Effect of Weight Training and Whey Protein on Atrial Natriuretic Peptide, Brain Natriuretic Peptide and Galactin-3

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Abstract

Background and Objective: Clinical studies have indicated that exercise activity and supplementation may have different effects on heart function and health. The aim of this study was to assess the effects of weight training with whey protein supplementation on ANP, BNP, and Galactin-3 in 60-65 years old sedentary men.

Material and Methods: This study was based on a quasi-experimental design. The sample population was selected from volunteers out of the Kahrizak charity sanitarium of the Tehran province. Forty men were randomly assigned to 4 groups (control, exercise, whey protein, and exercise + whey protein). Whey protein and exercise + whey protein groups were asked to consume whey protein. Training protocol lasted for 6 weeks. Blood levels of ANP, BNP, and Galactin-3, before and after the study were measured. Normality and variance heterogeneity was assessed using Shapiro-Wilk and Levene's tests respectively. The significance of differences between training and supplementation groups was assessed by ANOVA and LSD hock post-test.

Results: The results showed that weight training program and in combination with whey protein supplementation significantly reduced Atrial natriuretic peptide (P=0.001), Brain natriuretic peptide (P=0.001), and Galactin-3 (P=0.001), compared to the control group (P<0.01).

Conclusion: The reduction of cardiac injury indices and structural and physiological recovery and the increase of cardiac function have been affected by the synergy of his training and protein.

Keywords: Natriuretic Peptides; Whey Protein; Galactin-3
Introduction
Mechanization of life has led to lifestyle changes and increased prevalence of cardiovascular disease (1). These changes, and health programs have been designed with lifestyle modifications, through exercise, proper nutrition, avoiding stress, and quitting smoking (2, 3). Protein is indicated as the key nutrient for the health of the elderly, aiding in a better performance of the organism and in a better quality of life. However, many studies indicate that the elderly population has an insufficient intake of proteins of high biological value. The use of whey protein may be a food strategy to increase protein intake. The whey protein fraction is obtained from the whey resulting from cheese-making process through classification. Whey proteins have around 15% to 20% of the total milk proteins (4). Lack of physical activity along with obesity and diet have been reported to be important cardiovascular risk factors (5). Also, regular physical activity and proper diet have been introduced as the main interventions to prevent cardiovascular diseases (6). Cardiac biomarkers play important roles in acute coronary syndrome and congestive heart failure (7).

Adolescence is marked by biological changes, such as cortex maturation, reorganization process, and cognitive performance and development. Some researches indicate that during this period physical exercises can stimulate the brain-derived neurotrophic factor. Brain-derived neurotrophic factor is understood to be an essential neurotrophin that modulates angiogenesis and strengthens neural connectivity (8). The biological activities are crucial in the development of learning and memory and contribute effectively to better academic performance and brain health (9). The number of heart failure is higher among elderly men than women.

Studies have shown that atrial wall tension is the main stimulus for cardiac hormone secretion (10). The effect of endurance activity on heart hormones in healthy people and heart patients has shown the positive effects of this type of exercise (11). Aerobic exercise has increased the ability, respiratory capacity and duration of physical activity of myocardial infarction patients (12). Some of the studies showed, six weeks of very intense intermittent training significantly reduced BNP and ANP (13). The effect of whey protein supplementation on myofibrillar protein synthesis and performance recovery in resistance-trained men was investigated by Davies et al (2020) (14).

The effect of exercise with supplements on reducing the risk of cardiovascular disease has been studied by some researchers (15, 16) Galectin-3 has also been used as a biological indicator for the early detection of cardiac abnormalities (17). High-intensity endurance training has been reported with biochemical abnormalities and increased galactin-3 and cerebral natriuretic peptide (18). Plasma galactin-3 levels in endurance athletes increased significantly after running, but were not associated with the role and function of the heart muscle, and other cardiac biomarkers (19, 20). Also, the effect of 8 weeks of regular endurance training on galactin-3 changes after intense aerobic

Highlights
Confirmation of the effect of Whey protein on improving the condition of the heart
Metabolic effects and protection in the immune system
The use of this supplement can be useful for athletes and ordinary people heart patient, and sports coaches.
exercise (21) and an exhaustive aerobic exercise were investigated (22).

Researchers showed that plasma levels of galactin-3 before and after a debilitating aerobic training were not significantly (23). Bosnjak (2015) showed that heart failure and decreased myocardial function are related to changes in galactin-3 levels (5). However, plasma levels of NT-Pro BNP did not change significantly after one session of exhausting endurance exercise, but significant changes were observed in galactin-3 levels (24). Experiments show that whey protein has a significant effect in preventing cardiovascular pathogens (25). Some of the results showed that supplementation with whey protein activated Akt/mTOR, Erk1/2 signaling pathways and expression of PGC1-α gene in skeletal muscle fibers (26). Therefore, in this study, the effect of weight training and whey protein on the atrial natriuretic peptide, brain natriuretic peptide and galactin-3 inactive men aged 65-60 years have investigated.

