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The Effect of Eight Weeks of Aquatic Aerobic Training on ABCA1 and ABCG1 Genes Expression in the Blood Mononuclear Cells in Women After Coronary Artery Bypass Grafting

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Abstract

Background: The aim of this research was to investigate the effect of aquatic aerobic training on regulatory factors related to Reverse Cholesterol Transport in women after coronary artery bypass grafting (CABG).

Methods: 24 middle-aged women were studied after coronary artery bypass grafting (12 were in control group and 12 in aquatic aerobic training group). The aquatic aerobic training program was performed in a pool of 1.20 m depth for eight weeks (three sessions per week with 50-75% intensity of the maximum heart rate). Furthermore, 48 hours before initiating the training program as well as 48 hours after the last training session, blood samples were taken in a fasting state. Then, Leukocytes were isolated, total cellular RNAs were extracted and complementary DNAs were synthesized. Gene expressions of ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette sub-family G member 1(ABCG1) were evaluated at messenger RNA levels using real-time PCR method. The amounts of ApolipoproteinA-1(Apo A-1), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were measured in plasma using an enzyme-linked immune sorbent assay method. Statistical analysis was performed using an independent-sample t-test and covariance, with a significance level accepted at $P < 0.05$.

Results: The findings showed that aquatic training was able to express ABCA1 and ABCG1 gene in women after coronary artery bypass grafting.

Conclusion: The data pointed to the possibility that aquatic training during the cardiac rehabilitation period can improve the reverse cholesterol transport and can be an alternative exercise program to achieve physical preparation and rehabilitation objectives in individuals who may have trouble doing exercises on the ground.

Introduction

Coronary artery disease (CAD) is one of the most common cardiovascular types of diseases, whose main cause of development is the formation of atheromatous plaque in the walls of coronary arteries, as well as atherosclerosis, and eventually coronary artery stenosis (1). Nevertheless, the clinical manifestations of this disease are different in men and women; the burden of atherosclerosis, as well as its resulting

mortality, is higher in women than in men (2). Among the factors involved in the development of coronary artery disease and atherosclerosis are gender (male), advanced age, high serum concentrations of lipid, hypertension, smoking, diabetes (or even mild degrees of glucose intolerance), obesity, low vital capacity, and certain ECG (Electro Cardio Graphy) (3).

Extensive studies have indicated that there is an inverse relationship between high-density lipoprotein (HDL) concentration and the risk of coronary artery disease. It has been estimated that every 1 mg/dl increase in HDL levels reduces the risk of coronary artery disease by 3% in women and 2% in men (4). HDL plays a significant role in the reverse cholesterol transport (RCT) process through which extra cholesterol is removed from the peripheral arteries and transferred to the liver (4). Indeed, one characteristic of atherosclerosis is accumulation of fats and cholesterol in the subendothelial space of arteries as well as intimal foam cells. Several ATP binding cassettes (ABC) have been identified which play an important role in the propagation and cellular flow of fats and cholesterol. Therefore, the impairment of some ABC transporters including ABCA1 and ABCG1 leads to increased atherogenesis. In contrast, purposeful activation of these mediators may provide new therapeutic options against atherosclerosis-induced cardiovascular disease (5).

On the other hand, apolipoprotein A-1, which is considered as the main protein of HDL (6), has been identified as a key regulator of cellular cholesterol propagation through ABCA1 available across the cell (7). ABCA-1 transports these materials to fat-free lipoproteins, causing the formation of primary HDL, and playing a significant inhibitory role in the development of macrophage foam cells which are important in the incidence and development of atherosclerosis. Further, ABCG-1 is responsible for transferring cholesterol to mature HDL (8).

Although a major part of treating cardiovascular disease consists of pharmacotherapy and following a special diet, in many patients, in order to resolve the problems caused by this disease, sometimes nonpharmacological methods including percutaneous coronary intervention (PCI) or even coronary artery bypass grafting (CABG) are called for. Although the cardiac function improves after CABG, this surgical operation alone is not sufficient for reversing the effects of this disease, which means that even patients who have undergone CABG could relapse into the disease symptoms if they make no changes in their lifestyles (9,10) .

