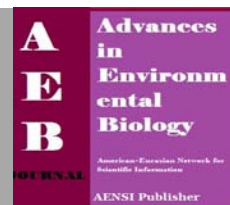




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Effect of Creatine and Ginseng Supplements on Level of the Selected Growth Factors After a Period of Simulated Karate Competition in Young Boys

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ABSTRACT

The overall objective of this study is to investigate effects of creatine and ginseng supplements on expression of the selected growth factors in male karate players after a period of simulated competition. Considering important role of the selected growth factors in reaction to sport exercises and efficacy of creatine and ginseng on athletes' performance, the present study tries to examine the corresponding actions after a period of competitive and training program in young, male karate players. The present study is a quasi-experimental double-blind research with a pretest-posttest control group. Out of the population volunteering to participate in this study, 24 karate players with minimum 3 years of experience are selected as the statistical sample and are randomly divided to three groups with 8 members. The experimental group 1 is creatine group, the experimental group 2 is ginseng group and the third group is a control group of placebo; each group receives 5 mg of their assigned medication on a daily basis. One day before starting to consume the supplements and placebo, as a pretest, weight, body fat percent and maximum heart rate are measured based on scientific methods in all groups. Then, for laboratory analyses, 5 cc of blood is put in the test tubes containing anticoagulant and its DNA is extracted in a standard method for identifying levels of growth factors including IGF-1, MGF mRNA and myostatin. Statistical analysis of the findings is done using one-way ANOVA test, repeated measures test and Tukey's test. So, physical activity associated with creatine intake can play a major role in the signal transferring pathways in several ways through growth factors and hormones present in supplements.

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INTRODUCTION

Karate means empty hands and refers to struggle with a competitor without using any combat equipment [18]. This sport field is full of various acts, for performing which all parts of the body are involved. Beauty, harmony, fluency and using all mental and physical abilities in performing skills are all essential features of a superior technique [17]. One of the most common supplements is creatine which has been widely used after Barcelona 1992 Olympic Games so that, in recent years, its usage has been prevalent in most sports among competitive athletes. Out of every four athletes who received medals in Atlanta 1996 Olympics, three had used creatine supplement. Creatine is a supplement which has not been identified as an illegal chemical by international sport organizations [15, 16]. Over time and with advancement of different sport sciences, skill levels of athletes in different fields have made great progress. Athletic records have reached new levels and the borderline between success and failure has been minimized. Nutritional and medical supplements are among the substances used by most professional athletes to maximize their performance. Hence, these materials may enhance their athletic performance [5].

After creatine monohydrate, another doping which is commonly used by athletes is ginseng. It is one of the largest plants which exists in nutritional supplement (diet) all over the world and is also called plant of youth or panacea. Ginseng is a plant from Aralias family and its scientific name is *Panax ginseng*. This plant is available in two main kinds of Chinese or Korean species and is one of the most expensive and rarest plants in the world with a role in sustaining health and harmony of vital bodily activities. Research has shown that ginseng affects various parts and indices like hypothalamus, parathyroid, brain adrenaline, cortisol, heart and veins, performing endurance training, concentration, weight loss and metabolism [19]. Given the important role of growth factors

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in response to sport exercises and efficacy of creatine and ginseng, the present study tries to investigate the corresponding actions after a period of competitive and training program among young, male karate players.

Statement of the Problem:

There is conflicting information about effect of creatine and ginseng supplementation on athletic performance. Increasing and widespread use of this material, especially by youth and adolescents who are recently very interested in sports, have been propagated with a belief in their very positive impact. Nevertheless, there are still many questions about effects of creatine and ginseng and their consumption method, the response to which could help athletes in maximizing their athletic performance and saving cost. Today, creatine is one the most popular and most widely used nutritional supplements among athletes of different fields including bodybuilding, wrestling, martial arts, track and field and so on. But, ginseng is a cool and warm plant which is somehow appetizing and leads to secretion of digestive juices. It is a fortifier and stimulator which decreases blood sugar and blood cholesterol and strengthens and stimulates immunity system. This plant is a good medicine for lungs and cures chronic lung diseases very quickly. Ginseng is a plant used in food diets and medical prescriptions in different countries of the world. Some people also call it the plant of energy or elixir of youth. Panaxin ginseng from Aralias family which is known in Asia as Korean ginseng is more well-known than other species which have been used in medicine since long ago and has a wider usage [1].

