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The Role of Ghrelin in Blood Pressure Reduction Induced by Exercise in Patients with CABG

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ABSTRACT: The effect of exercise on cardiovascular function was one of the main interested topics of researchers in the field of exercise physiology in the last decade and it also is on the focus of many researchers right now. The researchers in the present study are looking for examining the potential role of exercise-induced ghrelin leakage on cardiovascular efficacy in the CABG patients. 16 CABG patients were chosen by the Available sampling method and randomly assigned in two groups of cardiac rehabilitation and control groups in the javad al-aemeh hospital. Ghrelin gene expression in lymphocytes, arterial stiffness, brachial-ankle index and the mean blood pressure were measured.

The results showed that cardiac rehabilitation was the reason of a slight increase in ghrelin gene expression, but it was not significant (p=0.185 and F=1.68). Also Brachial-ankle index that reflects the efficacy of peripheral arterial function, as a result of rehabilitation exercises showed a significant improvement (p=0.041 and F=3.44), and the mean blood pressure in the rehabilitation group decreased significantly (p=0.0008 and F=7.64).

Researchers clearly showed improvement in blood pressure and peripheral vascular resistance as a result of rehabilitation training in CABG patients in present study. Even though several mechanisms are proposed for ghrelin dilating effect, but changes in the blood pressure were independent of ghrelin in this study.

Keywords: Ghrelin, Lymphocytes, Rehabilitation Training, Arterial Stiffness

INTRODUCTION

The effect of exercise on cardiovascular function was one of the main interested topics of researchers in the field of exercise physiology in the last decade and it also is on the focus of many researchers right now. Cardiac rehabilitation is a supervised exercise program with the overall objective of improving the quality of life. This program is designed to limit the mental and physical effects of cardiovascular diseases, reducing the risk of sudden death or re-stroke to stabilize or reverse the atherosclerosis trend and it is typically recommended to the people who have done a coronary artery bypass surgery. Cardiac rehabilitation is not only useful and effective for people with heart failure but also it is a way for all people who are suffering from of obesity, hypertension and diabetes or smokers. Researching in the field of the positive effects of cardiac rehabilitation exercises on cardiovascular function in CABG patients can map bright horizons for mortality control and quality of life improvement by providing new information for these patients.

The reviewed studies of researchers show that few objective researches is conducted in this field in Iran and other countries.

Ghrelin peptide, with 28 amino acids, is a peptide secreted by the stomach that was discovered in 1999 first one. The authors reported the existence of ghrelin in other tissues such as intestine, pancreas, pituitary, kidneys and placenta (Katugampola *et al.,* 2002). After discovering Ghrelin, it was observed that this substance is effective on appetite, energy consumption, weight and body composition that all of these cases helps to regulate energy balance of the central body. Apart from the ability to stimulate of growth hormone releasing and the regulatory effects on appetite and body metabolism, the evidence increasingly reveals that ghrelin has direct independent of GH effects on the cardiovascular system. The reason of this hypothesis is existence of Ghrelin and GHS-R in the heart. It has showed that ghrelin has a protective effect in cardiovascular system by inhibiting and apoptosis of endothelial cells and it protects the left ventricle during heart tissue damage on the stage of pre heart attack.

Ghrelin produced dilating effect in blood vessels. This may be due to the anti-inflammatory effect of ghrelin on the arteries which prevents from progression of arteriosclerosis. Previous studies suggests this point that ghrelin regulates vascular resistance. In summary ghrelin protects heart function and reduces the vascular resistance in patients with chronic heart failure. Hathory and colleagues showed R-GHS and ghrelin gene expression in all immune cells that the gene expression was different for everyone, and it was shown that ghrelin gene expression is greater in lymphocytes. And they concluded that the wide distribution of ghrelin and GHS receptor in human immune cells may indicate other unknown biological functions in addition of increasing GH secretion in the human immune system (Kojima *et al.,* 1999, Hattori *et al.,* 2001). The effect of physical activity on ghrelin secretion at the levels of genomics and Proteomics has been tested and shown that training causes increase in ghrelin gene expression and its levels in plasma. Therefore the present study was designed to investigate the effects of cardiac rehabilitation on ghrelin gene expression in patients with CABG to determine that whether this consistent occurs in this particular society which are expose of greater risk of cardiovascular disease also, and leads to take its advantages or not.

