

# The Effect of Combined Training Courses on the Expression of ABCG4 Gene and Interleukin-4 Plasma Level in Middle-Aged Men Undergoing Coronary Artery Bypass Grafting

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## Abstract

**Introduction:** Atherosclerosis (AS), as a significant cardiovascular disease, is the main factor of death in the world. There has been an association between plasma lipoproteins and AS. Reverse cholesterol transfer (RCT), in which ATP binding cassette (ABC) adenosine triphosphate transporters are major contributors, prevents AS incidence by reducing the accumulation of cholesterol on the walls of blood vessels. Our purpose was to investigate the effect of a combination training session on the gene expression of ABCG4 and plasma level of interleukin-4 (IL-4) in nucleated blood cells of middle-aged men after coronary artery bypass grafting (CABG).

**Methods:** The statistical population consisted of 20 middle-aged men who had previously undergone CABG. After selection, these individuals were randomly divided into two groups of n=10; control and combined exercise. At the beginning and end of training sessions, blood samples were obtained to isolate mononuclear cells and extract mRNA. Real-time polymerase chain reaction (PCR) was used for gene expression analysis, and IL-4 was determined by ELISA. SPSS software version 16 was used for data analysis.

**Results:** The training period remarkably increased ABCG4 expression and IL-4 plasma level compared to the control group.

**Conclusion:** Combined training, as a part of cardiac rehabilitation in those undergoing CABG may improve the RCT process by affecting the gene expression of ABCG4 and IL-4 production, which are involved in fat burning and metabolism.

**Keywords:** Combined training, Coronary artery bypass grafting, ABCG4, Interleukin-4

## Introduction

Atherosclerosis (AS) is the most common reason of death in industrialized countries, and it has been associated with plasma lipoproteins<sup>1</sup> because these lipoproteins trigger the deposition of cholesterol on the wall of vessels.<sup>2</sup> Phospholipids and cholesterol are key metabolic elements for the body; however, their excessive accumulation of cholesterol may lead to AS.<sup>3</sup> Reverse cholesterol transfer (RCT) is an anti-atherosclerotic process which is characterized with the accumulation of excess cholesterol from the surrounding tissues, such as arterial wall macrophages, and their return to the liver, along with

the formation of HDL.<sup>4</sup> ATP binding cassette transport (ABC) proteins, which belong to a superfamily of membrane proteins, contribute a prominent role in RCT.<sup>5</sup> This superfamily includes ABC-A to G groups<sup>6</sup>; of which the ABC-G group has five members (ABC-G1, -2, -4, -5, and -8).<sup>7</sup> ABC proteins play an important role in AS because they are essential players in cholesterol metabolism, vascular inflammation, modulation of endothelium function, regulation of blood pressure, and platelet differentiation and accumulation.<sup>8</sup> The G-type adenosine triphosphate transporter protein is involved in the RCT process in plasma and tissue cholesterol

metabolic pathways.<sup>9</sup> ABCG4 is similar in structure and function to ABCG1, which transports cholesterol from macrophages to the liver and regulates cerebral cholesterol metabolism.<sup>10</sup> During the RCT process, cholesterol is derived out of the intracellular space by some transporter proteins such as ABCA1, ABCG1, and ABCG4, and delivered to the protein which eventually results in HDL formation. ApoA1 is synthesized by the small intestine and liver and enters the bloodstream.<sup>11</sup>

Cytokines have been identified as the molecules produced and released by immune cells, triggering immune responses against pathogens.<sup>12</sup> There are two main classes of cytokines, including pre- and anti-inflammatory; while the first class is involved in the onset and continuance of inflammation, the second class, which includes interleukin-4 (IL-4), is released in response to inflammation, limiting and reversing the inflammatory process.<sup>13,14</sup> Elevated IL-4 is seen in chronic inflammatory conditions, contributing a main role in the progression of the disease. This cytokine has been shown to be highly elevated in atherosclerotic patients.<sup>15</sup>

The health benefits of exercise, especially its positive effects on cardiovascular function, have long been known; so far, no research has been done on the impacts of combined aerobic and resistance training in cardiovascular patients after coronary artery bypass grafting (CABG) on the expression of ABCG4 gene and IL-4 plasma level.

## Materials and Methods

### Subjects

The present study was a quasi-experimental research on 60 middle-aged men who had previously undergone CABG surgery in the Javad Al-A'meh Cardiovascular Hospital of Mashhad. As the control group, 20 male eligible volunteers in the age range of 50-60 years were chosen by the convenience sampling method.