### Materials and Methods

The present study was an interventional study and post-test design in four groups.

**Ethical Statement and Study Design**

The subjects were all inactive men aged 65-60 years in Kahrizak charity sanitarium (143 people). Participants were informed of the risks and benefits before providing written informed consent. Ethical approval was granted by the University of Varamin Pishva Ethics Committee. From which 40 people were selected according to Cochran’s formula and in a double-blind parallel-group design, participants were block-randomized (n=10 per group) into 4 groups: exercise, protein Whey, exercise + protein Whey and control.

The first, 10 cc of blood were taken (Table 1). Blood samples were poured into 5 cc micro tubes encoded for galactin-3 and 5 cc into micro tubes encoded for natriuretic peptide.

**Table 1. Mean ± SD of descriptive characteristics of subjects**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variable</th>
<th>Control</th>
<th>exercise + protein Whey</th>
<th>exercise</th>
<th>protein Whey</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>63.2±2.1</td>
<td>62.8±2.26</td>
<td>61.46±1.12</td>
<td>62.71±1.08</td>
<td>0.516</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.58±0.3</td>
<td>171.13±0.1</td>
<td>175.10±1.18</td>
<td>172.08±0.5</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.2±4.1</td>
<td>78.45±3.6</td>
<td>79.41±1.15</td>
<td>75.15±2.26</td>
<td>0.488</td>
<td></td>
</tr>
<tr>
<td>BMI(%)</td>
<td>24.02±0.33</td>
<td>25.52±0.12</td>
<td>24.08±0.11</td>
<td>23.42±0.13</td>
<td>0.582</td>
<td></td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>30.04±0.39</td>
<td>27.09±0.78</td>
<td>31.46±0.01</td>
<td>29.04±0.58</td>
<td>0.243</td>
<td></td>
</tr>
</tbody>
</table>

**Dietary Assessment, Exercise Protocol and Supplementation**

Before starting the study, participants completed habitual dietary intake, feeding pattern, exercise training, and activities of daily living. Intensity training (6 weeks±3 days per week) performed by training and training + protein groups (Table 2). All participants were informed about the aims of the study and gave written consent. Major inclusion criteria before randomization over the past six months, they have not consumed any supplements or medications. It does not exclude any history of common chronic health problems and diseases, such as respiratory, metabolic, cardiovascular, renal...
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, and hepatic diseases, history of psychiatric or neurological disorders, smoking, medication, obesity, and competitive sports. Age conditions were also considered. Protein whey and protein whey + exercise groups consume 35 grams after exercise (27, 28). After 6 weeks of training protocol and supplementation and 24 hours after the last training session, the second blood sample was taken.

Table 2. Weight training protocol with course, repetition and rest between sets (18)

<table>
<thead>
<tr>
<th>Week</th>
<th>Set</th>
<th>Repetition</th>
<th>Maximum Repetition(1RM)</th>
<th>Rest min</th>
<th>Exercise content</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>4</td>
<td>10-12</td>
<td>%60</td>
<td>1</td>
<td>Chest and forearm: Chest press, upper chest press, chest with dumbbells on a flat surface, chest with dumbbells on a sloping surface, forearm and standing, forearm Lari, forearm with alternating dumbbells</td>
</tr>
<tr>
<td>Second</td>
<td>4</td>
<td>8-10</td>
<td>%65</td>
<td>1.5</td>
<td>Back and back of the arm: horizontal, rowing armpit, lap money, cable back of the arm, lying on the back of the arm with a barbell, sitting on the back of the arm with dumbbells</td>
</tr>
<tr>
<td>Third</td>
<td>5</td>
<td>6-8</td>
<td>%70</td>
<td>2</td>
<td>Legs, shoulders and abdomen: squat front thigh with machine, back thigh with machine, standing behind leg, shoulder with barbell from behind, dumbbell head, shrug, lifting, dumbbells on both sides, crunch.</td>
</tr>
<tr>
<td>Fourth</td>
<td>4</td>
<td>8-10</td>
<td>%65</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fifth</td>
<td>4</td>
<td>10-12</td>
<td>%60</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Sixth</td>
<td>4</td>
<td>10-12</td>
<td>%60</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Blood Sampling and Laboratory Measurements

The concentration of ANP was measured by ELISA and by a specialized kit made by Biomedica with a sensitivity of 7.8 pg/ml. The concentration of Ntpro BNP was measured by ELISA and by a specialized kit made by Mitsubishi of Japan with a sensitivity of less than 125 pg/ml and HUMAREADER device, which had a coefficient of variation for BNP of 3.5%. The concentration of galactin-3 was measured by ELISA method and by a specialized kit made by EASTBIOPHARM Company in China with a sensitivity of 49.2 pg/ml.