Studies performed on coronary artery patients have also indicated the positive effects of physical activity following CABG (11). In addition to improving the symptoms of the disease, regular exercise leads to the amelioration of the physical, psychological, and social conditions of the patients (9). In this regard, Moholdt et al. reported that exercise following an operation can cause improvements in the cardiovascular function of patients along with their functional abilities (12). Adhering to exercise is of course affected by various factors including the type of exercise prescribed. It seems that organized exercises which are suitable and enjoyable and can be practiced easily can support increased participation of patients in long-term exercise programs. In this regard, exercises in water can be one of these methods (13).

Over the past two decades, aerobic exercise in water or even aquatic training has developed as an alternative exercise program to achieve physical preparation and rehabilitation objectives in individuals who may have trouble doing exercise on the ground. Extensive studies have indicated that exercise in water can contribute to the important components of physical preparation such as flexibility, muscle balance, muscle power, cardiovascular stamina, and reduction of fat percentage in patients as well as the elderly and disabled people. Therefore, it

has various positive physical and physiological effects (14). So far, few studies have dealt with the role of exercise and especially aquatic aerobic training in the cardiac rehabilitation period and the expression of the genes involved in the reverse cholesterol transport process (15-17). Most studies have investigated the role of exercise training on improving depression, functional capacity, strength, body composition, and quality of life of patients following CABG (18-23). According to previous research, at the time of coronary artery operation, women experience a worse situation than men and are more severely affected even after the surgery (23-24). Proper exercises at the time of cardiac rehabilitation significantly reduce these risks (25). By suitable exercise training during the rehabilitation period, these risks can be mitigated. Given the important role played by the factors involved in RCT in the course of atherosclerosis and cardiovascular disease (5,26), in the present research, the effect of aquatic aerobic training in women after CABG during the cardiac rehabilitation period was tested on the ABCA1 and ABCG1 genes expression, plasma levels of Apo-A 1 and the ratio of total cholesterol to HDL, bearing in mind that postoperative mortality is more probable in women compared to men (27).

Materials and Methods

Participants

The statistical population of the present research consisted of middle-aged women in Kerman who had previously undergone CABG and referred to Shafa Hospital in Kerman. Early examinations and interventions by a cardiologist for patients participating in this plan included comprehensive medical evaluation, initial cardiovascular examination, electrocardiography, echocardiography (40% to 50% injection rate) and exercise tolerance test to assess functional capacity. Maximum heart rate was determined and cardiovascular status was assessed, which was the basis for determining the duration and intensity of each session. Among them, 24 volunteers who had the inclusion criteria were chosen through available sampling method. They also used the same drugs. After completing the consent form regarding the procedure, they were chosen and randomly assigned into one of the two groups: the aquatic aerobic training group (n=12) and control group (n=12). Further, permission for conducting the research was obtained from the ethics committee of Mashhad University of medical sciences with the code of IR.MUM.FUM.REC.1396.08. Nutritional status and daily activity of the subjects were assessed by 24-hour diet recall, including a three-day food test and activities of daily living (ADL) scale, respectively. The exclusion criteria were unstable angina pectoris, decompensated heart failure, previous infarcts over the past month, ventricular arrhythmias that caused problems (19), or any other limitations as investigated by the relevant physician. Table 1 presents some of the primary anthropometric characteristics of the participants. Exercises started with moderate intensity so that 50% of the patients' maximum heart rate at the time of exercise was considered as the target heart rate for the patients, with the duration and intensity of the exercises accordingly adjusted. The intensity and duration of the exercises gradually increased with accordance to the patients' ability to reach 70% of their maximum heart rate during the last sessions.

