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The Effect of Whey Protein and Creatine Supplementation on the Physical Fitness Indicators, Velocity and Muscle Hypertrophy of Untrained Women during a Resistance-Training Period

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Abstract

Background and Objectives: A few studies have conducted on the effect of protein and creatine supplementation combined with resistance training on the fitness, speed, and strength and muscle hypertrophy of untrained young women. The purpose of the present study was investigating the effect of whey protein, creatine supplements and resistance training on fitness, velocity and muscle hypertrophy in untrained young women. Materials and Methods: The present study was as a quasi-experimental. Thirty-sixth non-active healthy women (weight 61.39±5.92 kg, age 23.91±1.89 year) volunteered to participate in this research. They were randomly assign into three groups: Experimental group 1: Supplementation (protein 1260gr and creatine 450gr) + 6 weeks of strength training, Experimental group 2: placebo + 6 weeks of strength training, and control group. Duration of training was 6 weeks; 4 sessions per week, and each session lasted 50-70 minutes with the intensity of 60-75 percent of maximal reserve heart rate. Data analysis with use of inferential statistics (repeated ANOVA, and Bonferroni post hoc test, when necessary) and employing the SPSS software (version 18). Significance level of $p \leq 0/05$ considered. Results: No significant changes in speed, percent body fat, and maximum oxygen uptake have occurred. A significant increase has observed in the maximum power in both groups of supplementation and placebo, and there was a significant increase in the Supplementation group in the variables of weight, BMI and muscle hypertrophy compared to the pre-test and the control groups, as well as the placebo group compared to the pre-test group. The variables of power and muscular endurance have increased no significantly in both groups of supplementation and placebo. Conclusion: Strength training alone can increase power, but to further increase the strength, weight and hypertrophy in heavy exercises, long-term consumption of supplements of creatine and protein recommended. A greater undersetting of these pathways and consequences of concurrent consumption protein and creatine supplementation in females might certainly boost their performance and could use as a dietary supplement to optimize and enhance health outcomes for women.

Key words: Protein whey, Creatine, Resistance training, Speed, Muscle hypertrophy.

Introduction

One of the issues considered by athletes since long time ago is improving sport performance. Hundreds of special nutritional supplements have introduced for athletes in the market in recent years. Creatine and whey protein are among these supplements. Extensive studies have investigated the effects of supplements on sport performance. (Kilduff *et al.*, 2003; Keri Marshall, 2004; Chrusch *et al.*, 2001; Aguiar *et al.*, 2013; Spillane *et al.*, 2009) The commercial supplements consumption and high-intensity resistance exercises are increasingly to increase muscle mass with the goal of improving the physical appearance, success in the competitions, or gaining strength in women. Inactive people may participate in resistance exercise programs to improve physical body appearance, but many people start the programs of exercising with weight with the aim of improving general health fitness. (Bemben *et al.*, 2001; Jackson, 2000) Whey protein is a part of the milk protein. This protein includes a high concentration of essential amino acids and a rich source of branched-chain amino acids (BCAA), especially leucine. (Cribb *et al.*, 2007; Delecluse *et al.*, 2003; Biwer *et al.*, 2003) It is said that whey protein acts as an antioxidant, antihypertensive, anti-tumor, fat-lowering agent, antiviral and antibacterial agent. (Boone

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et al., 2005; Jon and Sub, 2003) Whey is a rich source of calcium and other minerals. (Antonio and Ciccone, 2013) Various products of this protein differ in terms of value of protein, carbohydrate, immunoglobulin, lactose, minerals and fat. These variables are crucial in selecting different Whey products for special nutritional applications. (Biwer *et al.*, 2003; Boone *et al.*, 2005; Jon and Sub, 2003) Creatine is a nutritional supplement used to increase strength and muscle mass, access to instant energy in the muscles, increase athlete performance, and help for fatigue delay in performing high-intense and short-term strength exercises.

Creatine has been widely used in recent years, especially in weight lifting and bodybuilding athletes. Creatine is similar to an amino acid, which produced in liver and stored in muscles. (Aguiar *et al.*, 2013; Madureira *et al.*, 2007) Creatine is an amino acid found naturally in the skeletal muscle of the body. When a high-intensity and short exercise is performed (such as weight lifting), a specific chemical reaction occurs to provide enough energy to the muscles. Creatine plays a major role in this reaction. (DeNysschen *et al.*, 2019; Pal *et al.*, 2010) As the major part of the energy provides by Adenosine triphosphate (ATP) and creatine phosphate during heavy activities and before the start of anaerobic glycolysis process, it seems that an increase in creatine phosphate stores may increase the amount of energy produced during high intense activity. (Traverso *et al.*, 2010; Ha and Zemel, 2003)

Limited studies have been conducted and different results have been reported on the effect of combination of resistance exercises and protein supplement and creatine on weight control, muscle growth, strength, and body composition. (Bemben *et al.*, 2001; Baechle *et al.*, 2000; McBride *et al.*, 2003) However, review of the studies conducted in this regard suggests a contradiction in the results of the studies.