### Exclusion Criteria

Patients with ventricular arrhythmias, myocardial infarction over the past four weeks, uncompensated heart failure, and unstable angina pectoris or any other restrictions for exercises were excluded.

### Inclusion Criteria

1. Systolic and diastolic blood pressure of <160 and <100 mm Hg, respectively.
2. Having cognitive, visual, and auditory health
3. Not using neuroleptics
4. Not using walking aids such as canes and walkers
5. At least 2 months passing since their operation
6. The operating capacity of at least five metabolic equivalent of task (MET), each Met representing 3.5 mL oxygen consumption per kg of body weight per minute, which was estimated based on the Bruce's modified test.<sup>16</sup>

### 7. Age of 50 to 60 years

Ten people in each group participated in this study. After screening, the subjects were randomly divided control (n = 10) and combined aerobic and resistance training (n = 10) groups.

### Exercise instructions

Tests included Timed Up and Go (TUG) (i.e., unassisted sitting down and standing up in 5 seconds),<sup>17</sup> Berg Balance Scale (BBS) (3-meter go and back),<sup>18</sup> and a cardiorespiratory function (6-minute walk) test.<sup>19</sup> Combined training, which included aerobic and resistance exercises, were performed simultaneously by patients. The subjects performed combined sports activities in the same environment and conditions.

### Aerobic Exercise

Patients in this group, during a 24-session period, exercised three days a week. Each session lasted 90 minutes. The training program included: walking on a treadmill (20 to 30 minutes), pedaling on a stationary bike (10 to 12 minutes), and using a manual ergometer (8 to 10 minutes). All members of this group performed the above exercises during each exercise session. In each session, stretching exercises were used to warm up at the beginning and gradual cooling down was performed at the end of the program. Exercises started with moderate intensity. Thus, in addition to the rate of fatigue and the occurrence of cardiac symptoms, 60% of patients' heart rates during exercise testing were considered as the target heart rate; according to which the duration and intensity of exercises were adjusted. The intensity and duration of training gradually increased according to the patients' abilities so that during the 7<sup>th</sup> to 10<sup>th</sup> sessions, the patients' heart rates reached 80%.<sup>20</sup> The details of the aerobic exercise program are listed in Table 1. Based on the patients' initial conditions and the results of the exercise test, the range of beats, the level and intensity of treadmill speed, and the resistance of manual ergometers and stationary bike were recorded for each patient on a sheet. Between the exercises, patients rested for 5 to 10 minutes, depending on their conditions.

### Resistance Training

The sport movements were performed for eight weeks (3 sessions/week) with eight repetitions in initial sessions increasing to 15 repetitions in two sets at late sessions. The movements included Scott with physio ball,<sup>21</sup> shoulder flexion,<sup>22</sup> shoulder abduction,<sup>23</sup> elbow flexion,<sup>24</sup> thigh flexion,<sup>25</sup> thigh abduction,<sup>26</sup> plantar ankle flexion<sup>5</sup> and Dorsey ankle flexion.<sup>6</sup> The movements were initially based on the individual's ability, body or limbs weight, and over time, with a weak Traband and finally with a very light weight.<sup>27</sup> The movements were initially performed with eight repetitions using a weak yellow Traband. Then during each session, two repetitions were

added to each movement to reach up to 15 repetitions. Then the power of Traband (pink) increased, and again the movements increased initially by eight repetitions and gradually to 15 repetitions in subsequent sessions. Details of the resistance training program are listed in Table 2.

### Blood Sampling and Laboratory Measurements

Forty-eight hours pre-intervention and 48 hours after the final exercise session, all the subjects underwent fasting blood sampling 5 mL (from the brachial vein into test tubes with EDTA anticoagulant. Plasma samples were used to determine IL-4 level using a specific ELISA kit (Cusabio ELISA Kits, China).

The separation of mononuclear cells was done using the Ficoll solution. Mononuclear cells were submerged in liquid nitrogen and crushed completely by a mortar and pestle for mRNA purification. In order to obtain mRNA, the damaged tissue was homogenized in buffer RLT, and then the tissue powder and liquid nitrogen were poured into a 2-mL RNase-free microcentrifuge tube, and the liquid nitrogen was allowed to evaporate while the lymphocytes stayed frozen. Sufficient buffer RLT was added. Lysate was transferred directly to the QIAshredder spin column in a tube and centrifuged at high speed for 2

minutes. After isolating mononuclear cells and extracting mRNA, gene expression analysis was performed using real-time polymerase chain reaction (PCR).<sup>28</sup> The sequence of the primers has been shown in Table 3.