In this study, the enzyme immunoassay (ELISA) was used to measure ANP. The test was performed using enzymatic and hormonal activity that binds to the enzyme peroxidase. If the substrate (reactant) of this enzyme is in the environment, this enzyme converts it into a pigment product. The amount of product produced was determined by the colorimetric method, in other words, the amount of labeled hormone was determined. In the enzyme immunoassay, the amount of hormone in the plasma sample was determined using a standard curve. To separate the plasma, the samples were centrifuged at 3500 rpm for 15 minutes at 4 °C and the plasma was stored at -80°C. Sandwich was measured using special kits for these parameters. The coefficient of change within the test was 2.4% for ANP and 3.5% for BNP.

Statistical Analysis

All results were presented as mean ± standard deviation. The normality of the data was assessed using the Shapiro-Wilk test and the homogeneity of variances was assessed by the Leven test, and one way analysis of variance was used to examine the differences between groups. LSD post hoc analysis for multiple comparisons was used to determine any significant changes between groups (P < 0.05, SPSS statistical software, version 21).
Result

Participants and Dietary Compliance

Baseline characteristics are presented in Table 1. There were no differences between groups for: age, height, body mass, and weight. Participants were weight-stable. After measuring and recording the descriptive characteristics of the subjects, the mean and standard deviation of the results of descriptive statistics of atrial natriuretic peptide concentration were measured and recorded (Figure 1).

The mean and standard deviation of the results of descriptive statistics of brain natriuretic peptide were measured and recorded (Figure 2).

The mean and standard deviation of the results of descriptive statistics of galactin-3 was measured and recorded (Figure 3).

![Figure 1](image1.png)

**Figure 1.** Comparison of mean ANP changes between four groups of inactive men aged 60-65 years and significant changes between groups

- #, P ≤ 0.05 significant decrease compared to the all group.
- **, P ≤ 0.05 significant decrease compared to the control group.

![Figure 2](image2.png)

**Figure 2.** Comparison of mean BNP changes between four groups of inactive men aged 60-65 years and significant changes between groups

- ##, P ≤ 0.05 significant decrease compared to the exercise group.
- **, P ≤ 0.05 significant decrease compared to the control group.
- ++, P ≤ 0.05 significant whey group compared to the control group.
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Figure3. Comparison of mean Galectin 3 changes between four groups of inactive men aged 60-65 years and significant changes between groups

<table>
<thead>
<tr>
<th>pmol/ml</th>
<th>Galectin 3 before</th>
<th>Galectin 3 after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>whey+exercise</td>
</tr>
<tr>
<td></td>
<td>exercise</td>
<td>whey</td>
</tr>
</tbody>
</table>

##, P ≤ 0.05 significant decrease all groups compared to the control group.

**, P≤ 0.05 significant decrease exercise + whey group compared to whey group.

Shapirovillek test showed that the variables were normal. Homogeneity of variances was also confirmed by the Leven test. The results of statistical analysis of one-way analysis of variance of atrial natriuretic peptide showed that there was a significant difference between the experimental and control groups. The results of the LSD post hoc test showed that there was a significant difference between the atrial natriuretic peptides in the exercise + whey protein group compared to other research groups (P=0.001). But there was no significant difference between the exercise group and the whey protein group. There was a significant difference between the exercise group and the control group (P=0.001). The results of one-way analysis of variance related to galactin-3 showed that there was a significant difference between the exercise and control group (P=0.001, F=1.25).

The results of the LSD post hoc test showed that galactin-3 in the exercise + protein Whey group was significantly different from the whey protein (P=0.041) and the control group (P=0.002). But there was no significant difference between his exercise + whey protein group and the exercise group. There was a significant difference between the exercise group and the whey protein group and the control group (P=0.043). But there was no significant difference between whey protein group and the control group (P=0.05).
Discussion

