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ORIGINAL ARTICLE

Effect of Eight Weeks Aerobic Exercise and Green Tea on Atherogenic Ratio and ABCG8 Gene Expression of PBMC Globules in Overweight Women

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Abstract

Objective: ABCG8 plays a crucial role in the reverse transfer of cholesterol from macrophages to the liver. The herbal compliments and regular physical improve the lipid profile and metabolic disorders. The aim of this study was to evaluate the effect of eight weeks aerobic exercise and green tea on the ABCG8 gene expression of peripheral blood mononuclear cell (PBMC) and the atherogenic ratio in overweight women.

Materials and Methods: The studied population was the overweight women in Mashhad referring to athletic and aerobic gyms. Among the eligible population, 45 women were divided randomly into three groups of exercise, exercise with green tea supplement and control. Aerobic exercise sessions were held three times a week (8 week, per session 45 minutes), and the cases were provided with 500 milligrams of green tea supplement. After the separation of mononuclear blood globules, the changes in gene expression was performed via RT-PCR and the results were analyzed using SPSS version 16 software with the significance level of 0.05.

Results: As a result of aerobic exercise, with or without the use of green tea supplement, ABCG8 gene expression has increased significantly in PBMC cells (*P*-value:0.001) and the atherogenic ratio has decreased significantly (*P*-value:0.001).

Conclusion: Based on the findings of the present study, we may conclude that eight weeks of aerobic exercise along with usage of green tea supplement leads to an increase in ABCG8 gene expression and a decrease in the atherogenic ratio in overweight women compared to the aerobic exercise and placebo groups.

Keywords: Aerobic exercise, ABCG8, Peripheral blood mononuclear cells, Green tea

Introduction

Overweight and obesity are the risk factor of many diseases as cardiovascular disorders, diabetes, hypertension and others (1). Interventions to improve diet and increase physical activity are the priority in the obesity prevention and cohesive control plans in many countries. The reversed relationship between the plasma high

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density lipoprotein (HDL) and the risk of atherosclerosis is indicative as the role of HDL and its receptors (generally Apo A-1 and Apo A-2) in the absorbance and transfer of cholesterol (1).

The additional cholesterol remove from macrophage foam cells via HDL and its basic lipoproteins Apo A-1 is one of the key protective mechanisms of HDL against atherosclerosis (2). The reverse transfer of cholesterol is a process against atherosclerosis and refers to the collection of excess cholesterol from peripheral tissues, including the macrophages in the artery walls, and returning them to the liver, along with the formation of HDL (3). One of the main features of reverse cholesterol transport (RCT) is the regulation of the genes of ABC transferor family by LXR. One of the most important transferors is ABCG8 (3,4).

ABCG8 is also considered as the main transfer of biliary cholesterol and it is expressed in different body tissues such as liver, intestines, visceral fat and blood cells (5). Regular physical activity is an effective method for the improvement of blood lipid profile, specially increasing HDL and expansion of RCT mechanism in order to prevent the atherosclerosis of coronary arteries (6).

In addition, these days the herbal medicine or complements have come into attention as main variables controlling the RCT in order to improve the lipid profile and prevent metabolic syndrome (7). Green tea is made from the leaves of Camelia Sinensis plant which has caffeine, catechin, flavonoids, glycoprotein, polyphenol, vitamins B, C, E, fiber, lipid and carotenoids (8). The green tea extract is involved in the lipid emulsion process, the inhibition of the activity and synthesis of fatty acids, thermogenic properties and through acting on intracellular C-AMP level leads to increase energy consumption (9), acute increase in lipid oxidation and considerable increase in the 24 hours energy consumption (10).

Ghanbari-Niaki et al. (2012) studied the effect of six weeks of endurance exercise and Pistacia atlantica extraction on the relative expression of ABCG4, ABCG8, tissue ghrelin and nesfatin genes in female rats (11). On the expression of this gene in human subjects, Jafari (2016) analyzed the effect of six weeks of cardiac rehabilitation on the ABCG8 gene expression in the cells of middle-aged men after CABG (12).

Shimotoyodme et al. (2005) in a study on rats realized that the body fat in rats reduces more when the usage of green tea is accompanied with exercise compared to each method separately (13). Furthermore, studies on lipid metabolism in animals, tissues and cellules indicated that the usage of tea and catechin decreases triglycerides and total cholesterol concentrations, and prevents body and liver fat accumulation as well as stimulating thermogenesis (14).