Exercise instruction

Aquatic aerobic training

An aquatic aerobic training program was implemented in a shallow pool (depth: 1.2 m) with the water temperature at 28-30°C. The individuals first participated in a four-session briefing session to get familiar with the activities in the water. The aquatic aerobic training was implemented for eight

weeks/three sessions per week with 50-70% of the maximum heart rate for 1 hour per session. Each training session involved warm-up (10 min) with very low intensity in the form of activities like walking, ankle mobilizations, active shoulder, circumduction, pelvis, and wrist. These exercises were executed for different organs of the body for maximal preparation to do the main movements of the exercise. Five exercises performed in the lower limbs in a standing position were hip extensions and flexions (with extended knee), leg knee extensions and flexions, lower limb abductions/adductions, lateral side bounces, and calf raises. The five movements of the upper limbs included shoulder flexions/extensions, upper limb abductions/adductions, forward and lateral pushes, and shoulder abductions/adductions on transverse plane. These 10 movements constituted the main component of the exercise for the purposes of this study, lasting for around 45 minutes.

Table 1. Some of the primary anthropometric characteristics of the participants in each group (mean ± SD)

Variables	Weight (Kg)	Age(yrs.)	Height(m)
Aquatic aerobic training Group	54.2±3.61	87±3.43	159±6.09
Control group	55.6±4.06	82±4.40	158±4.82

The pressure of the exercise was exerted using movements shortening rest time between sets and increasing the number of repetitions from 8 to 10. In the design of exercises, attempts were made to cater for variety and enlivening exercises (28,29). Finally, about 10 minutes was devoted to cooling down, using 6 stretching statuses with the duration of 60 to 90 seconds, at the end of each training session. Muscular regions involved were shoulders, upper and lower back chest, hamstrings, and quadriceps. Stretching intensity was kept at moderate intensity, as suggested by the American College of Sports Medicine guidelines (30).

Laboratory measurements

24 hours before the study period, subjects had no physical activity. 48 hours before initiating the plan and 48 hours after the last training session, 10 ml of venous blood was taken from the brachial vein in EDTA anticoagulation tubes after overnight fasting (12 h) from all subjects. The samples were then centrifuged for 5 min at 2500 rpm, and around 0.5 ml of plasma was separated to measure the plasma levels of apo A-1, LDL, and HDL through the ELISA method. Then, leukocytes were separated using the ficoll from the residual of the venous blood present in the test tube. The total RNA and cDNA were synthesized immediately after leukocyte isolation, and cDNA and plasma were immediately stored at -70°C for analysis in the future. The subjects then underwent the exercise program, while none of them were allowed to have more physical activity exceeding the program set for them (29). Eight weeks after training, blood samples were taken just like before. Samples were taken for the analysis of plasma, leukocyte isolation, total RNA extraction, and cDNA synthesis and were saved thereafter. Gene expressions of ABCA1 and ABCG1 were evaluated at the messenger RNA (mRNA) levels using real-time PCR method. The amounts of plasma levels of apo A-1, LDL, and HDL were measured using enzyme-linked immune sorbent assay (ELISA) method, as described below (all laboratory methods were adapted from current protocols in molecular biology book).

RNA, cDNA synthesis, and real-time PCR

To extract total RNA, Trizol reagent (Bioneer, South Korea) was used. mRNA was extracted according to the manufacturer's instructions. The uniformity of RNA was confirmed by Agarose gel electrophoresis (1.5%). The RNA concentration and its purity were controlled by NanoDrop device (AmpliQuant AQ-07). The OD260/280 ratio was then calculated and values between 1.8 and 2 were defined as acceptable purity.

Next, using the oligo primers (dT) and synthesis kit of cDNA (Takara Co., Japan), cDNA was synthesized from RNA according to the manufacturer's instructions. The sequence of ABCA1 and ABCG1, as well as β. actin are presented in Table 2. The expression of the genes of interest was tested using real-time PCR via master mix containing SYBR green (Takar, Japan) in real-time PCR device (Corbet, Australia) using real-time PCR program including primary denaturations at 95°C for 5 min, 45 cycles including denaturation in each cycle at 95°C for 10 s, and annealing/ extension at 60°C for 30s. Note that β.actin was employed as the reference gene to measure the relative expression of genes and to control the specific proliferation of the product from the melting curve analysis. In the end, the expressions of genes before and after the exercise were computed using the $2^{-\Delta\Delta CT}$ formula.