The main role of the complex of Ghrelin - GHSR is dilating the blood vessels. Moakley and colleagues showed that ghrelin injection to healthy subjects decreased the mean blood pressure without changes in heart rate. They stated that ghrelin implements its Hemodynamic properties independent of GH in reducing the workload of the heart. Similar results were reported in subsequent studies. Another effect of ghrelin is increasing in cardiac output. Bacy and colleagues were observed the injection fraction increase of the left ventricle as a result of the injection of ghrelin in healthy subjects and patients with HDL deficiency. These changes were independent of blood pressure reduction. Newer studies also have demonstrated positive effects of ghrelin on improving cardiac output (Katugampola and Davenport 2003, Bisi *et al.,* 1999, Nagaya and Kangawa 2003).

It has been shown that the Protective effects of ghrelin are in inhibition of apoptosis of the heart and endothelial cells. The studies have shown that ghrelin applies its anti-apoptotic properties through the Mpk and PI3K / Akt. Newer investigations have also reported anti-apoptosis properties in other tissues such as the endothelial cells and pancreas cells. So far Researches which examined the effect of exercise (one session / long-term) on the ghrelin amounts mostly focused on aspects of metabolism of this peptide, and they investigated its changes on appetite, weight gain and insulin homeostasis scientific knowledge around the impact of physical activity on the Ghrelin – GHS complex and the secondary effects of cardiovascular protection is very little and uncertain (Granata *et al.,* 2007, Ghanbari-Niaki *et al.,* 2011, Hedayati *et al.,* 2012, Ukkola *et al.,* 2012).

Also the majority of studies have reported the constructive role of ghrelin on the cardiovascular system benefited clinical injection method, and there is no study which has paid to direct effect of exercise in induction of effects of ghrelin in the cardiovascular system especially about hemodynamic benefits. The researchers in this study have attempted to examine the potential role of ghrelin secretion resulted by exercise on induction of cardiovascular productivity in the risky society of patients who have CABG.

METHODOLOGY

The present study is accounted such a semi experimental research with a control group. Sampling was by applying the available sampling method and the subjects were patients who had done a coronary bypass, they were homogeneous from the viewpoint of disease level, they had the adequate fitness for doing their exercises in terms of physical stamina, and they had to past at least one month after their operation time. Exclusion criteria of the study were congestive heart failures, the pacemakers / permanent defibrillators, volvuli diseases, hernia or aneurysms and physical disabilities that limited the activity on the treadmill or ergometer, respectively. Individuals after screening were randomly divided into control group (n = 8) and rehabilitation group (n = 8). An intervention of The study was approved by the ethics committee of Javad-al-Aemeh hospital of Mashhad and written consent was received from all participants.

A. The exercise protocol

Circular rehabilitation program lasted two months. Each week consisted of three one-hour sessions of combined aerobic exercise with treadmill, stationary bike and manual ergometer devices. The subjects' Heart rate was monitored during training, and to control the intensity of exercises perceived exertion scale of "Borg" was used (Borg, 1990). The concept of Borg was explained to the participants in the study before starting exercises and they were asked to hold the intensity rate of their activities between 11 (fairly light) and 13 (somewhat hard). Also, an expert has changed the difficulty rate of the devices to more or less to remain at the desired range according to the perceived exertion of each subject. Each exercise session was including a 5 minute of warm up, 15 minutes of brisk walking on the treadmill, stationary bike for 10 minutes and then 10 minutes manual ergometer performance as well as a 3-minute non-active rest between each stage.

The assessment of brachial-ankle Indicator and the average blood pressure. Continuous-wave Doppler device was used to assess brachial-ankle Indicator and pressure gauges with a blood pressure cuff were used also. As well as the following formula was used for the subjects of average blood pressure.

Mean arterial pressure = 2/3 diastolic pressure + 1/3 systolic pressure

B. Blood samples Collecting

all subjects in both groups had been taken 10 ml of the brachial artery blood for sampling which they were in fasting state since 48 hours before and 48 hours after the first exercise session. The participants would be asked to be present at the site of sampling at 8 am for similarity of pre and past time of sampling. Sampling started at 8 o'clock and finished to 9:30 am for the both phases. Gene Blood samples collected in test tubes by using EDTA anticoagulant and transferred to biotechnology laboratory of Ferdowsi University of Mashhad. The separation of lymphocytes was done by ficole method at this step. To assessment of ghrelin gene expression amounts qRT-PCR method was used.

C. The process of qRT-PCR

Total RNA was extracted from blood samples using the guanidinium thiocyanate kit (Roche, Germany). Subsequently, 1 microgram isolated RNA was used to produce primer cDNA related to oligo dT built by AccuPower ® RocketScript ™ RT (Bioneer, Korea). To calculate the relative ghrelin gene expression the qRT -PCR method by the QIAGEN device with TaqMan probes was used. The cyclic program was as follows: initial denaturation at 95°C for 5 minutes followed by 40 cycles of temperature and 20 seconds slow annealing for each stage. The device was set to read fluorescence signal at extension stage. PCR reactions were performed in triplicate in a final volume of 20µl. Finally, levels of ghrelin mRNA gene expression has obtained compared to GAPDH respectively. The sequences of used primers are shown in Table 1.