The obtained evidence has shown that this plant can help remove signs of fatigue and bodily and mental weakness. Findings have also demonstrated that use of creatine and ginseng supplements could enhance ability and power of the middle-aged and elderly [7]. Mechano growth factor is a new string form of insulin-like-1 growth factor which is identified as IGF-1Ec in humans and IGF-1Eb in rodents. In fact, this factor is called Mechano since its RNA form in muscular tissues is expressed in response to training overload and muscular damage. Studies have shown that increased expression of insulin-like growth factor and Mechano growth factor in combination with decreasing regulation of myostatin is necessary for efficacy of exercise-induced hypertrophy. However, these special effects are not still completely clear [8]. Studies have demonstrated that, after endurance sport exercises, IGF-1 sticks in a binary form to MGF and local restoration of damaged muscles through activating in-depth muscles as an anabolic process considerably reduces muscle volume loss at old age and special diseases caused by injuries and failure in MGF expression [10]. In such a situation, it seems that in-depth muscles cannot be sufficiently recompleted [19]. Some studies have considerably attributed effect of sport training on animals suffering from chronic heart failure to myostatin expression [10]. On the other hand, experiments by Katja Bulmier *et al.* (2007) for preventing colon cancer on IGF-1 gene expression in rats who had 12 weeks of training showed that training programs caused decreasing demonstration of IGF-1 serum levels (concentration) compared with the control group. During this test with the aim of decreasing body size by training, sport exercises could play an effective role in the main chain of preventing from colon cancer through decreasing IGF-1 serum level (concentration) [11].

Bish L.T. *et al.* (2011) tested long-term systemic inhibition of myostatin through hepatic gene transfer in muscular dystrophy. The results showed that inhibition of myostatin increased muscle mass. It also became clear that hypertrophy of type IIA fibers was largely responsible for the increased muscle mass and that decrease in serum creatine kinase and muscle fibrosis was accompanied by long-term inhibition of myostatin [12]. In 2007, Ates K. *et al.* found that MGF binding species of IGF-1 increased stem cells in dystrophic muscle and healthy muscle [13]. Sarami *et al.* (2010) studied effects of oral creatine and resistance training on serum myostatin and GASP-1. The results showed that myostatin was one of the catabolic regulators of skeletal muscle mass. Accordingly, resistance training considerably decreased serum myostatin levels and increased GASP-1. It seems that creatine supplementation in combination with resistance training resulted in further reduction of myostatin. Effects of resistance training on serum myostatin levels could justify increased muscle mass, aggravated by creatine supplementation [4]. In 2011, Ahtianin *et al.* studied effects of a two day recovery after heavy resistance training on androgen receptor protein concentration in skeletal muscle, AR mRNA insulin-like growth factor and expression of mechanical growth factor. The results showed that expression of MGF and IGF-1Ea increased compared with the pre-training period [5]. Studies by H. J. Engels *et al.* (2008) on effects of short-term intense sport activity on 24 healthy active women demonstrated that long-term use of ginseng had ergo-metric effect on improving their athletic performance [6]. Hwang H.J. *et al.* (2007) showed that effects of swimming combined with ginseng supplementation delayed lymphocyte function despite ROS production decrease and DNA damage after acute exercise [7].

Therefore, considering different research results, the present study attempts to find effect of creatine and ginseng supplementation on level of the selected growth factors after a period of simulated karate competition in male karate players; also, the following question is supposed to be answered: which of these two supplements have the highest influence on level of the selected growth factors?