In addition, most of the previous studies evaluated the effects of supplements alone and limited information have observed on the relationship between the use of Whey protein along with creatine and strength exercises. As bodybuilding used in all sport fields and many athletes used non-allowed and harmful supplements, conducting such research can be an effective scientific step in recognizing the effects of supplements in enhancing the performance of athletes in all sport fields. The aim of this study was to evaluate the effect of six weeks of resistance exercise along with whey protein and creatine on the fitness, strength and muscle hypertrophy indicators in young women.

Methodology

This is a quasi-experimental study due to the lack of control of some of the intervening variables. The research design has pre-intervention, post-intervention, and a control group. The population of this study included young girls of Sari. At first, a public call installed in the sports gyms of the city. Thirty-Six people aged 20-25 years have selected as the statistical sample of the study. The subjects' information on the history of disease, drug use as well as using supplement have reviewed. They selected through randomized purposive sampling method. All subjects were completely healthy according to the medical information questionnaire, had no specific complication, and did not have regular exercises over the past one year. Completing the consent form, they declared their willingness to participate in the test. All subjects attended a session to gain knowledge on the protocol and the correct performance of the movements. During the exercise period, the principle of overload and increasing resistance observed. The control group evaluated only in the pre-test and posttest stages, they did not use any substance, and no exercise program considered for them. They sked to avoid performing heavy activities during the exercise period. The subjects started using supplements since the morning after the pre-test. Both supplements were prepared in a powder form. They used 450 g of creatine monohydrate, produced by Eksir-e Badansazan-e Javan Company and approved by the Ministry of Health, and 5 g glucose supplement as a placebo for 5 consecutive days in each meal (a total of 100 g during loading period) with breakfast, before lunch, before exercise and after exercise.

Then, they did not use it for two days until the creatine-loading period. Then, they used it in two 5-gr meals for 5 weeks (on exercise days, one meal 30 minutes before the exercise and one meal immediately after the exercise and before lunch and dinner in non-exercise days).

The 1260g of isolated Whey protein produced by Eksir Company, starch used as placebo. Synthetic orange syrup powder was equally added to supplement and placebo so that both of them to be same in terms of taste and color. The method of protein in the subjects during the exercise days: they used a 35g package (30 g protein or placebo and 5 g orange syrup) divided into two equal parts and consumed in two meals (one meal along with lunch and one meal during exercise in the exercise days and along with lunch and dinner in non-exercise days). The supplements used and exercises were performed under the monitoring of the researcher, who was also the coach and manager of the club. At the end of each week, the number of packets consumed by the subjects was also controlled. The creatine supplement product was 100% creatine monohydrate and the food composition in creatine and whey protein on the product label have shown in Tables 1 and 2.

Tables 1. The combination of nutrients in the Supplements creatine monohydrate (Compounds: (every 100 grams)

Calories (g)	0.0 g
Protein (g)	0.0 g
Carbohydrate (g)	0.0 g
Fat (g)	0.0 g

Tables 2. The combination of nutrients in the protein

Calories	93
Protein (g)	22
Total fat (g)	0.4
Saturated fat (g)	0.0
Cholesterol (mg)	0.0
Total carbohydrate (g)	0.3
Dietary fiber (g)	0.0
Sugar (g)	0.3
Sodium (mg)	30

A scale in kg measured the weight of subjects. Subcutaneous fat measured by USA Lafayette Model caliper. In this research, physical fitness factors including muscle strength (One repetition maximal foot press and chest press), muscular endurance (horizontal bar) and maximal oxygen consumption used. In their maximal strength session, in the chest press movements, standing barbell curl, triceps extension machine, snatch-grip behind-the-neck, the foot press and foot squat were measured by one repetition maximal (1RM) using the Brzycki formula (Madureira *et al.*, 2007) so that the intensity of work to be controlled during the exercise sessions based on percentage. The subjects have randomly divided into three equal groups of supplement, placebo, and control. A six-week program, four sessions per week, designed for the subjects. It has been presenting in Table 3. The intensity of the exercises varied from 60 to 75 percent of one repetition maximal. (Eliot *et al.*, 2008) In order to measure the speed index, the subjects have asked to a pedal ergometer in 10 turns with a maximum speed of 6 seconds (6 × 10 seconds) after 5 minutes of warming. The subjects had an inactive rest for 30 seconds between each repetition. The ergometer used in this research was a Techno Gym model (made of Italy). The maximum speed recorded every 6 seconds as rounds per minute. The mean of 10 numbers, each of which indicates the maximum speed at one turn (6 seconds) of pedaling, was the mean of maximum speed of pedaling. (Cribb *et al.*, 2007) The body mass index calculated by formula of weight (kg) on the square of height (m2). (Kraemer *et al.*, 2002) To measure strength, the maximum strength in the upper extremities, the chest press movements considered, and for the lower extremities, foot squat movements considered. (Tartibian, 2006)