In order to reduce some interfering and effective confounder factors in research and to curb the short-term effects of diet on the desired indicators, in this session, the subjects were asked, to refrain from eating fast foods as well as caffeinated beverages for at least 24 hours before exercising and blood sampling.

### Statistical Analysis

The Shapiro-Wilk test was used to determine the normality of data distribution, showing a normal distribution for the variables of age, height, weight, as well as ABCG4 gene expression and IL-4 level in the control and intervention groups at the pre-test and post-test stages. Means and standard deviations were used to describe individuals' characteristics. Paired *t* test was used to evaluate the differences in the levels of variables before and after training in each group. Independent *t* test was used to evaluate the differences in the levels of variables before and after training between the two groups. All statistical analyses were performed in SPSS (version

Table 1- Description of Aerobic Exercise Program

Protocol	Device Type	Variables*	Intensity	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Aerobic Exercise	Navigator	Intensity (%)	60-80	60	60	65	65	70	75	75	80
		Duration (min)	20-30	20	20	22	24	26	28	30	30
	Manual ergometer	Intensity (W)	30-50	30	30	35	35	40	45	45	50
		Duration (min)	8-10	8	8	9	9	9	9	10	10
	Stationary bike	Intensity (W)	30-50	30	30	35	35	40	45	45	50
		Duration (min)	10-12	10	10	10	11	11	11	11	12

\*The intensity of the sport activity on the treadmill (with variation of km/m/h) was adjusted to watts according to the heart rate obtained during the exercise and the intensity of the manual ergometer and stationary bike through the resistance applied to the device in watts. Control subjects did not perform any exercise for two months.

Table 2 - Description of resistance training program

Week	The Color of Traband	Exercises	Number	Repetition
First	Yellow		3	8
Second	Yellow	Foot movements Scott with a physio ball, Thigh flexion, Thigh abduction, Plantar flexion of the ankle,	3	10
Third	Yellow		3	12
Fourth	Yellow		3	15
Fifth	Pink		3	8
Sixth	Pink	Hand movements Dorsey ankle flexion, Shoulder flexion, Shoulder abduction, Elbow flexion	3	10
Seventh	Pink		3	12
Eights	Pink		3	15

Table 3. The Sequences of the Primers Used in the Study

Gene	Forward Primer	Reverse Primer
ABCG4	5'-CCGAGACCAGCCGCTTC-3'	5'-TCCCAAAGACTGGGCAACTAAG-3'
B-actin	5'-CCT ATG TTC TCA GCA GCT TC-3'	5'-GAA TTT CCT GGC TGT CCC TG-3'

16). A *P* value of <0.05 was considered as a statistically significant observation.

### Results

Baseline anthropometric characteristics of the participants are presented in Table 4. The mean age of the participants was 58.08 ± 3.5 years for the control group and 55.5 ± 3.6 years for the experimental group. The mean height was 173.5 ± 3.69 cm for the control group and 172.8 ± 3.5 cm for the experimental group. The mean weight was 76.16 ± 5.23 kg for the control group and 74.1 ± 6.4 kg for the experimental group.

Eight weeks of combined training significantly increased ABCG4 expression (*P*=0.001) and IL-4 level (*P*=0.001) compared to the control group. The results are demonstrated in Table 5.

### Discussion

The aim of the present study was to investigate the effects of combined (aerobic and resistance) training sessions on the gene expression of ABCG4 and IL-4 plasma level. The results of this study represented that combined training for eight weeks during cardiac rehabilitation significantly increased the expression of ABCG4 and plasma level of IL-4. In line, a study suggested that exercise can be a promising therapeutic strategy that can increase the functional capacity of patients with heart diseases.<sup>29</sup>

AS is a chronic inflammatory disorder linked with several inflammatory mediators.<sup>30</sup> Many inflammatory regulators, including macrophage-derived proteins and cytokines, play a key role in the progression and development of the disease.<sup>31</sup> All four members of the mammalian ABCG proteins (ABCG1, ABCG8, ABCG5, and ABCG4) participate in sterol membrane transportation.<sup>32</sup> ABCG4, ABCG8, and ABCG5 play essential roles in the RCT process.<sup>32</sup> The former (i.e., ABCG4) has been reported to be expressed in various human tissues such as the bone marrow, spleen, brain, eyes, and the liver.<sup>33</sup>