ELISA method

In order to measure the plasma concentration of Apo A-1, LDL, and HDL via ELISA method (Enzyme-linked immunosorbent assay) which is based on colorimetry, an ELISA reader (Awareness Start Fax2100) was aligned with the specific instructions of the ELISA kit (R & D Systems, Minneapolis, MN, USA). The absorption of the samples was read at 450 nm, and eventually, based on the standard curve, the concentration of unknown samples was measured.

Statistical analysis

To measure the number of copies of the target and reference genes, the threshold cycle comparison method (Livak) was used (31). The data were then analyzed by descriptive and inferential statistics which were carried out using an independent-sample t-test (SPSS 23), where the significance level considered was $p \leq 0.05$.

Table 2. Oligonucleotide Primer Sequences and Real-time PCR Amplification Parameters Temperature

Gene	Forward and reverse primer sequences	Annealing temperature	Amplicon size (bp)
ABCA1	F.5-GCAAGGCTACCAGTTACATTTG-3 R 5-GTCAGAAACATCACCTCCTG-3	60 °C	205 bp
ABCG1	F.5- CAGGAAGATTAGACACTGTGG-3 R.5-GAAAGGGGAATGGAGAGAAGA-3	60 °C	177 bp
β-Actin	F.5-AGCCTTCCTTCCTGGGCATGG-3 R.5-AGCACTGTGTTGGCGTACAGGTC-3	60 °C	109 bp

Results

Data analysis revealed that differences between the pre-test and post-test scores of ABCA1 and ABCG1 genes were clearly expressed in leukocytes (Figure 1) (Table 3). Given that the calculated P-value turned out to be less than the significance level of 0.05, the null hypothesis of the research ($H0: \mu 1 \leq \mu 2$) was rejected, which meant that the mean difference between the post-test and pre-test of gene expression in the experimental group was significantly higher than in the control group.

Moreover, a significant difference was found between aquatic aerobic training group and the control group in APO-A1 and plasma HDL concentrations (Table 3). Therefore, it can be concluded that eight weeks of aquatic aerobic training affected the expression of these genes in leukocytes and plasma levels of APOA-1 and HDL in CABG patients. Changes in

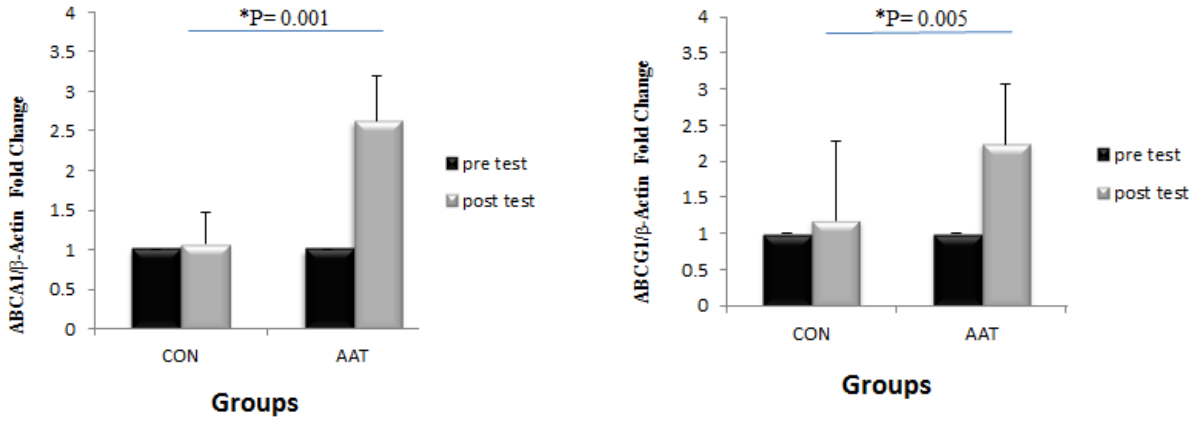
plasma LDL concentrations in response to aquatic aerobic training were not significant (Figure 2, Table 3). However, the results showed that training lowered the plasma LDL concentrations (Figure 2, Table 3).

