ORIGINAL ARTICLE

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

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Abstract

Objective: Atherosclerosis of coronary arteries is the most significant cause of disability and mortality worldwide and the most efficient physiological process reducing the risk of this disease is RCT. ABCG8 plays a crucial role in the reverse transfer of cholesterol from macrophages to the liver. The aim of this study is to study the effect of eight weeks aerobic exercise on the atherogenic ratio and the ABCG8 gene expression in peripheral blood mononuclear cells globules in women with overweight.

Materials and Methods: The statistical populations were overweight women in Mashhad who were referred to gyms. Among the eligible population, 30 women were included and divided randomly into two groups of exercise and control. Aerobic exercise sessions were three times a week. A blood sample was taken from all cases 48 hours before the first exercise session and 24 hours after the last exercise session. After the separation of mononuclear blood globules, the gene expression was studied via RT-PCR and the atherogenic ratio was measured by special kits.

Results: The data demonstrated that exercise has a significant effect on the increase of ABCG8 gene expression in PBMC globules (P-value:0.001, t=5.40), and on the decrease of atherogenic ratio (P-value:0.01, t=-2.86).

Conclusion: Based on the findings of the present study, eight weeks of aerobic exercise could lead to an increase in ABCG8 gene in overweight women and improve the function of reverse cholesterol transportation.

Keywords: Aerobic exercises, ATP binding cassette transporter, PBMC polymer, HDL, LDL

Introduction

The high prevalence of obesity caused increase of obesity management studies and its related cardiovascular diseases and metabolic risk factors. Disorders in lipid metabolism, specially increased cholesterol and triglycerides and decreased concentrations of plasma high density lipoproteins (HDL) are factors predisposing patients to cardiovascular diseases. In addition, the sedentary lifestyle causes obesity and cardiovascular diseases (1,2). The lipid profile and atherogenic ratio (the ratio of low density lipoprotein (LDL) to HDL) are important markers predicting dyslipidemia, atherosclerosis and

cardiovascular diseases. Any alteration in normal levels of lipids predisposes people to the risk of cardiovascular diseases and abnormal function of endothelial cells. The reverse transfer of cholesterol is a process in which cholesterol is transferred to HDL in peripheral cells and HDL transfers cholesterol to other blood lipoproteins (LDL and VLDL) or to the liver to be excreted in feces or bile (3,4). The cholesterol accumulation reduction in artery walls prevents development of atherosclerosis. The cholesterol movement which is a part of RCT (Reverse Cholesterol Transport) process considered as the process of the macrophages inside artery walls excrete cholesterol out of the cell in order to reach HDL and its main apolipoprotein, Apo A1 (5). This stops the macrophages containing cholesterol from attaching to artery walls, as well as maintaining cell cholesterol homeostasis and preventing atherosclerosis (6,7). It is generally believed that HDL plays its role in the prevention of cardiovascular diseases through the reverse transfer of cholesterol, and the process of reverse cholesterol transfer is mediated by ABC transporters. ABC transporters are proteins that hydrolyze ATP and consume the energy to stimulate the transfer of different molecules through cell membranes (8). ABCG (ATP binding cassette type G), is a big family of this set including subsets such as ABCG1, ABCG2, ABCG3, ABCG4, ABCG5 and ABCG8. ABCG8 is considered to have an important role in the reverse transfer of cholesterol from macrophages to the liver (9,10). ABCG8 is also considered as the main transferor of biliary cholesterol and its expression was shown in different body tissues such as liver, intestines, visceral fat and blood cells (11). Mutation in ABCG5 and ABCG8 lead to an increase of intestinal cholesterol absorbance and herbal esterol and a decrease in their biliary excretion which increases the concentration of herbal esterols in plasma over 200 times (12). They secrete herbal esterols and cholesterol from enterocytes into the intestines and excrete them from hepatocytes

into the bile, and thus reduce their absorption and through this action, they participate in the excretion of excess cholesterol from coronary artery walls and the reduction of blood cholesterol levels and reduce the risk of atherosclerosis and heart stroke. In fact, esterols are the main substrates from ABCG5 and ABCG8 (13).