The recent studies performed on the effect of endurance exercise on ABCA1 gene expression showed increased expression of this gene in the liver and intestinal tissues.<sup>34</sup> Also, low-intensity exercise (eight weeks, walking 10,000 steps three times per week) significantly increased ABCA1 and ABCG1 genes' expressions.<sup>35</sup> In addition, ABCG1 and ABCG4 have been shown to be highly similar in amino acid sequence<sup>33</sup>; therefore, it is possible that the increase in ABCG4 gene expression in the present reflects elevated ABCA1 and ABCG1 following exercises. The overexpression of ABCA1, ABCG1, ABCG4, ABCG5 and ABCG8 genes predicts elevation of lipoprotein lipase, hepatic lipase, pre-beta HDL, and lecithin cholesterol acyltransferase, and increased levels of these markers can prevent the development of

**Table 4.** Demographic and Anthropometric Characteristics of Subjects

Variables	Groups	Pre-test		Post-test	
		Mean ± SD	<i>P</i>	Mean ± SD	<i>P</i>
Age (years)	Control	58.08 ± 3.5	0.364	-	-
	Combined training	55.5 ± 3.6	0.237	-	-
Height (cm)	Control	173.5 ± 3.69	0.661	-	-
	Combined training	172.8 ± 3.5	0.419	-	-
Weight (kg)	Control	76.16 ± 5.23	0.986	76.18 ± 5.23	0.824
	Combined training	74.1 ± 6.4	0.769	72.9 ± 6.2	0.635
ABCG4 expression (fold-change)	Control	0 ± 1	-	1.04 ± 0.47	0.29
	Combined training	0 ± 1	-	3.52 ± 0.68	0.67
IL-4 (pg/mL)	Control	2.4 ± 0.75	0.74	2.41 ± 0.8	0.96
	Combined training	2.45 ± 0.71	0.75	3.6 ± 0.9	0.32

**Table 5 -** ABC G4 and IL-4 values separately in different groups

Variables	Groups	Pre-test Mean ± SD	Post-test Mean ± SD	Changes			
				Within-Group		Between-Group	
				<i>T</i>	<i>P</i>	<i>T</i>	<i>P</i>
ABCG4 (fold-change)	Combined training	0 ± 1	3.52 ± 0.68	6.3	0.001*	5.021	0.001**
	Control	0 ± 1	1.04 ± 0.47	1.2	0.25		
IL-4 (pg/ mL)	Combined training	2.45 ± 0.71	3.6 ± 0.9	11.1	0.001*	9.47	0.001**
	Control	2.4 ± 0.75	2.41 ± 0.8	0.8	0.43		

Significance of within-group changes using paired samples t-test.\*

Significance of between-group changes using independent samples t-test.\*\*

cardiovascular diseases.<sup>36</sup> Alterations in cytokines' levels are not only seen in inflammatory diseases, but acute exercise also affects cytokine responses and inflammation in healthy individuals.<sup>37</sup> Furthermore, the physiological factors induced by exercise such as oxidative stress, heat, acidosis, and stress hormones can affect the release of cytokines.<sup>38</sup> In addition, cytokine responses may vary in type, duration, recovery period, and extent based on exercise frequency and intensity.<sup>37</sup>

So far, studies have been conducted to investigate the effects of physical activity on gene regulation mechanisms during the RCT process, particularly for the family of ABC transporters including type G proteins in animals.<sup>39</sup>

In a study, Zeiaadini Dashtkhaki et al examined the changes of ABCG8 in peripheral blood mononuclear (PBMN) cells following eight weeks of water and dry resistance training in middle-aged women following CABG and showed that both types of exercises induced similar optimal adaptations to ABCG8 gene expression, so this may directly prevent cholesterol deposition on coronary arteries' walls.<sup>40</sup>

In the study of Hosseini et al, it was shown that one session of aerobic and resistance training induced the gene expression of ABCG1 and ABCA1 genes, as well as HDL-C plasma level while decreased plasma LDL-C concentration in athlete women. These findings indicated the positive effects of both training methods in preventing AS.<sup>32</sup>

In another study, it was shown that a change in ABCG4 gene expression after exercise on a treadmill (8 weeks/5 days per week, 1 hour per day, 25 m/min) significantly altered plasma HDL-C level, and a significant correlation was observed between HDL-C fluctuations and ABCG4 gene expression in the small intestine and liver.<sup>9</sup>

Regarding the effects of exercise during cardiac rehabilitation, the study of Moosavi et al, on the effects of 24 sessions of combined training on ABCG1 gene expression in mononuclear cells in middle-aged males undergoing CABG significantly improved the RCT process, offering beneficial effects for patients with cardiovascular problems.<sup>41</sup> These results are consistent with the present study, suggesting a positive correlation between the overexpression of ABCG4 transmitter and exercise, improving cardiovascular function.