Necessity and importance of the research:

Given the important role of growth factors in sport exercises and affectability of athletes' performance by nutritional supplements, especially simulated competitions, various studies have been done with this approach. Among the existing supplements, creatine and ginseng have attracted attention of many researchers and have pushed mind of investigators toward studying effects of this supplement on body composition, muscular power and physiological metabolisms [8]. Results of the studies by Bryne *et al.* (2008) confirmed 4-fold increase of IGF-1 expression after 72h of damaging contraction DOMS activity and its increase 120h after training. Researchers believe that mRNA expression of mechanical growth factor happens 48 h prior to increase in IGF-1. But, MGF expression decreases within 72 h after the contractile activity. Nevertheless, MGF increasing trend to levels more than the pre-training trend and its remaining at this level have been observed both 72 and 120 h after training. Researchers attribute increased expression of MGF earlier than IGF-1 to its distinctive role in muscle repair after contraction-caused injury and damage [19]. Given the research background in different fields of using creatine and ginseng supplements, there have been many studies on growth factor levels. Also, use of creatine and ginseng supplements is very common among athletes of many sport fields like bodybuilding, weight lifting, wrestling and martial arts and all segments of society, especially the youth, are interested in their consumption. It seems that there is some uncertainty about the amount of effect of creatine and ginseng supplements among researchers. Therefore, further research is required in this area.

Overall goal of this research:

The overall objective of this study is to investigate effect of creatine and ginseng supplements on levels of the selected growth factors among experienced male karate players after a period of simulated competitions.

Specific objectives:

- 1- Effect of creatine supplement on IGF-1 levels in experienced male karate players after a period of simulated competition
- 2- Effect of creatine supplement on myostatin levels in experienced male karate players after a period of simulated competition
- 3- Effect of ginseng supplement on IGF-1 levels in experienced male karate players after a period of simulated competition
- 4- Effect of ginseng supplement on myostatin levels in experienced male karate players after a period of simulated competition

Research questions:

- 1- Does one month consumption of creatine supplement affect IGF-1 levels in male karate players after a period of simulated competition?
- 2- Does one month consumption of creatine supplement affect myostatin levels in male karate players after a period of simulated competition?
- 3- Does one month consumption of ginseng supplement affect IGF-1 levels in male karate players after a period of simulated competition?
- 4- Does one month consumption of ginseng supplement affect myostatin levels in male karate players after a period of simulated competition?

*Defining special terms**Creatine supplement:*

Creatine is a nitrogenous compound that can crystallize and is an amino acid which is stored in muscles as creatine phosphate. During intense training, creatine phosphate is decomposed by a specific enzyme and is turned into creatine plus phosphates and energy. The energy released from decomposition of creatine phosphate is used for regenerating ATP (adenosine triphosphate), which is a fuel that causes muscle contraction [15,16].



Fig. 1: Creatine monohydrate.

Ginseng:

It is a plant from Aralias family, with scientific name of *Panax ginseng*. The two most common forms of this power generating plant are Korean and Chinese ginseng. It is one of the most expensive and rarest plants in the world with an important role in sustaining health and harmony in vital bodily activities [6].



Fig. 2: A sample of ginseng root and stem.

Insulin-like growth factor:

It is a peptide hormone involved in many physiological processes and is identified as a biological indicator which reflects health and metabolic condition. It is often used in increasing IGF-1 concentration. Also, it should be emphasized that measuring IGF-1 concentration provides important insights into physiological processes [12]. Mechanical growth factor: is a member of the large family of IGF-1 which depends on both training and aging. MGF also called IGF-1 Ec has a unique E region in humans which alters reading frame during IGF-1 gene translocation for producing an adult isoform. MGF-E peptide increases spectrum of proliferative life and raises aging of stem cells in newborn and young people, but not in the elderly [2, 4].

Myostatin:

It is a member of a large family of TGF- β . Myostatin is mostly found in muscle cells, which greatly reduces muscular power. When myostatin is bound to a natural receptor, several signaling cascades are started that prevent muscular growth and promote muscle atrophy. Myostatin prevents muscle growth in the first step by inhibiting formation of a new muscle fiber, known as myogenesis [3, 5].

Placebo:

Refers to a material that looks like the original material; in other words, it is identical to creatine in terms of size, form, color, taste and other characteristics; however, as far as material type is concerned, it is made of maize powder or other substances and has no effects on dependent changes.