Fathi et al. (2015) performed a study on non-athletic obese women and realized that use of green tea may lead to decrease total cholesterol rate. However, if green tea combined with aerobic exercise the mentioned effects are reinforced (15). In addition, Haghighi et al. (2013) established that the combination of green tea supplement and aerobic exercise leads to a significant decrease in body weight and fat rate (16).

In the present study, we intended to eveluate the effect of aerobic exercise and green tea supplement on ABCG8 gene expression and the atherogenic ratio in the RCT of overweight women.

Materials and Methods

The present study design is quasi-experimental and includes three pre-test and post-test groups. After case selection, medical examinations were carried out in order to ensure health. Complete information was given on the method of the study and consent forms were collected from subjects. The menstrual status was controlled and their diets were matched. A fasting blood sample was then drawn from each subject. Two sessions of exercise were held in order for the subjects to get acquainted with the exercise protocol.

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Then the exercises were continued for eight weeks.

The studied population included overweight women in Mashhad going to fitness and aerobic gyms. Amongst the studied people, 45 subjects participated in the study and were randomly allocated in three groups of exercise, exercise with green tea supplement and control. It should be noted that all subjects participated in the study voluntarily and filled out the questionnaire consisting of personal information, medial history and assessment of physical activity. The inclusion criteria were the age range of 30-40, body mass index higher than 25 and fat percentage more than 30. The exclusion criteria were menopause, cardiovascular disease or use of certain supplements or medications.

Aerobic exercise sessions were done three times a week. Each session included 10 minutes warm up and then 30 minutes of different aerobic movements exercise. The length of exercise sessions was 30 minutes in the first weeks and increased gradually to 45 minutes in the last week. Every week, 2 minutes were added to the total time of the main exercise and in the final week the time length of the main exercise reached to 45 minutes. The exercises started with 55 percent of maximum heart rate in the first week and gradually the intensity was increased and in the final week reached to 75 percent of the maximum heart rate in the subjects, and at the end of each session, there was 10 minutes cool down. The scholar controlled the intensity of exercise sessions using polar heart rate sensor. Subjects in the experiment group used 500 milligrams of green tea and the placebo group used similar capsules containing wheat flour an hour before the exercise sessions (17).

After matching the diet of subjects by a nutritionist, a fasting blood sample of all subjects in resting position in 48 hours before the first exercise session and 48 hours after the last session were taken. The samples were then moved to vials containing anticoagulants (EDTA). The separation of PBMC was done using the Ficoll solution. Peripheral blood mononuclear cell PBMC cells were submerged in liquid nitrogen and crushed completely by a mortar and pestle for mRNA purification. In order to reach mRNA, the damaged tissue was homogenized in buffer RLT and then the tissue powder and liquid nitrogen were poured into a 2-milliliter RNase-free micro centrifuge tube and the liquid nitrogen was allowed to evaporate while the lymphocytes stay frozen. Sufficient buffer RLT was added. Lysate was transferred directly to the QIA shredder spin column in the tube and was centrifuged in high speed for 2 minutes. For the synthesis of cDNA, 200 nanograms of mRNA were analyzed using Oligo (dT) primer and a special kit. In order to study the relative expression of ABCG8 gene, the RTPCR method was used. In the end, PCR products underwent electrophoresis and placed on Agarose gel to be photographed. Finally, the results were obtained using the UV Tech system and the Beta-actin sums were obtained for each subject, the numbers were divided by the Beta-actin values and multiplied by 100 to reach mRNA amounts related to ABCG8 for each subject as percentage (18).

In addition, the amounts of atherogenic factors (LDL/HDL) were measured using Pars Azmoon kits and ELISA method. (IR.IAU.NEYSHABUR.REC.1397.010).

In order to ensure the homogenization of the data the normal distribution of data were checked by the Leven test and the Shapiro-Wilk test. Considering the abnormality of the data, the one-way analysis of variance (ANOVA) and the Dunnett's tests were used. The data was analyzed by SPSS version 16 software with the significance level of 0.05.

Results

As a result of aerobic exercise, with or without the use of green tea supplement, ABCG8 gene expression has increased significantly in PBMC cells (*P*-value:0.001) and the atherogenic ratio has decreased significantly (*P*-value:0.001). Significant changes were observed in the exercise and green tea

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supplement compared to the placebo group (Table 1 and figures 1-2).