Table 1: The sequences of used genes in the study.

Hum-GAPDH-F	GGAAGGTGAAGGTCGGAGTC-3'-5'
Hum-GAPDH-R	CGTTCTCAGCCTTGACGGT-3'-5'
-F Hum- Ghrelin	CAAGGGGTAGGAGAAAGAGACGC-3'-5'
Hum- Ghrelin -R	CTCAGCCAGCACCCCCAG-3'-5'
Hum-GAPDH-Probe	TTTGGTCGTATTGGGCGCCTG-3'-5'
Hum- Ghrelin -Probe	CCAGCCACGGCGTCCCTGCTGT-3'-5'

D. Statistical Methods

Mean indications and standard deviation were used to describe the data. 2*2 ANOVA test was used for comparison between groups with two factors of time and exercise. Data were analyzed by using SPSS version 16.

RESULTS

Primary measured anthropometric characteristics by the researchers are shown in Table 2. The results showed that cardiac rehabilitation exercises had slight and insignificant increases in ghrelin gene expression (p =0/185 and F =1/68).also Brachial-ankle index that reflects the performance of body peripheral vascular showed a significant improvement as a result of rehabilitation exercises (p = 0/041 and F = 3/44). The average blood pressure in the rehabilitation group decreased significantly compared to control group (p = 0/0008 and F = 7/64). The results of changes in the research factors are shown in Table 2.

Table 2: Anthropometric characteristics of subjects.

Variable	**The control group (N =8)**	**The Rehabilitation Group (N =8)**
Age (years)	52/83±2/33	54/66±3/30
Height (cm)	174±6/85	172±7/99
Weight (kg)	74/33±3/65	77/25±2/31
Body Mass Index (2kg / m)	22/88±1/49	25/80±1/22

Table 3: The change amounts of study variables in the control and rehabilitation exercise groups.

Group / Variable	control group (N = 8)		Rehabilitation Group (N = 8)		Repeated measures test
	Pre test	Past test	Pre test	Past test	
The relative amounts of ghrelin mRNA gene expression	1/54 ± 0/04	1/55 ±0/05	1/56 ± 0/05	1/68±0/15	F= 1/68 P= 0/185
Ankle-brachial blood pressure index	0/87 ± 0/07	0/83 ±0/03	0/79 ± 0/04	0/94±0/10	F= 3/44 P= 0/041
The average of blood pressure	105/2 ± 7/3	107/8 ±11/2	110/79 ± 14/7	96/14 ±11/5	F= 7/64 P= 0/0008

DISCUSSION AND CONCLUSIONS

It was shown that cardiac rehabilitation exercises caused a slight increase in ghrelin gene expression in CABG patients in this study, but this reduction was not significant statistically. The long-term effects of exercise on ghrelin and its metabolic effects were examined in several studies (Mager *et al.*, 2008, Buss *et al.*, 2014), but less studied about its cardiovascular effects.

Shyya and colleagues investigated the effects of moderate exercise on plasma levels of ghrelin and ghrelin without acyl ghrelin and other molecules assess the relationship between hormonal and metabolic parameters were evaluated during the exercise. In this study, healthy male (25.2 ± 0.5) years old a period of 60 minutes at 50% of maximal oxygen uptake, their own. Plasma levels of ghrelin, without acyl ghrelin, GH, norepinephrine, epinephrine, dopamine, insulin and glucose were measured. Plasma ghrelin and ghrelin levels unchanged total. Levels of norepinephrine, epinephrine, dopamine and plasma GH increased significantly during exercise. Plasma insulin levels significantly decreased during exercise, plasma glucose levels during exercise remained stable (Shiiya *et al.*, 2011). The results are consistent with the results of the present study.

But a number of studies have reported different results with this study. The Drabble and colleagues have found that the total amount of ghrelin in the control group of participating rats in resistance exercise (five times per week for 5 weeks) at the end of the experiment was lower than the experimental group (Ebal *et al.*, 2007). Magyar and colleagues also reported an increase in Ghrelin mRNA expression and its receptor in lymphocytes caused by resistance exercise with 60-70% of intensity and four days a week, respectively. Foster and colleagues studied the effect of regular moderate aerobic exercise for 45 minutes in each session and for 5 days a week on plasma ghrelin response in menopausal women. After the implementation of the Protocol, ghrelin in subjects who reduced more than 3 kg was increased 18 percent.