Table 3: Resistance training program for groups

Training sessions	Exercises movements			
shoulder	Snatch-Grip Behind-The-Neck, dumbbell shoulder, extending the sides with dumbbell, dumbbell lift, barbell upright row, Barbell Shrug, barbell-wrist curl			
Chest and biceps	Chest press, chest press with dumbbell, upper chest press, cable crossovers, Standing Barbell Curl, Dumbbell Preacher Curl, Dumbbell Barbell Curl			
Back and triceps	Horizontal bar, Lat Pull-down, Seated Cable Row, Barbell Bent-over Row, machine triceps extension, lying triceps extension, Dumbbell triceps			
Feet and abdomen	Machine leg extension, machine lying leg extension, seated calf raise, hack squat machine, push-up, crunch			
The first week	3 courses	Repetitions 1214	1RM %60	1.5 minute rest between sets
second week	3 courses	Repetitions 1012	1RM %65	1.5 minute rest between sets
The third week	3 courses	Repetitions 1012	1RM %65	1.5 minute rest between sets
forth week	4 courses	Repetitions 8-10	1RM%70	1 minute rest between sets
Fifth week	4 courses	Repetitions 8-10	1RM %70	1 minute rest between sets
Sixth week	5 courses	Repetitions 6-8	1RM%75	2 minute rest between sets

The muscle volume of the subjects measured and recorded in the chest, arm and thigh areas through the arm and thigh circumference at the right of the body and chest using a flexible tape meter by McBride *et al.* method. (Kinugasa *et al.*, 2004) Horizontal movement used to assess the muscular endurance Sargent Jump test used to measure the strength of subjects. (Candow *et al.*, 2008) The measured items in the previous research before and after the intervention also measured in this study. Given the random distribution of the subjects in the three groups, after confirming the normality of data (using Kolmogorov– Smirnov test), repeated measures of variance analysis were used to investigate the changes of the three groups before and after the intervention. If the effect of the period was significant (intra-group changes), paired t-test would be used, and if the effect of the group was significant, one-way ANOVA test and Bonferroni post hoc test would be used. The significance level considered P≤0.05. (Bemben *et al.*, 2001) SPSS18 software used to analyze the data, all calculations and statistical operations.

Results

The individual characteristics of the subjects based on the mean and standard deviation have shown in Table 4.

Table 4. Demographic characteristic of subjects

Variable	group	Mean±SD	P_value
Weight (Kg)	Supplement	62/22±6/22	0/541
	placebo	60/42±4/55	
	Control	62/30±6/72	
Height (cm)	Supplement	161/62±5/22	0/211
	placebo	159/59±4/64	
	Control	163/32±3/91	
Age (years)	Supplement	23/35±3/78	0/084
	placebo	21/84±6/14	
	Control	23/24±1/13	
BMI (Kg/m^2)	Supplement	26/25±4/34	0/320
	placebo	27/28±8/25	
	Control	25/37±2/20	

Based on the results of one-way ANOVA, it has found that the subjects of all three groups at the beginning of the study showed a significant difference in terms of age, height, weight, and body mass index (Table 5).

Table 5. Change variables to the subjects before and after the test

group	Variable	Pre-test	Post test
Weight (Kg)	Supplement + Resistance training	62/22±6/22	69/22±5/44 *¥
	Placebo + resistance training	60/42±4/55	63/74±0/46*
	Control	62/30±6/72	62/20±3/04
BMI (Kg/m^2)	Supplement + Resistance training	24/25±4/34	27/33±0/77 *¥
	Placebo + resistance training	23/80±8/25	24/12±6/07 *
	Control	23/37±2/20	23/02±4/63
Body fat percent (BF %)	Supplement + Resistance training	23/42±2/16	23/21±2/52
	Placebo + resistance training	21/13±0/75	21/13±0/75
	Control	22/22±8/62	22/22±2/5
Vo_2 Max	Supplement + Resistance training	72/22±2/52	72/1±8/42
	Placebo + resistance training	73/54±0/55	73/55±4/22
	Control	72/34±4/32	72/34±1/93
Speed	Supplement + Resistance training	155/512±5/419	156/804±6/200
	Placebo + resistance training	157/020±5/222	157/21±3/854

