrine, norepinephrine, growth hormone, and cortisol to enhance fat oxidation and the increase in the call for free fatty acids the need for extra energy is met and body fat mass shrinks (Kelley, 2009). Results from this study demonstrated that density of MDA as an index for lipid plasma peroxidation and total antioxidant density decreases 24 hours after 12 weeks of aerobic exercises in both groups receiving omega-3 rich eggs; however, the decrease was significant only in the group not doing aerobic exercises (Table 2). Regarding the effects of strength training on lipid peroxidation of blood, various results have been reported. On the one hand, in spite of the significant decrease in density of MDA in athletes after receiving omega-3 supplements, there are evidences showing that these indices are higher in groups doing strength training in comparison to those not doing any physical activities (Teixeira *et al.*, 2009) or at least were in similar levels (Timothy, 2013). Concurring the findings from the present study, McAnulty *et al.*, (2005) revealed that one session of strength training does not have a significant effect on oxidative stress indices and plasma antioxidant capacity in athlete men. Besides, Farzanegi *et al.*, (2012) demonstrated that consuming omega-3 increases serum antioxidant biomarker and decreases resting biomarker levels of oxidation and lipid profile in trained individuals; nevertheless, this was not enough to alleviate the oxidative stress induced by sports.

However, findings from this study contradict with findings of Teixeira *et al.*, (2009) who reported that lipid peroxidation index and muscular damage in elite rowers were higher compared to control group. They also showed that one session of intense strength training significantly increases plasma Malondialdehyde as in index for oxidative stress in trained men. In another similar study, Guzel *et al.*, (2007) investigated the effects of two different strength training protocols on oxidative stress indices in

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inert men and found that high-intensity sports exercises produce more free radicals compared to low-intensity activities; therefore, lipid peroxidation index significantly increases. Furthermore, Viitala *et al.*, (2004) and Dominic *et al.*, (2010) do not agree with findings of the present study. One of the reasons for this contradiction may be the difference in the type of training whether strength or endurance, type of omega-3 supplement added to the diet, dosage of fish oil consumed, type of food eaten, intensity of the exercises, and the age of the subjects. Moreover, this contradiction may come from nutrition, intensity of exercises, level of exercises, physical fitness and various measuring methods utilized to measure oxidative stress. In any case, two points should be taken into account: firstly, capacity of antioxidant system and its efficiency are two different things. It is demonstrated that regular physical activities increase activity of antioxidant enzymes like Glutathione, Peroxidase and Superoxide dismutase and this creates a preventive factor against oxidative stress (Martin, 2013). Secondly, there is a probability that density of enzymatic and non-enzymatic antioxidants changes without affecting total antioxidant capacity (Atashak *et al.*, 2013). It seems as if adjustments made in antioxidant defense system of the body is the result of regular aerobic exercises and the reason for this may be the cell's constant exposure to ROS after physical activities (Eftekhari *et al.*, 2013).

Considering the drop in density of Malondialdehyde and total antioxidant as main indicators of lipid peroxidation in both experimental groups of the present study and significant decrease in lipid peroxidation in the group receiving omega-3 rich eggs without doing any physical activities, it could be concluded that aerobic exercises can increase oxidative stress. However, omega-3 supplementing may hinder peroxidation process and reduce the risk of damages to the cells and cell damage induced by free radicals released after doing aerobic exercises.

Conclusion

The present study revealed that groups receiving omega-3 enriched eggs with and without endurance exercising possess a better lipid profile compared to control group. As a result, it could be said that considering common features of omega-3 fatty acids and endurance exercises in lowering the level of blood cholesterol, inflammation and body fat mass, and considering the clear advantages of omega-3 for cardiovascular system and lipid metabolism, supplementing eggs with omega-3 can decrease blood fat and body mass and improve aerobic performance (Table 2) in aerobic athletes. Furthermore, decrease in lipid peroxidation index shows that regular aerobic exercises coupled with consuming omega-3 fatty acid supplements added to eggs can prevent formation of oxidative stress in an active muscle.

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