Meanwhile, before making any statistical inferences about the effects, the validity of the statistical test was examined. According to the P-values calculated in the Shapiro-Wilk test, the assumption of normal distribution in the expression of the mentioned lymphocyte genes was not rejected, while in the Levene's Test, the assumption of equal variances between groups was rejected. In order to correct the degree of freedom, the independent-sample t-test was used for unequal variances.

Table 3. ABCA1 and ABCG1 gene expression and Plasma Variables Concentration (mg/dL) in the Control group (CON), and aquatic aerobic training (AAt)

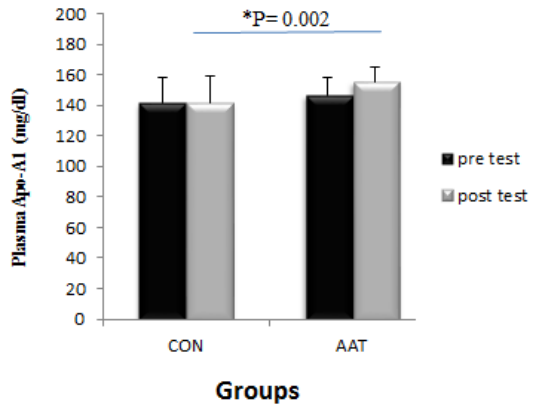
variable	Group	Pre-test	Post-test	t	df	Mean Difference	Std. Error Difference	p
ABCA1	CON	1	1.07±0.41	7.58	22	1.55	0.20	0.001*
	AAT	1	2.62±0.57					
ABCG1	CON	1	1.19±1/08	3.084	22	1.10	0.35	0.005*
	AAT	1	2.24±0.82					
ApoA-1 (mg/dl)	CON	141.5±16.44	141.23±18.28	3.64	18	9.16	2.51	0.002*
	AAT	146.75±11.35	155.64±9.63					
HDL-C(mg/dl)	CON	46±4.52	45.5±5.12	3.11	18	6	1.92	0.006*
	AAT	39.7±2.21	45.2±3.48					
LDL-C(mg/dl)	CON	67.4±14.75	69.4±16.93	-1.43	18	-3.8	2.64	0.16
	AAT	70.4±9.47	68.6±10.92					

Significant difference within and between groups: * $p < 0.05$

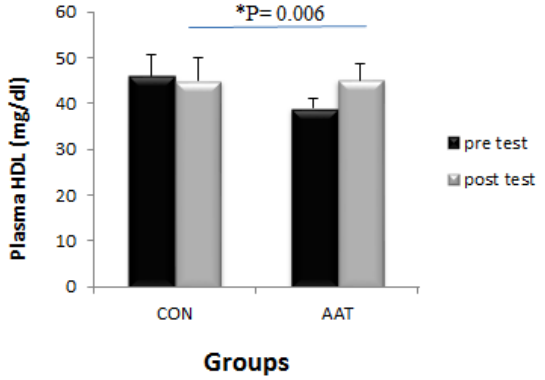


(a) (b)

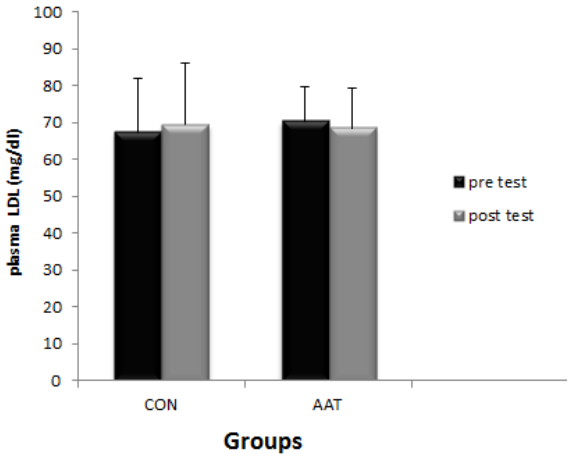
Figure 1. Bar graph showing the measured changes in gene expression before and after eight weeks of aquatic aerobic training on ABCA1 and ABCG1 genes expression in women after coronary artery bypass grafting (n = 12). The data were reported as mean ± SEM. * $p \leq 0.05$ was considered significant. a) Real-Time PCR of ATP-binding Cassette Transporter A1 (ABCA1) Relative mRNA expression, b) Real-Time PCR of ATP-binding cassette sub-family G member 1(ABCG1). (CON: Control group. AAT: Aquatic aerobic training).



