Effect of Eight Weeks of Cardiac Rehabilitation Training on PPAR-alpha Gene Expression in CABG Patients

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ABSTRACT

Background and Objectives: Heart disease is a leading cause of death in today's society. This study examined the effects of cardiac rehabilitation exercise on lipid profile and expression of PPAR-α gene in patients with CABG.

Methods: this is a quasi-experimental study and the research sample was selected after screening among CABG patients randomly divided into two groups: control (n=12) and experimental (n=12). The experimental group would do the rehabilitation exercises for two months, after the surgery and passing the period of hospitalization. The control group was discharged after surgery and passing the initial phase of rehabilitation. To study the biochemical variables as well as measure PPAR-α gene expression of lymphocytes, the blood samples were collected before and after the last training session, while all subjects were fasting. To measure the gene expression of PPAR-α, qRT-PCR method was used. Statistical analysis was done using repeated measures by the SPSS software, Version 20.

Results: In following up of a two-month cardiac rehabilitation exercises, a significant increase in PPAR-α gene expression and plasma HDL levels in the training group was observed compared with the control group (p<0.05). But concentrations of LDL and triglycerides in the rehabilitation group were decreased, compared to the control group; however this reduction was not statistically significant (P> 0.05).

Conclusion: This study showed that conducted protocol by the researchers for cardiac rehabilitation can be used as a strategic way to improve lipid profile and improve cardiovascular function in CABG patients have a higher risk of cardiovascular disease heavily.

Key words: Cardiac Rehabilitation, Gene Expression, PPAR, open heart surgery

INTRODUCTION

Studies show that Cholesterol and triglycerides congestions and decreased HDL-C levels are factors that predispose individuals to involve in disease of atherosclerosis. On one hand, the machinery life which pushes the people to the inactive lifestyle exacerbates these diseases. Prevalence studies where weight loss with diet and exercise would be used, suggests that each increase in HDL-C unit and decrease in LDL-C help to improve the functioning of the cardiovascular system and prevent diseases associated with it. HDL particles as well as the antioxidant and anti-inflammatory role are effective in the prevention of cardiovascular disease through the reverse cholesterol transport(1).

The reverse cholesterol transport is called by the collection process of excess cholesterol from peripheral tissues, including macrophages in the artery wall and returning it to the liver, along with deformation to HDL (2). Since low levels of HDL is usually one of the risk factors for atherosclerosis, raising levels of HDL and its positive effects on coronary heart disease has always been a concern to the researchers. Strategies to increase HDL levels include lifestyle changes such as incorporating regular exercise, quitting smoking, weight loss and a diet is of lower in saturated fatty acids (3).

Peroxisome proliferation-activated receptors (PPARs) would be a member of nuclear receptors family that binding to fatty acids, regulate gene expression and determine the cell fate (4). Having PPAR-alpha activated, key genes occur that are involved in the metabolism of HDL and reverse cholesterol transport process (5).

Although studies have shown that physical activity can lead to improvements in some key steps of the process of reverse cholesterol transport (6), so far there are few studies on the impact of physical activity on the expression of receptor gene PPAR-alpha which was the stage upstream and key in the process of reverse cholesterol transport and its function as a transcription factor is one of the key factors in
metabolism and maturation of HDL-c (7, 8). Also checking out this seems essential in human samples and clinically high-risk. This study is also designed considering the important role of physical activity and regular exercise for a public health to conduct a scrutiny at genomics level in the process of reverse cholesterol transport. Recent studies show that Physical activity increases HDL size and make HDL species more buoyant. Exercise also appears to increase apolipoprotein composition. All these would facilitate the RCT process (9).

This study investigated the role of exercise on the PPAR-alpha gene expression in quasi-experimental method and the exercise protocol performed on CABG patients and the objective is to examine the possible effects of exercise in protecting the cardiovascular system by relying on the trends of reverse cholesterol transport pathway, headed by increased PPAR-alpha in high-risk groups. With more accurately understanding of PPARs and the concurrent role of and exercise, it can be rather than one-sided medicine treatment, the complementary and exercise treatment are taken advantage to maximize the cardiovascular function improvement of the individuals.

**MATERIAL AND METHODS**

The study was quasi-experimental with control group. Sampling was of the available one and patients and the subjects are who already have coronary bypass surgery and were homogeneous in terms of disease level and are relative readiness to do exercises in terms of physical strength and at least one month had passed since their surgery. Exclusion criteria from the study of heart failure included the permanent defibrillator/pacemaker, heart failure, history of hernia or aneurysm disease, history of regular exercise and physical disabilities that limit the movement of the treadmill or ergometer,. After screening subjects () were randomly divided into control group (n=12, age= 52.83±1.33) and rehabilitation group (n=12, age=54.66±1.30). Interventions in the study were approved by the ethics committee of Javadolaemeh Hospital of Mashhad and written consent was received from all participants of the study.