It should be noted that there was not any change in food intake and calorie intake behavior of the subjects. They showed that weight losing due to regular the aerobic exercise was not only associated with a progressive increase in the plasma ghrelin levels compared with baseline trends but also the increase in ghrelin was significant statistically. On the other hand, there was no significant difference in the group that only did stretching movements. Also the researchers by using statistical methods to examine the relationship between variables found that there was no significant relationship between the changes in levels of physical fitness, more oxygen intake and plasma ghrelin levels. The researchers suggested a hypothesis (Foster-Schubert *et al.*, 2008).

Plinta and colleagues evaluated of the effect of average short-term exercises, aerobic, compressed fitness and fast activities for three months in the preseason course on the levels of ghrelin, leptin, and visfatin and adipockine of 50 healthy females' basketball and handball professional players in a study. Estradiol serum and the levels of leptin, adiponectin, visfatin and ghrelin plasma was determined at the beginning and end of a period of three months moderate aerobic exercises. In addition, the Plasma levels of adiponectin and ghrelin was determined after 2 hours of moderate aerobic exercise, fitness fast and compression exercises. Long-term moderate aerobic exercise showed a significant reduction on plasma ghrelin and leptin levels, but visfatin and plasma adiponectin remained unchanged. There was no change in plasma levels of leptin and ghrelin after short-term moderate aerobic exercise or after fast and compressed fitness exercise. The plasma visfatin concentration increased significantly after short-term moderate aerobic exercised increased, while adiponectin increased after compressed and fast fitness exercises. According to the study reportage, regular moderate aerobic activity at the time of preparation for the competitive season caused reduction in leptin and ghrelin levels of blood circulation (Plinta *et al.*, 2012).

The reason of inconsistencies in the results of this study with the mentioned researches is probably related to 2 logics: at primary, the ghrelin was kept at average level and there was not adequate intensity to suppress the secretion of ghrelin in the target tissues as the result of exercise intensity with regarding the nature of the subjects in the present study. At secondary, Foster and colleagues' hypothesis suggest that weight reduction is a reason for the increase in ghrelin levels in the blood. In fact, this increase is known as a part of the adaptations to the lack of energy. Based on this hypothesis, unchanged ghrelin may be related to the constant weight of subjects resulted by exercising.

In this study, it was shown that the implementation of two-month cardiac rehabilitation exercises led to improvement in peripheral vascular resistance index and blood pressure reduction. Ankle-brachial blood pressure index is a non-invasive method and a criteria for evaluating the environmental arteries performance. The application of this method is in evaluation of the success rate of interventional surgery in cardiovascular patients. The normal ankle-brachial index varies between 0/91 to 1/30. Mild to moderate Peripheral arterial disease usually makes ankle-brachial index at the range of 0/41 to 0/90 (Grenon *et al.,* 2009). The benefits of different types of exercising are clearly demonstrated in blood pressure improvement and peripheral vascular resistance reduction by the researchers. But the present study researchers had not been found a study examining the effects of physical activity on changes in ghrelin and its interaction with cardiovascular factors. The Studies that have examined the interaction of ghrelin and blood pressure are as follows. In Hasoda and colleagues study a dose of injected ghrelin was significantly reduced blood pressure. Many mechanisms can be considered for ghrelin vasorelaxation effect. The first study around this issue is that ghrelin acts contrary of endothelin-1 effect that causes narrowing the isolated arteries (Cornelissen and Smart 2013, Hosoda *et al.,* 2006).

Experiments had done on the mice aorta which have been treated by ghrelin due to lack of growth hormone shows that an increase in acetylcholine causes the vasodilatation. Studies show that the effects of coronary artery narrowing effect caused by ghrelin has no relationship with stimulation of muscarinic and cholinergic receptors and it is through beta-adrenergic receptors which causes nitric oxide release (Grossini *et al.,* 2007). Another effect of ghrelin is regulation of vascular tone that causes reduction in sympathetic nervous activity and blood pressure, it was obtained by injecting ghrelin in the mice.

In this study, renal sympathetic nerve activity and arterial blood pressure were measured by injection of ghrelin in the peripheral vascular and brain of vigilant mice. The researcher believes that intravenous injection of ghrelin in the central nervous system causes arterial blood pressure and sympathetic nerve activity decrease finally (Kemp *et al.,* 2014).

Previous studies have shown the effects of ghrelin on the metabolism, appetite, energy expenditure, and weight and body composition. However, the role of ghrelin on the cardiovascular system is an entirely new field of researching about ghrelin. The researchers in the recent study showed blood pressure improvement and peripheral vascular resistance as a result of exercise rehabilitation in CABG patients clearly. On the other hand, even though there are different mechanisms proposing vasorelaxation action of ghrelin that the most important one is inhibition performance of endothelin-1, the changes in blood pressure was independent of ghrelin in this study.

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