Ngo Sock et al, in their study investigated the expression of the ABCG5 gene in female rats following moderate-intensity aerobic exercise on a treadmill (6 weeks of incremental running at 15 m/s and a 0% incline for 15 minutes (2 weeks) to 60 min/d with the speed decreasing by 26 meters per minute with a slope of 10% (4 weeks) and five times a week).<sup>42</sup> They showed a decrease in ABCG5 gene expression that was not consistent with our findings. This may be due to differences in the nature of subjects, given that animals were used in the recent study, as well as the duration and intensity of training.

Also, Suzuki et al in a study in 2014 noted that endurance

exercise augmented plasma levels of IL-4 and IL-12 p40, which may suppress the cellular immune response and increase susceptibility to infections.<sup>43</sup> Schiøtz Thorud et al showed that moderate-intensity exercise positively regulated IL-4, -5, -6, -10, -2, tumor necrosis factor alpha (TNF- $\alpha$ ), and transforming growth factor beta in the rat's horseshoe muscle.<sup>44</sup> In general, IL-4 may be involved in AS pathogenesis through several pathways, including the modulation of lipoprotein metabolism by regulating lipoxygenase 15,<sup>45</sup> cluster of differentiation 36 (CD36),<sup>46</sup> and class A scavenger receptor.<sup>47</sup> By influencing the function of endothelial cells,<sup>48</sup> as well as macrophages and smooth muscle cells,<sup>49</sup> IL-4 can affect the course of inflammatory diseases via reducing the production of inflammatory cytokines.<sup>50</sup> Rahimi and Shoker Nejad reported a decrease in inflammation with an increase in IL-4 following resistance training.<sup>51</sup> These results were in agreement with our observations. Another study by Fu et al mentioned that six months of aerobic exercise (four times a week, 60 minutes per session) decreased serum levels of IL-4 and TNF- $\alpha$ .<sup>52</sup> However Boyd et al, noticed no significant shift in IL-4 and IL-5 serum levels following exercise in humans.<sup>53</sup> This difference may be related to differences in the type, intensity, and volume of exercises, as well as the nature of subjects.

Significant increase in ABCG4 gene expression and IL-4 plasma level in this study following a combined training session confirmed the antiatherogenic role of IL-4 through upregulation of ABCG4. Inflammation can downregulate HDL and suppress the RCT process. Structural modifications of HDL during inflammation leads to the production of acute phase HDL, which is relatively rich in apolipoproteins A-IV, serum amyloid A, triglycerides, and fatty acids. This is while anti-inflammatory enzymes (e.g., paraoxanase-1) and cholesterol esters are reduced. Furthermore, myeloperoxidase, an enzyme that converts apolipoprotein A1 and impairs its ability to absorb cholesterol, is increased during inflammation. Finally, inflammation negatively regulates the genes participating in the consumption, secretion, and excretion of cholesterol in the liver (e.g., ABCG5, ABCG8).<sup>54</sup> So, probably one of the mechanisms by which IL-4 increases ABCG4 is inhibiting the synthesis of inflammatory cytokines such as TNF- $\alpha$  and IL-1 by monocytes.<sup>55</sup> Therefore, physical activity seems to be a known and practical method to prevent cardiovascular diseases. In fact, moderate-intensity physical activity may have anti-inflammatory effects and modulate the production of inflammatory markers.<sup>56</sup>

## Conclusion

In general, it can be said that aerobic and resistance training can improve the RCT process by increasing the gene expression of ABCG4 and plasma level of IL-4 in middle-aged men undergoing CABG, offering many cardiovascular benefits. Thus, despite elucidating the

effects of exercise on ABCG4 transmitter and IL-4 level, it is necessary to conduct research to examine other key factors in cholesterol uptake from peripheral tissues in high-risk people such as those who suffer from AS or those with a history of open-heart surgery.

### Ethical Approval

This research was approved under the ethics code of IR.IAU.NEYSHABUR.REC. 1399,016 by Islamic Azad University, Neyshabour Branch.

### Conflict of Interest Disclosure

None.

### Authors' Contributions

AR and RKH conceived and designed this study. FY collected the materials, performed the experiments, analyzed the data and wrote the manuscript. ABY improved the quality of the paper.

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