Discussion

The ABCG8 gene is expressed in different tissues such as the liver, small intestine, lungs and also white blood cells. The reason for selecting PBMC as the target tissue in the present study was its accessibility compared to other tissues. It has also been reported recently that the ABCG8 transferor expression level is an independent risk factor for the prediction of atherosclerosis (19).

In general, the findings showed that aerobic exercise with or without green tea supplement led to a significant increase in ABCG8 gene expression in PBMC cells and a significant decrease in the atherogenic ratio, and the fact that the ABCG8 gene expression was elevated

Significantly in the exercise and supplement group compared to the exercise group demonstrates the reinforced effect of combining exercise and supplement.

Cunha et al. (2013) studied the effect of 8 weeks of using green tea extract on obesity and lipolysis pathways in rats with high fat diet. Their results showed that the daily usage of green tea extract (400 milligrams for each kilogram of body weight) leads to weight loss and decreased adipose tissue and improvement of lipolysis pathways, does not change the amount of total cholesterol and triglycerides, but increases HDL concentration and adiponectin (20). Furthermore, Fathi et al. (2015) in a study on non-athlete women realized that green tea usage solitarily may help the reduction of total cholesterol and LDL-C levels but if combined with aerobic exercise, the mentioned effects will be reinforced (15). In this regard, Haghighi et al. (2013) concluded that a combination of green tea supplement and aerobic exercise is able to

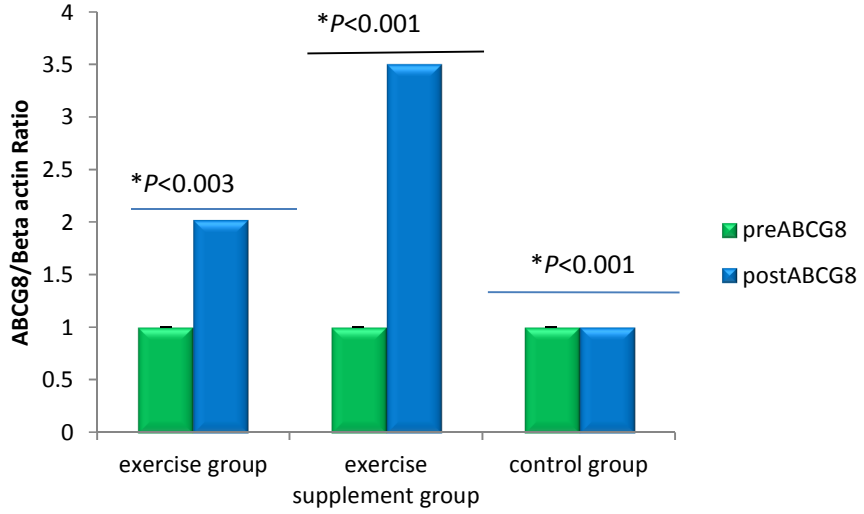

Figure 1. Changes in ABCG8 gene expression amounts in different groups before and after the exercise protocol and use of supplement

Table 1. Changes in ABCG8 gene expression in PBMC globules and the Atherogenic ratio in overweight women using paired T-test

Groups	Exercise with green tea	Exercise with green tea	*P*-value	Exercise group	Exercise group	*P*-value	Control group	Control group	*P*-value
Pre/Post test	Post test	Pre test		Post test	Pre test		Post test	Pre test	
ABCG8 gene expression	0.96±3.51	0.001 ±1	0.001	0.61±2.04	0.001±1.0	0.001	0.001±1	0.001±1.0	0.096
Athrogenic factor	0.35±1.87	0.6±2.67	0.001	0.39±2.20	0.49±2.44	0.001	0.32±2.55	0.4±2.54	0.847