It seems that in the present study, the atrial natriuretic peptide has been modified due to adaptation to exercise and protein intake. One possibility is increased adaptation, stimulation of the sympathetic apparatus, and secretion of stress hormones. There has also been a period of structural and physiological recovery and improvement of cardiac function (29). The compatibility of exercise with the effect on the secretion and function of chemical peaks have also been suggested, which has caused the control and modulation of cardiac hormone secretion in trained individuals compared to people without training history. Exercise adaptation has been reported to modulate nitric oxide and produce guanylyl cyclase. Therefore, cyclic guanosine monophosphate is produced to a lesser extent and the activity of the enzyme phosphodiesterase is less, and then less calcium channel is closed, causing the heart muscle to relax and reduce the production of atrial natriuretic peptide. In this regard, nitric oxide is also indirectly involved in affecting blood vessels. As a result, cyclic adenosine monophosphate, which increases cardiac contractility, degrades phosphodiesterase less to the inactive adenosine monophosphate nucleotide, resulting in less activated protein kinase G enzyme (30). Exercise and supplementation may also affect secretory function by altering the number and the activity of natriuretic peptide receptors. Lipari (2010) reported a significant change in atrial natriuretic peptide after resistance training (31). But Ahmadizad (2011) showed that resistance training did not produce changes in resting levels of the cerebral natriuretic peptide, atrial natriuretic peptide (11). The discrepancy in the results may be due to differences in the study group, training protocol, time of assessment of factors, age of subjects, assessment the method, intensity, and duration of training. They measured atrial natriuretic peptide in rats immediately after training, but Ahmadizad, 48 hours after training. The age of the subjects was under 30 years, while the age of the subjects participating in the present study was over 60 years. Also, the rate of change of plasma atrial natriuretic peptide during exercise depends on the intensity and duration of the activity, the amount of catecholamine secretion (32), body posture during exercise (33), altitude and hypoxia conditions, and exercise habits (34). The results of changes in brain natriuretic peptide showed that there is a significant difference between the exercise and supplement groups and the control group and were inconsistent with the results of Guazzi (2012), that reported an increase in brain natriuretic peptide (35). Exercise may reduce plasma brain natriuretic peptide by modulating the secretion of inflammatory cytokines (36). Another a possible mechanism could be the compatibility of exercise with angiotensin II modulation in the reduction of brain natriuretic peptide (37). Which has led to a decrease in the diameter of the end-systole and the dimensions of the left ventricular end-diastole and a decrease in brain natriuretic peptide (38). Increased brain natriuretic peptide after heavy resistance training has been reported by Natalie (2015) (39). But Bordbar et al. (2013) did not observe a significant change in the level of brain natriuretic peptide after one session of resistance training (40). In intense maximal exercise, increased contractility, and heart rate are associated with a decrease in the ventricular diastolic period. As a result, myocardial blood flow is temporarily impaired and ischemia occurs, leading to the secretion of cerebral natriuretic peptide, which did not occur in this study. Differences
arising from the research sample, can be due, practice method, and type of supplement.

The results of galactin-3 statistical analysis showed that there is a significant difference between the exercise groups and the control group. The relationship between the intensity of endurance exercise and plasma levels of beta-galactin-3 has been reported immediately after significant activity (19). Increased galactin-3 levels indicate a change in heart status that results from heart damage and changes the structure of the heart (41). Stressful exercise can be a risk factor for galactin-3 changes. The results of Kim et al. (2007) study showed an increase in galactin-3 levels after 60 km of running, which could be due to biochemical abnormalities and the production of macrophages from the myocardium. These macrophages can be secreted from different organs (kidneys, heart, brain, pancreas, and liver), but the level of galactin-3 in these organs does not follow a specific pattern (42). The decrease in galactin-3 levels in this study is a sign of the absence of these events and the reduction of heart damage, which is the result of the synergy of the positive effects of weight training and supplementation.

Whey protein is a milk-derived protein complex containing lactoferrin, alpha-lactalbumin, specific glycopeptide, and immunoglobulins that act as an antioxidant (43). It is possible that his protein could help regenerate glutathione through cysteine-rich compounds. Glutathione contains glycine, glutamate, and cysteine, and sulfhydryl contains the thiol group, which is used as a reducing agent to prevent oxidation and tissue damage (7). The role of his protein in reducing the production of oxidizing agents and structural damage to the heart has reduced the production of hormonal peptides.

**Conclusion**

The results of the present study showed that weight training with whey protein reduced cardiac natriuretic peptide, brain natriuretic peptide and galactin-3. The research innovation is that consuming whey protein and resistance training will synergistically reduce cardiac peptides. These indicators usually cause damage and lack of structural recovery of the heart in old age and in people without physical activity. Therefore, the inclusion of whey protein in the diet of these people and the use of weight training that does not require much space and mobility, can contribute to better health and quality of heart and body function, without side effects.

Some limitations related to sample collection and data analysis included: insufficient the sample size for statistical measurement, limited access to data, time constraints, conflicts arising from cultural bias, and other personal issues.

**Funding resources**

This research has been done using the personal financial resources of researchers.

**Conflict of interest**

All authors contributed equally to this work, and was conducted with personal financial support.

**Authors' contributions**

Authors’ Contribution: Laboratory studies and tests: SS. Study and review: FN and SS. Analysis and interpretation of data: FN and SS

**Conflict of Interests**

The authors declare that they have no conflict of interest.
**Ethical Approval**

Researchers received introduction letters from the Varamin Pishva Branch of Islamic Azad University with code IR.IAU.VARAMIN.REC.1399.013 and have IRCT 20171210037809N7. The trial ID is 52856.

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