Regular physical exercise is an effective method to improve blood lipid profile, specially increasing HDL and expansion of RCT mechanism in order to prevent the atherosclerosis of coronary arteries (14). The effect of exercise and physical activity on RCT features is a novel issue. Few studies have been performed on the effect of aerobic exercise on the ABCG8 gene expression in PBMC (Peripheral blood mononuclear cells), cells and atherogenic ratio (LDL/HDL) in overweight women. However, the ABCG8 gene expression has been studied in the intestinal cellules in rats after endurance exercises in multiple studies. Ghanbari-Niaki et al. (2012) analyzed the effect of eight weeks on the ABCG8 gene expression in the intestinal cellules of female rats. Ghanbari-Niaki et al. (2012) also studied the effect of six weeks of endurance exercise and Pistachio atlantica (Baneh) extraction on the relative expression of ABCG4, ABCG8, tissue ghrelin and nesfatin genes in female rats (15). On the expression of this gene in human, 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 (Liver X receptor), and ABCG5 in middle-aged men after cardiac bypass surgery (16). On the same subject, Jafari (2016) studied the effect of six weeks of cardiac rehabilitation on the ABCG8 gene expression in the cells of middle-aged men after CABG (17). The studies are indicative of the role of physical activity on key phases in the reverse cholesterol transfer process including increasing the amount of HDL, increasing the outflow of cholesterol from cellules, increasing the formation and size of ApoA-1, increasing pre-beta, plasma

HDL and increasing LCAT enzyme activity. Nevertheless, there have been limited studies contemplating the direct effect of regular exercise on ABCG8 gene expression as the primer in formation of HDL particles and the process of the reverse transfer of cholesterol in human cases. Thus, in the present study, we intended to analyze the effect of aerobic exercise on ABCG8 gene expression and the atherogenic ratio in the reverse transfer of cholesterol in overweight women.

Materials and Methods
This study has been performed in a couple of athletic complexes in Mashhad. Amongst the qualified people, 30 subjects volunteered to participate in the study and were randomly placed in two groups of exercise and control. It should be noted that all subjects participating voluntarily in the study filled a questionnaire consisting of personal information, medial history and assessment of physical activity. The main inclusion criteria were the age range of 30-40, with a BMI higher than 25 and fat percentage of more than 30, no menopause, complete health and lack of any cardiovascular disease or usage of certain supplements or medications.

Exercise protocol
Aerobic exercise sessions were held three times a week. Each session included a 10-minute 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. (Each 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 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 a 10-minute cool down. The scholar controlled the intensity of exercise sessions using Polar heart rate sensor.

Laboratorial methods
After matching the diet of subjects by a nutritionist, a fasting blood sample of 10 cc was drawn from the median cubital vein of all subjects in resting position (sitting) in 48 hours before the first exercise session and 48 hours after the last session. The samples were then moved to vials containing anticoagulants (EDTA). The separation of PBMC was done using the Ficoll solution. 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 microcentrifuge 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 QIAshredder spin column in the tube and was centrifuged in high speed for 2 minutes. For the synthesis of cDNA, 200 nanograms of mRNA was 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.

Statistical analysis
After the ensuring the normal distribution of the data with Shapiro-Wilk test, paired-t test was used for intragroup comparison and independent T-test for intergroup comparison.

The data was then analyzed by SPSS version 16 software with the significance level of 0.05.

Results

The results of this study indicate that aerobic exercise increases the ABCG8 gene expression of m-RNA in PBMC cells (t=5.409, P=0.001) and reduces the atherogenic ratio significantly (t=2.861, P=0.01).

Discussion

The data demonstrated that exercise has a significant effect on the increase of ABCG8 gene expression in PBMC globules and on the decrease of atherogenic ratio.

The ABCG8 gene is expressed in different tissues such as the liver, small intestine, lungs and also white blood globules. 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).

The atherosclerosis of coronary arteries is the most important reason of disability and mortality worldwide and the most important physiological process reducing the risk of this disease is RCT. In general, the results are indicative of the fact that aerobic exercise has led to a significant increase in ABCG8 gene expression in PBMC cells. On the subject of the expression of this gene in human subjects, 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

