




Original Article

## Comparison of Combined and Aerobic Training on ABCG1 Lymphocyte Gene Expression in Middle-Aged Men Undergoing Coronary Artery Bypass Grafting

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### ABSTRACT

**Background and objectives:** Cardiovascular disease is one of the most important causes of mortality worldwide. The present study aimed to compare two different cardiac rehabilitation protocols on ATP-binding cassette sub-family G member 1 (ABCG1) lymphocyte expression and blood lipid profile in middle-aged men undergoing coronary artery bypass grafting.

**Methods:** Forty five middle-aged men who had previously undergone coronary artery bypass surgery were randomly divided into three groups of control (C; n=15), aerobic training (AT; n=15) and combined training (CT; n=15). Blood samples were taken before the first and after the last exercise sessions. After isolation of mononuclear cells using Ficoll and mRNA purification, gene expression changes were examined by real-time PCR. Data were analyzed using one-way ANOVA and Bonferroni post-hoc tests.

**Results:** Eight weeks of training intervention resulted in a significant increase in ABCG1 expression as well as a significant decrease in plasma levels of LDL, triglyceride and total cholesterol in both training groups. However, there was no significant difference between the AT and CT groups. In addition, high-density lipoprotein was significantly increased in the AT and CT groups.

**Conclusion:** Both AT and CT can increase plasma LDL and increase ABCG1 expression and HDL concentrations, indicating the positive effects of both interventions on the prevention of atherosclerosis.

**Keywords:** Coronary Artery Bypass, Circuit-Based Exercise, ABCG1, Exercise.

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## INTRODUCTION

Atherosclerosis is a major cause of cardiovascular disease, including myocardial infarction, heart failure and stroke (1). Reverse cholesterol transfer (RCT) is a pathway by which accumulated cholesterol in the vessel walls transfers to the liver for excretion, thereby preventing atherosclerosis. Cholesterol delivery and excretion are regulated by the ATP-binding cassette (ABC) transporters superfamily membrane vectors. The human genome has 48 different ABC vectors, classified into seven ABCA to ABCG groups. *ABCG1* belongs to the G index of the ABC transporter family. Animal studies have shown that *ABCG1* plays a significant role in cholesterol outflow into high-density lipoprotein (HDL) and RCT. Overexpression of the *ABCG1* gene in transgenic mice may have a protective role against lipid accumulation in the liver and lungs (2, 3). Research has shown that *ABCG1* is highly expressed in endothelial cells. Partial vector plays a remarkable role in removing cholesterol from endothelial cells and protects against endothelial dysfunction as well as promoting cholesterol flow from endothelial cells to HDL and reduces the amount of existing cholesterol (4). Thus, regulation of RCT by *ABCG1* indicates its important physiological role. It has been reported that *ABCG1* and *ABCA1* can synergistically enhance cellular cholesterol efflux in vitro (5). In addition to clinical interventions, it has been shown that physical activity protects against cardiovascular disease through suppression of sympathetic activity, blood pressure and heart rate, increased blood flow and nitric oxide production, vascular clearance, reduction of inflammatory cytokines and formation of reactive oxygen species (6). Regarding cardiovascular diseases, it has been reported that physical activity significantly increases expression of *ABCG5* (7), *ABCA1* and *ABCG1* (8, 9). In regard to CABG patients, studies have shown a significant increase in ABCG gene expression following an aerobic training (AT) program (10). The role of combined training (CT) on *ABCG1* gene expression changes in CABG patients has received little attention. The positive effect of physical activity on *ABCG1* gene expression and the improvement of RCT provide a basis for their protective role in the process of atherosclerosis. However, studies comparing

the two types of AT and CT protocols on *ABCG1* lymphocyte gene expression changes in CABG patients are very limited, and most studies have focused on functional performance of these patients. Thus, the aim of the present study was to compare AT and CT protocols on *ABCG1* lymphocyte gene expression and blood lipid profile in middle-aged men undergoing coronary artery bypass surgery.