24 hours before the first practice session and 48 hours after the last training session, of all subjects in two groups who are fasting, 10 cc blood samples were taken from the brachial vein. Considering similarity to the time before and after sampling; the subjects would be asked to be present at 8 am at the sampling site and in both stages of sampling, the sampling began at 8 o'clock and ended at 9:30 am. To equalize the night diet before sampling, the regime suggested by the researchers have been prepared for them. Part of the blood samples related to the gene expression were collected in test tubes with EDTA anticoagulant and transferred to biotechnology laboratory of Ferdowsi University of Mashhad, isolated lymphocytes were carried out by ficole technique at this stage. As well, to measure the biochemical variables, samples were collected in tubes containing EDTA and centrifuged (at 3000 rpm for 10 min), respectively. To measure HDL-c and LDL-c levels and triglycerides amounts were used PARSAZMUN® (Iran) kits.

In this study, total RNA using guanidine thiocyanate kit (Roche, Germany) was extracted from blood samples. After that, 1 microgram RNA was isolated, for producing first cDNA related to oligo (dT) primer, by AccuPower® RocketScript™ RT (Bioneer, Korea). qRT -PCR method was used To calculate the relative expression of PPAR-α gene by QIAGEN device, with a TaqMan probe. Cycle program will be as follows:

5-minute initial denaturation at 95 °C followed by 40 cycles of temperature, 20-seconds slow annealing was considered for each stage. Device for reading the fluorescence signal was set at the extension stage. PCR reactions will be performed in triplicate in a final volume of 20μl. Finally, mRNA expression level of PPAP-α gene than GAPDH was obtained. Gene primer sequencing used is shown in Table 1.
Circular rehabilitation program four began weeks after the surgery, and lasted two months. Each week consists of three one-hour sessions of aerobic training with treadmill, stationary bike and handy ergometer. In addition to monitoring heart rate, before each exercise session, each subject was connected to ECG leads. In the study, to control the intensity of exertion, the scale of perceived exercises of the "Borg" was used (10). Before performing the practices, the concept of Borg was explained to the participants and they were asked to keep the intensity rate of their activities between 11 (relatively light) and 13 (somewhat hard).

The rehabilitation group in this study during a training session became familiar with the devices and practices and then exercised three times a week for two months. Each practice session includes a 5-minute warm-up, 15-minute walking fast on the treadmill, 10 minutes of stationary bike and finally 10-minute handy ergometer and each takes a 3-minute non-active rest between every device. The subjects’ pressure is examined continuously by the researchers under Borg scale. If Borg’s value was announced fewer than 11, the subjects were asked to make more efforts and if higher than 13, they were asked to reduce the pressure, also whilst doing exercises, the subjects connected to the ECG leads for heart rate monitoring. The control group had no physical activity for two months.

To describe the data, the indicators of mean and standard deviation were used. Having ensured normal data, using the Kolmogorov-Smirnov test to compare the variance between groups, "repeated measurements with between-group factor" was used. Data were analyzed using SPSS software version 18 and diagrams were drawn by Origin software depiction version.

RESULTS

Evidence suggests a significant increase in levels of gene expression PPAR-alpha in training group rather than control group (p=0.0006, F=19.407). The levels of HDL-C increased significantly (p=0.034, F=5.520). In addition, the amount of LDL-C (p=0.191, F=1.890) and TG (p=0.113, F=1.611) in training group reduces, while, this changes did not significant compare to the control group. This results show in table 2 and figure 1.
Table 2: Compare dependent variables the study in two groups before and after the study protocol

| Variables          | Groups          | Stage   | Mean±SD     | Paired t test | Repeated Measures |
|-------------------|-----------------|---------|-------------|---------------|-------------------|
| PPAR-alpha/GAPDH  | Control (n=12)  | Pre-test| 0.908±0.268 | t=0.320       | F=19.407          |
|                   |                 |         | 0.745±0.238 | p=0.759       | P=0.0006**        |
|                   | Training (n=12) | Pre-test| 0.970±0.492 | t=5.766       |                   |
|                   |                 |         | 2.209±0.683 | p=0.001**     |                   |
| HDL-C (mg/dl)     | Control (n=12)  | Pre-test| 45.75±1.76  | t=1.121       | F=5.520           |
|                   |                 |         | 43.87±1.41  | p=0.300       | P=0.034*          |
|                   | Training (n=12) | Pre-test| 43.37±1.63  | t=2.447       |                   |
|                   |                 |         | 46.37±1.91  | p=0.044*      |                   |
| LDL-C (mg/dl)     | Control (n=12)  | Pre-test| 61.75±2.80  | t=0.824       | F=1.890           |
|                   |                 |         | 63.77±4.66  | p=0.437       | P=0.191           |
|                   | Training (n=12) | Pre-test| 58.25±2.92  | t=1.701       |                   |
|                   |                 |         | 55.62±2.11  | p=0.133       |                   |
| TG (mg/dl)        | Control (n=12)  | Pre-test| 98.13±11.23 | t=1.775       | F=1.611           |
|                   |                 |         | 102.00±10.10| p=0.274       | P=0.113           |
|                   | Training (n=12) | Pre-test| 92.88±12.32 | t=1.412       |                   |
|                   |                 |         | 89.91±13.33 | p=193         |                   |

* significant p<0.05
** significant p<0.001

Figure 1: The levels of plasma PPAR-alpha expression in both groups before and after the study protocol
Figure 2: The levels of plasma HDL-C levels in both groups before and after the study protocol.