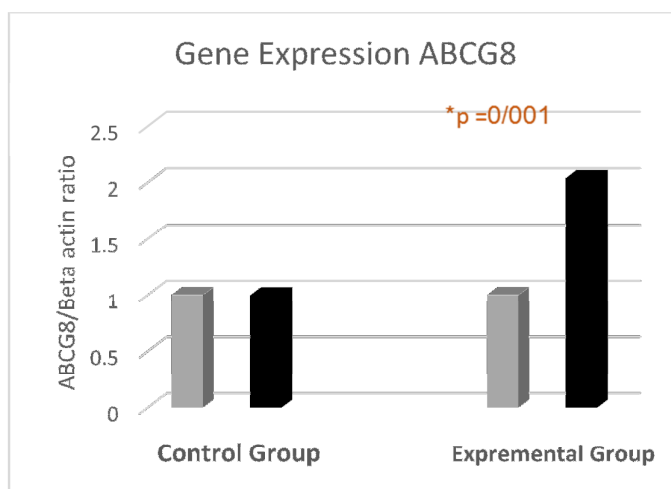**Figure 1. Changes in ABCG8 gene expression amounts in control and exersice groups before and after the exercise protocol**
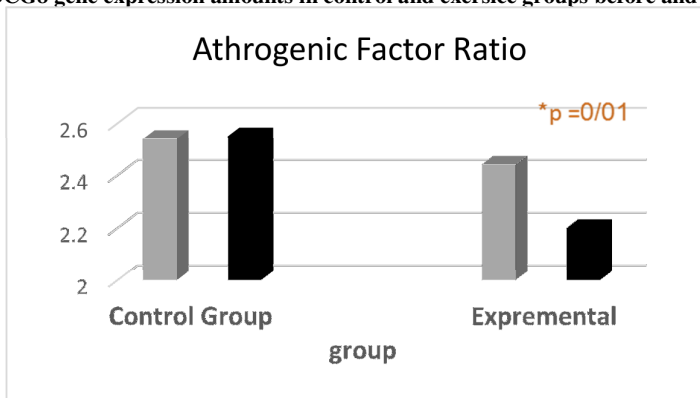

Figure 2. Changes in Atherogenic ratio in control and exercise groups before and after the exercise protocol

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 (16). On the same issue, Jafari (2016) demonstrated that 6 weeks of cardiac rehabilitation exercise increases ABCG8 gene expression in PBMC cells in middle-aged men after CABG (17). In addition, Ghanbari-Niaki et al. (2012) conducted a research on female rats, studying the effect of 6 weeks of endurance exercise and Pistachia atlantica (Baneh) extraction on the relative gene expression of ABCG 4, ABCG8, tissue ghrelin and nesfatin (15).

In general, the present study demonstrates that 8 weeks of aerobic exercise compared to the control group, leads to an increase in ABCG4, ABCG8 and tissue nesfatin gene expression. The results of this study prove the effect of aerobic exercises on the increase in the amounts of these two transporters which play fundamental roles in the reverse transport of cholesterol and increase of HDL production and are crucial in the health maintenance of heart and arteries. It has also been established that the increase in ABCG5 and ABCG8 gene expression is accompanied by an increase in the amounts of lipase lipoprotein, liver lipase, pre-beta HDL and LCAT, and that the increase in these markers may be of great importance in the prevention of cardiovascular diseases (20,21).

In this study, the ABCG8 gene expression has been demonstrated in PBMC cellules after exercise training. It may be expected that the ABCG8 in PBMC cell membrane along with ABCA1 and ABCG1 cooperate in the management of the cholesterol secreting from the cellule and reaching Apo-A1, forming HDL and reduce the risk of atherosclerotic wounds and heart attack (22). The reduction in cholesterol biosynthesis decreases the intracellular cholesterol content. In hepatocytes, the reduction in cholesterol content decreases the secretion of lipoproteins containing Apo-B and increases the activity of LDL receptor. Both these actions lead to a decrease in blood LDL levels (23). Increase in ABCG8 gene expression may also reduce the production of Apo-B, which is the main substrate for LDL production (24). Furthermore, the suppression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme in the cholesterol biosynthesis pathway, leads to a decrease in VLDL production and an increase in LDL receptor gene expression in liver. Both these changes lead to a reduction in plasma LDL levels (25). In general, the reduction of atherogenic ratio and increase of ABCG8 gene expression in PBMC cells noticed in this study, may decrease the risk of atherosclerosis among subjects. In this study, we only analyzed the effects of exercise on the mononuclear cellules of blood. The demonstrated effects are reflecting similar influences in cellules such as endothelial cells, lipid cells, liver cells and other cellules participating in lipid metabolism. The results of the effects of exercises on these cells could be added to the results of this study.

Conclusions

Gathering these tissues requires a complex and aggressive process. Despite the apparent effect of exercise on ABCG8 transporters, studies are required to establish the effect of exercise on this key element in cholesterol excretion from peripheral tissues in high-risk societies among the population of patients suffering from atherosclerosis and have gone through open-heart surgery. More research is essential for the clarification of the effect of exercise in these matters.