(a)



(b)



(c)

Figure. 2. Apo A-1, HDL, and LDL were tested via the ELISA method in plasma before and after eight weeks of aquatic aerobic training. The plasma levels of Apo A-1 and HDL changed after training (p =0.002, p = 0.006). LDL level dropped after aquatic aerobic training in patients, but not significantly (p=0.16). Data are reported as mean ± SEM. p<0.05 was considered significant. (a) Plasma Apolipoprotein A1(APO-A1 (mg/dl)) Concentration , b) High density lipoprotein (HDL) (mg/dl)) Concentration, c) Low density lipoprotein (LDL (mg/dl)) Concentration. CON: Control group. AAT: Aquatic aerobic training. DRT: Dryland resistance training.

Discussion

Human studies suffer from many limitations in analyzing proteins and genes involved in the reverse cholesterol transport process. Therefore, most studies deal with the study of these factors through mononuclear cells of the blood including monocytes or lymphocytes (32). Some scientific evidence has suggested that the lymphatic system has a direct association with atherosclerosis (33-37). It has been reported that without a functional lymphatic system, the outflow of cholesterol from the plaque macrophages cannot be well guided outside the vessel wall, and cannot, therefore, be cleared (36). The mechanisms responsible for the interaction between lymphatic function and initiation or progress of atherosclerosis are still under research and are yet to be fully explained. Nevertheless, adventitial lymphoid vessels have been accepted as important modulators of cholesterol transport between atherosclerotic

lesions and bloodstream (7). Against this background, the results of the present research showed that the aquatic aerobic training led to upregulation of expression of ABCA1 and ABCG1, as well as plasma levels of ApoA-1 and HDL in CABG patients. The findings of the present research have been in line with those of some other researches (38-41). For example, Rashidlamir et al. investigated the effect of two months of exercise during the cardiac rehabilitation period in men who had undergone CABG. They went through 15 min of rapid walking on a treadmill, 10 min of exercise on ergometer bicycle, 10 min of exercise on the manual ergometer, and 5 min of cooling down. The researchers observed increased expression of the ABCA1 gene and HDL plasma levels (40). Furthermore, in a study involving a six-month program of cardiac rehabilitation based on exercise (including gymnastics followed by 30 min aerobic exercise using ergometer bicycle as well as 3-5 sessions of walking per week for 30-60 min), Koba et al. observed elevated plasma levels of Apo A-1 and HDL (41).

The most important goal of cardiac rehabilitation is improving the level of patient's daily physical activity such that in addition to reducing mortality, it can offer an active lifestyle with positive effects on the most important cardiovascular risk factors including hypertension, lipid profile, and body composition (42,43). One of the most prominent effects of physical exercise on preserving cardiovascular health is enhancing the RCT process by affecting the factors involved in this process (44). RCT is an important anti-atherogenic mechanism resulting from HDL function associated with several essential factors including ATP-dependent transporter Type A1 and G1 (ABCA1 and ABCG1), Apo-A1, lecithin cholesterol acyltransferase (LCAT) and some other factors (44). In addition to the important role of HDL in removing extra cholesterol from the peripheral vessels and its transport to the liver for clearing it off the body, its other beneficial biological characteristics including anti-inflammatory, antioxidative, and anti-thrombotic effects act as enhanced protective effects of HDL against cardiovascular disease (4). Accordingly, concerning the elevated plasma levels of HDL following the implementation of aquatic aerobic training in the present research, it seems that the role of these exercises in the cardiac rehabilitation period should be more carefully investigated for patients undergoing CABG. On the other hand, in the present research, LDL levels dropped in aquatic aerobic training, though this reduction is not significant, and can be due to the intensity of the exercises. Scientific evidence suggests that moderate-intensity aerobic exercises can significantly elevate HDL levels. However, for reductions in triglyceride levels and LDL, higher intensities of aerobic exercise might be more suitable. Note that to enhance the intensity of aerobic exercise in individuals who have exercise capacity limitations or have other risk factors, more caution should be exercised (45). For example, O'Donovan et al. controlled the volume of exercise and examined the effect of intensities of 60% and 80% of the maximum oxygen uptake/three sessions per week for 24 weeks on the levels of the lipid profile of sedentary men. They concluded that total cholesterol and LDL levels significantly decreased only in the high-intensity aerobic exercise group (46).