## MATERIALS AND METHODS

This semi-experimental study was performed on middle-aged men (aged 40-60 years) undergoing coronary artery bypass surgery in the Javad Alaemeh Heart Hospital in Mashhad (Iran) in 2018. Blood samples, body measurements and cardiopulmonary fitness were collected before and after the training intervention. The subjects were randomly divided into three groups: AT (n=15), CT (n=15) and control (n=15).

A primary clinical evaluation specialist evaluated the severity and extent of heart disease. Subsequently, individuals who had previously undergone coronary artery bypass surgery who were homogeneous in disease level and physically fit to exercise, entered the study. Inclusion criteria were as follows: systolic and diastolic blood pressure of no more than 160 mmHg and 100 mmHg, respectively, cognitive, visual and auditory health, no use of walking aids like canes and walkers, at least two months have passed since their surgery, oxygen uptake of more than five metabolic equivalent of task (11, 12) and age of 40 to 60 years. Exclusion criteria were unstable angina, uncomplicated heart failure, myocardial infarction, ventricular erythema, neurologic medication (13). The study received approval from the Human Subject Committee of Islamic Azad University of Neyshabur (IR.IAU.NEYSHABUR.REC.1397.007).

Participants performed AT exercise three days a week for eight weeks. The exercise included treadmill walking (20 to 30 minutes), pedaling on a fixed bike (10 to 12 minutes) and arm ergometer (eight to ten minutes). Stretching exercises were used for warm up at the beginning of the session and for cool down at the end of the exercise session. Exercises began with moderate intensity. In addition to the amount of fatigue and cardiac symptoms,

the duration and intensity of exercise were adjusted at 60% of maximum heart rate. The intensity and duration of exercise gradually increased based on patients' ability to reach 80% of their maximum heart rate in the last seven to ten sessions (14).

In each session of CT, the patients first performed AT and performed simultaneous resistance training after a short rest as described below.

Resistance training consisted of specified exercises that were performed three sessions a week for eight weeks. The training was performed in two sets with 10 repetitions in the initial sessions and up to 15 repetitions in the next training sessions. Exercise included squat with physio ball, shoulder flexion, shoulder abduction, elbow flexion, hip flexion, hip abduction and plantar flexion and dorsiflexion of ankle.

Due to the ability of the participants, the exercises were initially performed without overload and merely by moving the limbs and gradually with a weak thera-band and finally with very light overloads (15).

The movements were initially performed with seven repetitions using a light yellow thera-band (set as lower intensities). Then, the number of repetitions

were gradually increased to 15 repetitions in the subsequent training sessions.

Blood sampling was performed in two stages, 48 hours before the first training session and 48 hours after the last training session, after 12 hours of overnight fasting. Blood samples (10 ml) were collected in test tubes with EDTA anticoagulant. After isolation of mononuclear cells by ficoll and centrifugation, mRNA was purified using a commercial kit. Quantitative real-time-PCR method was performed to evaluate the relative expression of the gene (16). Enzymatic method was used to measure lipid profile using Pars Azmoon kit (Tehran, Iran).

The mononuclear cells were embedded in liquid nitrogen, completely crushed, homogenized in RLT buffer and frozen for future use.

The lysate was transferred directly to the QIAshredder and centrifuged at high speed for two minutes. The 200 ng mRNA cDNA was synthesized using the Oligo primer (dT) and a kit (St. Leon-Rot Fermentas GmbH, Germany) according to the manufacturer's instruction.

Real-time PCR was performed to investigate the relative expression of *ABCG1* gene (11). The sequence of the primers and beta-actin are shown in [table 1](#).