References

1. Attie AD. ABCA1: at the nexus of cholesterol, HDL and atherosclerosis. Trends Biochem Sci, 2007;32(4):172-9.

2. Lange Y. Disposition of intracellular cholesterol in human fibroblasts. J Lipid Res, 1991;32(2):329-39.

3. Annema W, Nijstad N, Tolle M, De Boer JF, Buijs RVC, Heeringa P, et al. Myeloperoxidase and

serum amyloid A contribute to impaired in vivo reverse cholesterol transport during the acute phase response but not group IIA secretory phospholipase A2. J Lipid Res 2010;51:743-54.

4. Van der Velde AE. Reverse cholesterol transport: From classical view to new insights. 2010;16(47):5908-15.

5. Alexander ET, Weibel GL, Joshi MR, Vedhachalam C, De la Llera-Moya M, Rothblat GH, et al. Macrophage Reverse Cholesterol Transport in Mice Expressing ApoA-I Milano. Arterioscler Thromb Vasc Biol 2009;29:1496-501.

6. Tabas I. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest 2002;110(7):905-11.

7. Yao PM, Tabas I. Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. J Biol Chem 2001;276(45):42468-76.

8. Feng B, The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol, 2003;5(9):781-92.

9. Ji Y, Jian B, Wang N, Sun Y, Moya ML, Phillips MC, et al. Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. J Biol Chem, 1997;272(34):20982-5.

10. Yancey P G, et al. High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. J Biol Chem, 2000;275(47):36596-604.

11. Bailey JM. Lipid Metabolism in Cultured Cells. Iv. Serum Alpha Globulins and Cellular Cholesterol Exchange. Exp Cell Res, 1965;37:175-82.

12. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation. Physiol Rev 2009;89:147-91.

13. Woodward OM, Kottgen A, Kottgen M. ABCG transporters and disease. FEBS J 2011;278:3215-25.

14. Liem AH, Jukema JW, Van Veldhuisen DJ. Secondary prevention in coronary heart disease patients with low HDL: which options do we have Int J Cardiol 2003;90:15-21.

15. Ghanbari-Niaki A, Rahmati Ahmadabad S, Zare-Kookandeh N. ABCG8 Gene Responses to 8 Weeks Treadmill running with or Without Pistachia atlantica (Baneh) Extraction in Female Rats. Int J Endocrinol Metab 2012;10(4):604-10.

16. 16.Khajei R. (2016) The effect of 8 weeks of aerobic exercise on lipid profile changes, Apolipoprotein B to A1 ratio and the gene expression of ABCG5 and LXR-α of monocytes in middle-aged men following bypass surgery. Doctorate thesis. Hakim Sabzevari University.

17. Jafari M. The effect of 6 weeks of cardiac rehabilitation on the ABCG8 gene expression in PBMC cells and blood lipid profile after CABG in middle-aged men. Doctorate thesis. Tehran University 2015.

18. Rashidlamir A. Investigation of the Effect of Aerobic and Resistance Exercises on Peripheral Blood Mononuclear Cells ABCG1 Gene Expression in Female Athletes. JSSU 2012;20(1):1-9.

19. Demina E, Miroshnikova V, Rodygina T, Kurianov P, Vinogradov A, Denisenko A, et al. ABCA1 gene expression in peripheral blood lymphocytes and macrophages in patients with atherosclerosis. MolecularBiol 2011;45(2):258-62.

20. Ghanbari-Niaki A, Saghebjoo M, Hedayati M. A single session of circuit-resistance exercise effects on human peripheral blood lymphocyte ABCA1 expression and plasma HDL-C level. Regul Pept 2011;166(1-3):42-7.

21. Rashidlamir A, Ghanbari-Niaki A, Saadatnia A. Effect of eight weeks wrestling and wrestling-technique based circuit training on lymphocyte ABCA1 gene expression and plasma apolipoprotein I-A. World J Sport Sci 2011;4(2):144-50.

22. Baldan A, Bojanic DD, Edwards PA. The ABCs of sterol transport. J Lipid Res 2009;50:80-5.

23. Kajinami K, Brousseau ME, Nartsupha C, Ordovas JM, Schaefer EJ. ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. J Lipid Res 2004;45:653-6.

24. Chan DC, Watts GF, Barrett PHR, Whitfield AJ, Van Bockxmeer FM. ATP-Binding Cassette Transporter G8 Gene As a Determinant of Apolipoprotein B-100 Kinetics in Overweight Men. Arterioscler Thromb Vasc Biol 2004;24:2188-91.

25. Mohammadifard N. Sarrafzadegan N. Sajjadi F. Rafei M. Abdar N. The effect of cardiac rehabilitation on serum lipids. Scientific Journal of the medical council of Islamic Republic of Iran 2002;20(3):199-205.