A review of the research literature:

Nutritional supplements are materials and compositions that supplement athletes' food diet. Investigations have shown that usage of supplements has been increased among male and female athletes; but, only its optimal usage can lead to promotion of their athletic performance. Ginseng, creatine, caffeine and sodium bicarbonate are some of the supplements that probably lead to promotion of athletic performance. Ginseng: is a pharmaceutical plant native to East Asia (countries like China and Korea) and North America. It has been also used by Native Americans for reducing fatigue caused by long-term trips [6]. This herbal plant has a thick root (more than 2 cm in diameter) and length of about 7 cm with color range of yellow to brown in its American species; it has many medical applications owing to compositions like ginsenoside. The older the plant, the more the density of ginsenoside in its root would be. Ginseng is a late-growth plant and starts flowering after four years old and matures after four to six years. Its root is in the thickest during autumn. Thus, it is harvested at this time. With some structural changes, root of this plant can live for hundreds of years. This plant also has a stem with wide leaves and fruits similar to raspberry which has no medical use [1].

In human body, there is creatine in muscular tissue, heart, brain, nerves, testicles and blood plasma. The highest density of creatine is in skeletal muscles that contain about 95% of all creatine in the body. Creatine exists in two forms in skeletal muscles: free creatine or Cr and phospho-creatine or PCr. All the creatine concentration in skeletal muscles equals 120 MM per kg of dry muscle, 40% of which is in the form of Cr and 60% PCr [3].

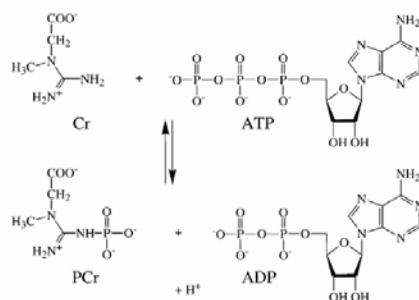


Fig. 3: Reaction of creatine kinase and phospho-creatine and creatine.

The amount of PCr in resting skeletal muscle is three to four times of ATP and this amount reaches four to six times in fast tension (FT) fibers. Main duty of phospho-creatine is preventing ATP concentration loss. In the related reactions, high-energy part of phospho-creatine restores ATP using creatine kinase (CK) enzyme applied to ADP. The reaction takes place in muscle fibrils. Creatine can also act in other ways. One of these ways is that phospho-creatine moves and transfers energy in the form of phosphate units from mitochondria to muscle fibrils. It happens during the reset period [16]. Researchers believe the increased expression of MGF earlier than IGF-1 can be attributed to its distinctive role in muscle repair after contraction injury and damage [10]. Some studies have considerably attributed effect of training on animals inflicted with chronic heart failure to myostatin expression [19]. On the other hand, in 2007, in a study for finding a way to prevent colon cancer on IGF-1 gene expression which was conducted on rats that did 12 weeks of different exercises, Katja Bulmier *et al.* found that training programs could demonstrate decreased IGF-1 serum level (concentration) compared with the control group. During the test which was conducted to reduce body size by exercising, exercise training could reduce serum level (concentration) of IGF-1 in main chain and play an effective role in preventing colon cancer [11].

Bish L.T. *et al.* (2011) tested long-term systematic myostatin control through hepatic gene transfer in muscular dystrophy. The results showed that inhibition of myostatin increased muscle mass. It also became clear that hypertrophy of type IIA fibers was largely responsible for increased muscle mass and decrease in serum creatine kinase and muscle fibrosis was accompanied by long-term inhibition of myostatin [12]. In 2007, Ates K. *et al.* found that MGF binding type on IGF-1 increased stem cells in healthy and dystrophic muscles [13]. In 2006, Tang L.L. *et al.* studied MGF expression osteobalsts in response to mechanical overload and found that cellular proliferation and expression of mRNA insulin-like growth factor and Mechano growth factor in ostebalsts were distinctively regulated in response to mechanical overload by mechanical signals at mRNA level. These findings proved that gene expression was regulated by mechanical stimulation and MGF expression in osteoblasts in response to pressure was probably related to an auto-crane mechanism [13].