Table 1- Oligonucleotide sequence of the primers used in the PCR experiment

| Gene name         | Type    | Sequences (5' to 3')       |
|-------------------|---------|----------------------------|
| <i>ABCG1</i>      | Forward | CCC AAC TGC AGC CAC TCT G  |
|                   | Reverse | GTG AAG AAA GGC CGC AGA GG |
| <i>Beta-Actin</i> | Forward | CCT ATG TTC TCA GCA GCT TC |
|                   | Reverse | GAA TTT CCT GGC TGT CCC TG |

After confirming the normality of the data distribution by Shapiro–Wilk test, one-way ANOVA and Bonferroni post hoc test were used to examine intergroup and intragroup differences, respectively. All statistical analyses were performed using SPSS software (version 24) and at significance level of 0.05.

## RESULTS

Subject's characteristics are presented in [table 2](#). There were significant main effects of time for *ABSCG1* expression ([Figure 1](#)). There

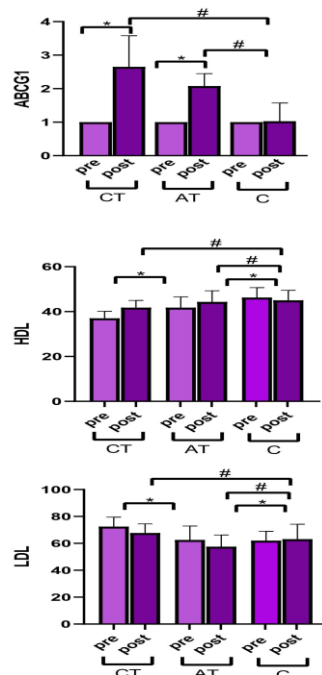
was a significant difference in *ABCG1* lymphocyte gene expression between the AT and C groups ( $P=0.002$ ) as well as CT and C groups ( $P=0.001$ ). However, no significant difference was observed between the AT and CT groups ( $P>0.05$ ). In addition, HDL and LDL levels differed significantly between the CT and C groups and between the C and CT groups ( $P<0.05$ ).

However, there was no significant difference between the AT and CT groups ( $P >0.05$ ).

Table 2- Subject characteristics

| Group | Number | Age (year) | Height (cm) | Weight (kg) | Duration of disease (months) |
|-------|--------|------------|-------------|-------------|------------------------------|
| AT    | 15     | 46.9±3.23  | 171.1±3.6   | 80.14±9.12  | 150±68.19                    |
| CT    | 15     | 47.4±3.23  | 170.2±3.5   | 80.16±8.99  | 150±69.01                    |
| C     | 15     | 47.4±2.75  | 170.9±3.9   | 80.1±8.8    | 149±69.12                    |

Figure 1- ABCG1, HDL and LDL values in different study groups. \* shows significant difference from baseline; # shows significant difference between groups.



## DISCUSSION

We aimed to compare the effects of AT and CT protocols on *ABCG1* expression in lymphocytes and blood lipid profile in middle-aged men undergoing coronary artery bypass surgery. The results showed that both AT and CT increased *ABCG1* gene expression in mononuclear cells. Yamamoto et al. (2015) showed that resistance training increased muscle strength, exercise capacity and mobility in middle-aged CAD patients (17). Gambassi et al. (2019) showed that acute response of AT in cardiovascular rehabilitation program after CABG improved cardiac autonomic control in patients undergoing CABG (18). Although there has been limited evidence about the effects of rehabilitation training programs on CABG patients, there is no significant consensus on the training intensity, duration, and even type of rehabilitation programs. In addition, researchers have sought to assess different training protocols such as AT, resistance training and CT to draw definite conclusions. The main findings of this study showed that *ABCG1* increased 108% in AT group and 165% in CT group. In addition, Gaieni indicated that eight weeks of CT is more effective than AT in improving functional capacity (cardiovascular fitness)

and muscle strength in CABG patients (19). However, Ghroubi et al. reported that resistance training would result in a greater increase in physical performance compared to AT (16).

Most of the common atherogenic abnormalities in humans appear to be due to damage to the ABC transporters or suppression of their expression. Diabetes and other metabolic disorders decrease the expression of ABC transporters via several mechanisms, which increases the risk of cardiovascular disease.