This study also found evidence supporting elevated plasma levels of ApoaA-1 and expression of the ABCA1 and ABCG1 lymphocyte gene following exercise. ApoA-1 has been known as a key regulator of the outflow of cholesterol through the ABCA1 receptor available across the cell (47-49). Although other apo-proteins such as AI, AII, AIV, CI, CII, CIII, and E also

have the ability to provoke cholesterol outflow, lipid deficient Apo-AI seems to be the major receptor of lipid outflow via ABCA1 (50).

One of the possible mechanisms for the overexpression of ABCA1 due to exercise might be associated with the upregulation of some nuclear receptors involved in fat metabolism in the RCT process including liver X receptor (LXR) and peroxisome proliferation activating receptors (PPARs). These receptors regulate the expression of some members of the ATP-binding transporter family including ABCA1 (50). Various animal and human studies have tested the effect of aerobic and resistance exercises on these receptors, reporting the overexpression of these genes (45,51-53). In this regard, Zeiaadini Dashtkhaki et al. investigated the effect of eight weeks of resistance training in both water and on the ground and found overexpression of PPAR-α of mononuclear cells in women undergoing CABG (16). Moreover, Kazeminasab et al. examined the effect of four weeks of aerobic exercise with an intensity of 28 m/60 min per session 5 days per week and observed overexpression of LXR (45). In the present study, the effect of physical activity on the expression of these receptors was not tested. Nevertheless, based on the results of previous studies, some of which were mentioned, it can be deduced that physical exercise, possibly through positive regulation of PPAR receptors, causes upregulation of LXR, thereby enhancing ABCA1 gene expression (50). Furthermore, given the role of ABCA1 in the HDL synthesis process (54), it seems that its overexpression also affects the increased production of HDL. The results of the present research showed significant difference in the expression of the ABCA1 and ABCG1 genes and HDL levels in aquatic aerobic training group. This could be due to the nature of aerobic exercise especially the implementation of movements in the water and, possibly, greater sense of joy and freshness, better motivation of subjects, better execution of the exercises and, therefore, greater effectiveness of aquatic aerobic training. In this regard, various studies have indicated that exercise in water can lead to improved components of physical preparation such as flexibility, muscle balance, muscle power, cardiovascular stamina, and reduction of fat percentage in patients as well as in the elderly and disabled people, all of which offer positive physical and physiological effects and important gains (55). On the other hand, some researchers have proposed that physical exercise, especially aerobic exercise, enhances AMP-dependent kinase expression (AMPK) and PGC1-α, where the interaction of these two can activate PPAR nuclear receptor. When the PPAR is activated, it moves towards the nucleus and binds to its promoter region, leading to transcription of genes associated with oxidative metabolism as well as lipoprotein transporters such as ABCA1, which may lead to improved RCT process (32). On the other hand, enhanced activity of PPAR can also increase the expression of lipoprotein lipase and Apo A-1 (56). All these factors support the prevention of atherosclerosis progression or relapse of the disease in individuals undergoing CABG, possibly through facilitating and enhancing the RCT process.

Conclusion

Generally, the results of the present research showed that the implementation of aquatic aerobic training during the cardiac rehabilitation program resulted in elevated HDL and ApoA-1 plasma concentration as well as overexpression of the genes involved in the RCT process in female patients undergoing CABG, thereby improving RCT. Thus, since

patients undergoing bypass surgery may experience a relapse of the disease, it seems that during the cardiac rehabilitation period, proper exercise thanks to its positive effects on the factors affecting the RCT would contribute to cardiovascular protection through preventing the relapse or progress of atherosclerosis process. In turn, it can protect those undergoing CABG against the consequences of the disease, especially its potential resulting mortality.

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