Figure 3: The levels of plasma LDL-C levels in both groups before and after the study protocol.
DISCUSSION

The main finding of this study is an increase in PPAR-alpha gene expression of lymphocytes in cardiac rehabilitation patients as the result of eight-week doing exercising. Although in the past a number of fundamental articles have repeated PPAR-alpha gene expression in multiple tissues of animal and humans models but there are few studies on the effect of exercise on the expression of these genes. The results of this study also showed a significant increase in HDL-C among rehabilitation training group, but no significant changes in LDL-C and triglyceride. Increased HDL-C is important because a role plays in reverse cholesterol transport. Previous studies have shown that PPARs receptors play a key role in two physiological functions, the first, its contribution to control the capacity of beta-oxidation of fatty acids and second, in reverse cholesterol transport (11).

McNeil et al investigated the effect of rehabilitation exercise combined with muscle rehabilitation on the capacity of the mitochondria Bayogens. In this study, 18 young subjects after passing two weeks of knee cast, they did exercise six weeks and three days a week, combined resistance-and-endurance practice. The training significantly increased the expression of genes involved in mitochondrial Bayogens (PGC-1α, PRC, PPAR α) and the capacity of the electron transport chain, too (12). Tantalite al examined the effects of nine days of cycling practice on factors of beta-oxidation of fatty acids, including α - PPAR and reported similar results (13).

In the field of effect of physical activity on lipid profile, Schwartz et al shows Following the 6 months of intensive endurance exercise (5d/wk) HDL-c increase (14 %, \( P = 0.01 \)) in healthy young men. In the older men, training was associated with a similar increment in HDL-c (15% \( P < 0.001 \)). A 21% decrease in plasma triglyceride concentration (\( P = 0.02 \)) and a 13% decrease in the ratio of LDL-c to HDL-c (\( P = 0.02 \)) were also observed only in the older subjects(14). Similar results were obtained in the research of park et al on obese middle-aged women (15).

The researchers have shown that a combination of aerobic/resistance exercise significantly increased PPAR-α after six months, and this is despite the fact that this gene of the present study only increased after eight weeks (16). Boucher et al. examined the effect of 8 weeks of low-intensity exercise on leukocytes LXR and PPAR gene. The results showed that the expression of these genes, as a result of the...
practice. They also suggested that PPAR ligands activating will lead to the initiative activation of LXR and finally LXR causes to both regulate positively and increase ABCA1 gene expression in both transmitters of ABCG1 and ABCA1 and all these factors lead to an increase reverse cholesterol transport process. The results of this study also suggest increasing the amounts of HDL-C and decreasing LDL-C plasma levels in the experimental group than the control group (7). Doorestin et al reviewed the effects of different protocols of exercise on levels of HDL and LDL within the plasma. Similar protocols under training program of the research have relatively similar effect on HDL and LDL levels (17)). This is so far unknown that the exercise by what mechanism can increase PPAR-alpha gene expression in leukocytes and macrophages, but there are possible mechanisms that we discuss about them. It was suggested that the regulative attribute of fatty acids is applied by PPARs. PPARs like LXR and RXR also were found to be nuclear receptors that adjust the expression of genes controlling lipid and glucose metabolism. Three isoforms form of PPARs (α, β, γ) are stated in metabolic tissues, including heart, liver, skeletal muscle, kidney and blood vessel cells such as Monocytes and macrophages (18, 19).

With the activation of PPAR-alpha, as a result of rehabilitation exercises, it is likely to take place expression of key genes that are involved in the metabolism of HDL and reverse cholesterol transport process and eventually leads to the protein making. These proteins are Apo A-I, Apo A-II, lipoprotein lipase, SRB1 and ABCA1. PPAR also by speeding up the release of cholesterol from peripheral cells and that is picked up by the liver, gives rise to facilitate reverse cholesterol transport and increase the synthesis of HDL (5).

**CONCLUSION**

This study is the first direct report which shows that eight weeks of cardiac rehabilitation exercises increase PPAR-alpha gene expression within the lymphocytes and this is associated with plasma HDL-C levels. The results show that protocols carried out for cardiac rehabilitation by the researchers can be utilized as a strategic way to improve HDL levels and improve cardiovascular function in CABG patients have a higher risk of cardiovascular disease.

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