CAD patients are relatively resistant to interventions designed to increase expression of ABC cholesterol transporters. Thus, mechanisms that impair ABC transducers or affect cellular pathways need to be specifically and directly targeted therapeutically (20).

One of the important components of RCT is the ABC transporter family genes by liver-x receptor. These transporters, notably ABCA1 and ABCG1, are key regulators of cholesterol and phospholipid removal from macrophage foam cells (21). However, ABCA1 transduces these substances into lipid-free lipoproteins, causing early HDL formation (22).

In an study by Dashtkhaki Lily et al. on ABCG8 alterations in peripheral blood

mononuclear cells, eight weeks of water-based and land-based resistance training in middle-aged women undergoing CABG induced similar adaptations regarding increments of ABCG8 expression that may indirectly prevent cholesterol accumulation in the coronary arteries (23). In this regard, Rashidelmir et al. (2012) investigated the influence of AT and RT on ABCG1 gene expression in peripheral blood mononuclear cells in female athletes, illustrating that CT could improve RCT and ABCG1 expression in peripheral blood mononuclear cells (20). Based on the results of the present study, both CT and AT improved cardiovascular function. In a previous study, 12 weeks of regular physical activity significantly increased the expression of ABCG1 in blood cells of sedentary obese women (24). Moreover, Ramezani et al. reported that eight weeks of AT could increase ABCG8 and improve RCT in overweight women (25). It has been also shown that ABCA1 and ABCG1 overexpression is associated with increased level of lipoprotein lipase, hepatic lipase, pre-beta HDL and cholesterol acetyltransferase (LCAT), which play important roles in the prevention of cardiovascular disease (11, 20).

ABCG1 is required for HDL to exert a protective effect against LDL. Physical activity and positive regulation of peroxisome activator receptors (PPARs) plays an important role in regulating the expression of genes involved in cellular cholesterol transmission and expression of the ABC transporter family. In addition, PPAR- $\alpha$  activity additionally increases the expression of lipoprotein lipase and apolipoprotein (apo A-I) A-V and simultaneously decreases the expression of lllc-apo in the liver. This process is known to be one of the inhibitors of atherosclerosis, which increases the expression of liver X receptor. In this regard, it has been reported that CT significantly increases PPAR after six and twelve months (26). In our study, LDL level decreased by 8.69% in the AT group and by 6.97% in the CT group. The increase in HDL was more profound in the CT group compared to the AT group (12.92% vs 6.18%).

Controlling lipid profile is essential for improving cardiovascular risk factors, which is very important for individuals with CAD. Cholesterol concentration can be reduced by taking fat-lowering drugs, which can reduce

risk of mortality following cardiovascular disease (27).

Regarding the effect of AT on reducing atherogenic factors, it can be argued that AT is associated with a decrease in body fat and LDL concentration as well as an increase in HDL. However, both environment and liver tissues and affect the increased activity of LCAT enzyme during short-term or long-term physical activity (28). These changes in lipid profile markers may be related to other mechanisms, including changes in lipoprotein lipase concentrations (29). The reduction of cholesterol biosynthesis decreases the intracellular cholesterol content. In hepatocytes, lowering cholesterol reduces the secretion of lipoproteins containing Apo B and increases the activity of LDL receptors, which both reduce blood LDL levels (30). Increasing the expression of the ABCG8 gene can also reduce the production of Apo-B, which is the main precursor to LDL production (31).

## CONCLUSION

Based on the results, CT may result in overexpression of the *ABCG1* gene via increasing the function of RCT in CABG patients. The results indicate that CT improves RCT function and expression of lymphocyte ABCG1 and reduces plasma LDL concentrations. A limitation of the present study was failure to accurately control the diet of the participants. However, they were instructed to maintain their previous dietary habits. Based on the results, performing AT as well as CT can improve muscle strength, functional capacity, blood pressure and lipid profile in CABG patients (27).

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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