	Control	156/312±0/562	157/954±2/430
Maximum power (1 RM)	Supplement + Resistance training	74/45±6/54	156/27±7/21*¥
	Placebo + resistance training	77/28±4/62	141/96±5/65*¥
	Control	73/93±7/49	82/02±3/43
Volume (muscle hypertrophy)	Supplement + Resistance training	124/43±3/79	132/75±5/63*¥
	Placebo + resistance training	123/25±5/53	129/53±7/88 *
	Control	121/52±3/40	126/32±0/76

*Significant difference from pre-test; ¥ Significant difference compared to the control group;

The two groups of supplement and placebo, the maximal strength (sum of chest press and foot squat movements) showed a significant increase compared to pre-intervention and the control group, and also the strength variable in the supplement and placebo groups increased by 34.22% and 21.54%, respectively, muscle endurance also increased in the supplement and placebo groups by 39.11 % and 24.86%. The results of these variables were not significant for the control group. In the supplement group, weight, body mass index, muscle volume (muscle hypertrophy for the sum of chest, thigh, and arm areas) showed a significant increase compared to the pre-intervention and in the placebo group, significantly increase only compared to pre-intervention and no significant differences was observed in the groups for maximal oxygen consumption, speed, and body fat percentage ($P < 0.05$).

Discussion

The results of this study showed that after six weeks of resistance exercise, only the maximal strength indicator in both supplement and placebo groups increased significantly compared to pre-intervention and control group and the variables of speed, maximal oxygen consumption and percentage of body fat in any of the groups did not change significantly. Moreover, in the supplement group, weight, body mass index and volume increased significantly compared to pre- intervention and control group, and in the placebo group, these variables increased significantly only in comparison to the control group. In similar studies, Jose Antonio *et al.* showed that four weeks of resistance exercises did not have a significant effect on weight and fat-free mass, but when creatine supplement consumed immediately after exercise, an increase in their strength observed. (Aguiar *et al.*, 2013) In a study conducted with the aim of evaluating the combination of creatine supplement and high-intensity resistance exercises on the body composition, Spillane *et al.* reported that the level of hypertrophy and maximal strength and muscle creatine levels increased. (Pal *et al.*, 2010) In addition, Cribb *et al.* examined the effects of isolated whey protein, creatine and resistance exercises on men's muscle hypertrophy in three groups after 11 weeks. Their results showed a significant increase in one repetition maximal strength, an increase in squat strength, and an improvement in muscle hypertrophy. (McBride *et al.*, 2003) Paul *et al.* evaluated the effect of creatine and protein supplement after resistance exercises on male bodybuilders and showed that the strength of foot press and squat was improved, and a significant increase was observed in muscle strength and muscle hypertrophy. (Wolfe, 2001) In a study inconsistent with the present study, Eliot *et al.* examined the use of creatine supplements and Whey protein on the men's body composition after resistance exercise. They found no significant changes for total calories, carbohydrates, and fat or protein intake in each of the groups before and after the exercise. (Hulmi *et al.*, 2009) The contradiction between the results of these studies can be attribute to genetic factors, lifestyle, physical fitness, gender, age of subjects as well as type, severity, duration of exercise and nutrition. In most studies, exercise investigated only with creatine supplement (Sinnott *et al.*, 2009; DeNysschen *et al.*, 2009) or only with protein consumption (Heyward, 2002) but in the study, both supplements were investigated. (Cribb *et al.*, 2007) Creatine monohydrat supplements and Whey protein are supplements that have considered by athletes of various sport fields. Using them causes no harm to healthy people. (DeNysschen *et al.*, 2009) For this reason, creatine supplement used as a dietary supplement to increase strength and muscle mass, access to instant energy in the muscles, to improve the athlete's performance, and help delay fatigue in performing high-intense and short-term strength exercises. Resistance exercises increases the synthesis and breakdown of protein. The use of amino acid known as a potent stimulant for protein synthesis. The use of amino acids before and during exercise increases protein synthesis and reduces protein breakdown. The increased amino may potentially increase transcriptional efficiency during protein synthesis. (Heyward, 2002) One of the main features of creatine use in this study is weight gain, so that we observed up to 3 kg of weight gain in the first week of creatine use. Its main reason is the movement of water from the blood into the muscle of the skeletal muscle, increasing the volume of the muscle. In general, creatine uses when we need for weight gain and increase the muscle mass, increase strength and help ATP production in the body. The creatine absorption with using protein is such that creatine needs insulin to enter muscle cells, and when people use creatine along with whey protein, they want to have experience higher level of insulin. A greater undersetting of these pathways and consequences of concurrent consumption protein and creatine supplementation in females might certainly boost their performance and could use as a dietary supplement to optimize and enhance health outcomes for women. As a result, this type of supplements has become popular among people especially for women.

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