Sarami *et al.* (2010) studied effects of oral creatine and resistance training on serum myostatin and GASP-1. The results showed myostatin as a catabolic regulator of skeletal muscle mass. Accordingly, considerable decrease in serum myostatin levels and increased GASP-1 were resulted from resistance training. It seems that creatine supplementation in combination with resistance training resulted in further reduction of myostatin. Effects of resistance training on serum myostatin justified increased muscle mass, intensified by creatine supplementation [8]. Ahtianin *et al.* in (2011) studied effect of a two day recovery after intense resistance training on concentration of skeletal muscle androgen protein receptors, AR mRNA insulin-like growth factor and mechanical growth factor. The results showed that expression of MGF and IGF-1Ea increased compared with the pre-training period [5]. In (2008), H. J. Engels *et al.* studied effects of short-term intense sport exercise on 24 healthy active women and found that long-term use of ginseng had no ergo-metric effect on improving short-term athletic performance [6]. Hwang *et al.* (2007) showed that effects of swimming exercises combined with ginseng supplementation delayed lymphocyte function despite decrease in ROS production and DNA damage after acute exercising [7].

MATEIALS AND METHODS

The present study is a quasi-experimental double-blind research with a pretest-posttest control group to define effects of using creatine and ginseng supplements during an intense 4 week exercise on level of the selected growth factors in male karate players during a simulated competition.

Statistical Population:

The statistical population was group of 80 male karate players in sari, all of whom is members of different sport clubs and voluntarily participates in the present study. The participants should have no background of

using doping or supplements and do not have any history of heart, liver, kidney and liver diseases or diabetes or any other problem.

Statistical Sampling:

Out of the population who volunteer to participate in this study, 24 karate players who have minimum 3 years of experience are selected as a sample and are randomly divided to three groups with 8 members; experimental group 1 as creatine group, experimental group 2 as ginseng group and the third group as control group with placebo.

Research Variables:

Independent variable are: Creatine, ginseng, karate; and Dependent variable are: Simulated competition, IGF-1, MGF mRNA growth factors and myostatin

Measurement Tools:

- 1- Questionnaire related to collecting personal (demographic) information of the participants.
- 2- Digital scale
- 3- Treadmill (for measuring maximum heart rate)
- 4- Caliper (a device for measuring skin thickness)
- 5- Questions related to background of sports and diseases

Method of measurement:

A day before starting to take supplements and placebo, weight, body fat percent (for estimating and calculating fat weight and fat free weight) and maximum heart rate of all the three groups are measured as the pretest.

Program of consuming the supplements:

For this purpose, first, the required amount of creatine monohydrate, wheat germ powder and ginseng powder are purchased from an authentic herbal pharmacy. Then, a large number of half-liter bottles of mineral water, a 2.5 g cup, wheat germ powder and some plastic bags are prepared for packing. Then, 2.5 g of creatine, 2.5 g of ginseng and 2.5 g of wheat germ powder (placebo) are separately prepared. The first experimental group is given creatine, the second experimental group ginseng and the third group placebo as the control. All stages of preparing and packing creatine, ginseng and placebo solutions are done by a researcher assistant. Based on the objective of this study, the participants and researcher do not know about the content of packages.

Methods and tools for data analysis:

For laboratory analysis, 5 cc of blood is kept in test tubes containing anticoagulant and then its DNA is extracted using standard method in order to identify growth factors levels of the present study including IGF-1, MGF mRNA and myostatin. Statistical analysis of the findings was being done using one-way ANOVA test, repeated measures test and Tukey's test.

RESULTS AND DISCUSSION

A one month administration of creatine and ginseng has a significant effect on the level of IGF-1 and Myostatin in karate sportswomen. IGF-1 mediated direct effect of growth factor on the increased strength and muscle hypertrophy. Physical activity induces secretion of growth factor. Somatomedin theory and direct effect of growth factor can both increase strength and the muscle size and facilitate glucose consumption in the absence of insulin through contraction of muscles. The research finding that physical activity is associated with significant decrease in IGF-1 within both groups and Myostatin decrease in creatine group was in accordance with a lower level of IGF-1 and Myostatin after exercise. So, physical activity associated with creatine intake can play a major role in the signal transferring pathways in several ways through growth factors and hormones present in supplements.

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