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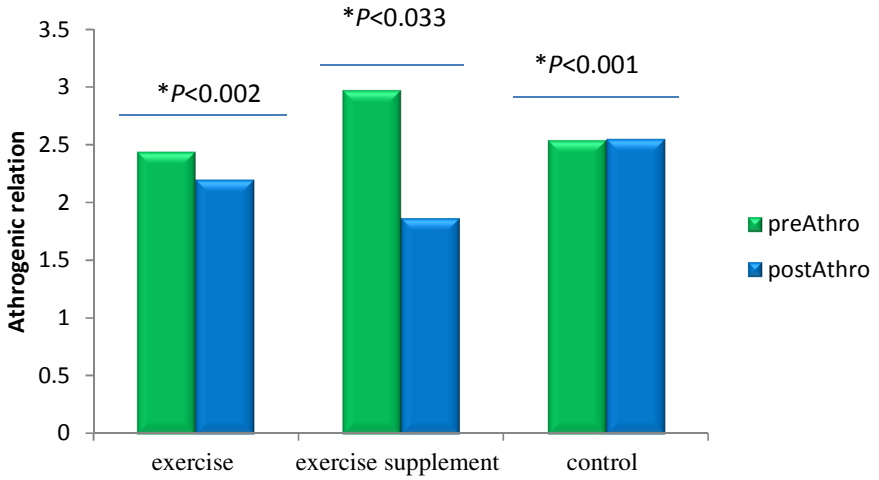

Figure 2. Changes in Atherogenic ratio in different groups before and after the exercise protocol and use of supplement

reduce body weight and fat percentage significantly (16). All the mentioned results are consistent with the present study.

The gen eral reduction mechanism of the atherogenic ratio (LDL/HDL) in the present study may be due to the catechin in green tea which prevents LDL oxidation by CuSO4 (21), and inhibits cholesterol synthesis by affecting the rate-limiting enzyme of cholesterol biosynthesis (22). Another possible mechanism for the LDL reduction is related to the micelles of cholesterol in the digestive system that form insoluble cholesterol and thus lead to the excretion of cholesterol in the feces and leads to lower absorption of cholesterol (21). Reduction in cholesterol absorption and reduction in liver cholesterol concentration causes the increased expression and activity of LDL receptors, and this increased activity in hepatocytes leads to the removal of cholesterol from the circulation (23). On the other hand, the polyphenol present in green tea decreases atherosclerosis by inhibition of LDL oxidation, increasing the serum antioxidant activity and increasing the HDL level (21).

With regard to the effect of exercise and supplement on the gene expression of ABCG8 and its family in rats and human subjects, Ghanbari-Niaki et al. (2012) conducted a research on female rats, studying the effect of 6 weeks of endurance exercise and Pistachiaatlantica (Baneh) extraction on the relative gene expression of ABCG 4, ABCG8, tissue ghrelin and nesfatin and demonstrated that physical acitvitt leads to an increase in the gene expression of ABCG4, ABCG8 and tissue nesfatin in the exercised groups compared to the control group (11). Regarding gene expression levels in human subjects, Jafari (2016) demonstrated that 6 weeks of cardiac rehabilitation exercise increases ABCG8 gene expression in PBMC cells in middle-aged men after CABG (12). Rambod Khajei et al. (2016) studied the effect of 8 weeks of aerobic exercise on lipid profile changes, Apoprotein B to A1 ratio and gene expression of monocyte LXR-α and ABCG5 in middle-aged men after cardiac bypass surgery and concluded that monocyte LXR-α and ABCG5 gene expression was increased significantly in the experiment group compared to the control group. In addition, TG and LDL levels and Apoprotein B to A1 ratio decreased in the experiment group whereas the plasma HDL increased (24).

It has been suggested that the regulatory effect of fatty acids is mediated by PPARs (Proliferator Activated Receptors). It has also been established that PPARs contain receptors such as LXR (Liver X Receptor) and RXR (Retinoid X Receptor), which regulate lipid

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controlling, and glucose metabolism genes. Three isofoams of PPARs (α, β, γ) are expressed in metabolic tissues including heart, liver, skeletal muscle, kidney and also cell in arterial walls such as monocytes and macrophages (25,26).

Conclusions

In general, the present study demonstrates that 8 weeks of aerobic exercise and green tea supplement compared to the exercise and placebo groups, leads to an increase in ABCG8 gene expression and more reduction of atherogenic ratio in overweight ratio. Considering the fact that cardiovascular diseases are increasing amongst the society and their prevention is a priority rather than their treatment, and since the reverse transfer of cholesterol is directly related to the prevention of atherosclerosis, it seems that based on the results of this study and combining aerobic exercise and green tea supplement, this protocol may be considered a positive step in the prevention of cardiovascular diseases and in the health of nations.

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