Unified Journals

4695-5206

Unified Journal of Sport and Health Science
Vol 1(1) pp. 001- 006 October, 2015.
http://www.unifiedjournals.org/ujshs

Original Research Article

The Effect of Eight Weeks of SSG and Resistance-Plyometric Training on Serum Level of Myostatin, GASP-1 and IGF-I in Young Football Players

Saeid Rostami[1], Mohammad Reza Kordi[2], Reza Gharakhanlou[3], Reza Nouri[4]

[1]PhD student of exercise physiology, university of Tehran, Kish international campus.
[2]Associated professor of exercise physiology, university of Tehran
[3]Associated professor of exercise physiology, university of Tarbiat Modarres Tehran
[4]Assistant professor of exercise physiology, university of Tehran, Kish international campus

Accepted 2nd October, 2015

Myostatin is one of the latest discovered cytokines, which regulates skeletal muscle growth negatively. The plan of research is so that two protocols of football-specific training and resistance-plyometric training on the serum level of myostatin GASP-1 and IGF-I are compared after young football players' 8 weeks training. 30 youth soccer players with a range of 14 to 17 years of age were divided randomly in three groups, SSG (n=8), resistance-plyometric (n=11) and control (n=11). Height, weight, BMI and VO2max of the subjects were measured. The next day, blood samples from the anterior brachial vein of subjects were taken. The experimental groups participated in their exercise program (8 week, 2 sessions per week plus three current sessions). All variables were measured again after 8 weeks. In order to compare data within groups' Dependent t-test was used and for comparison between groups dependent t-test was used by SPSS 17. The result of the research showed that eight weeks of resistance-plyometric training brought about a significant decrease of myostatin and a significant increase of GASP-1 and IGF-I. More studies of the interchange and relationship of these variables with growth, biochemical mediators in plasma can show us a clearer image of the role of these changes.

Keywords: resistance, plyometric, SSG, myostatin,IGF-1, GASP-1

**Corresponding Author's E-mail:* srostami.20@gmail.com

INTRODUCTION

Myostatin is one of the latest discovered cytokines, which regulates skeletal muscle growth negatively. After synthesis in skeletal muscle, myostatin is secreted into the blood circulation and then, it binds to the activin receptor of type IIB in the level of muscle cells (Joulia, D., 2006). The aim of binding myostatin in the skeletal muscle is inhibiting the satellite cells. Myostatin does this via setting down the regulatory factors of myogenic such as myogenin (Rios, 2002) and to the same extent, cyclin and cyclin-dependant kinases (cdk2) (McCroskery), and increasing inhibiting expression of cyclin-dependant kinases p21 (Rios, 2002). Myostatin biological activity in circulation in inhibited by binding to serum protein associated with the growth factor and differentiation factor 1 (GASP-1), the newest member of protein containing follistatin. GASP-1 is expressed in skeletal muscle and is secreted into circulation. In fact, GASP-1 is bound to myostatin through follistatin areas and it prevents its link to activin receptor. Moreover, it prevents the separation of propeptide area and mature myostatin (which activates myostatin) (Hill, 2003).

Myostatin expression increases during the periods of inactivity of the skeletal muscle (Joulia, D., 2006), or the inhibition of serum myostatin leads to the increase of muscle strength and mass (Whitemore, 2003). In spite of the importance of myostatin in regulating the mass of skeletal muscle, firstly, the response of an inhibitor factor of growth of skeletal muscle to special exercises, particularly periodic exercise, is not clear, and secondly, there is no research about GASP-1 response, specific binding protein of myostatin which can take a role in regulating its activity, to sport exercises. In reverse, IGI-1 is a positive regulator of the growth of skeletal muscle. This hormone is produced in the liver and skeletal muscle and acts as endocrine and autocrine/paracrine. It has been proven that in different conditions IGI-1 causes activation, proliferation, and differentiation of satellite cells. So, it has been proven that the increase of IGF-I expression plays an important role in the muscle hypertrophy (Goldspink, 2005). The biological effects of IGF-I are mediated by a series of binding protein in blood circulation. In serum IGFBP-3, IGI-1 is the most important binding protein, so that 75 percent of IGI-1 in blood is associated with IGFBP-3 (Borst, 2002). In fact, IGFBP-3 is intravascular reservoir of IGF-I, and harmonic activities of IGF-I are inhibited in the presence of IGFBP-3 through linking to the receptor. Therefore, the changes of this binding protein may influence IGF-I activity (Gharekhanloo,R., 2009) (Gharekhanloo,R.,2008), (Sale, 2003). By the way, there is no agreement about the changes of serum levels of IGF-I and IGFBP-3 after exercises and the issue needs more investigation to get clear.

The analysis of football game shows that players have only about 2 percent of ball possession and they run the rest of the game without ball based on the tactical method of team. The successful team technics depend on the players' ability in cooperation with other teammates in special zones of the field. So, special football drills usually include training games which are played by fewer numbers of people in a smaller-sized field. This special exercise is called Small Sided Game (SSD) (Rampinni E, 2006). SSG drills are often a part of adult players' training programs in different methods depending on the training objectives (Hill-Hass, 2008). It is clear that interval training using SSG training is effective on the aerobic fitness and special endurance in football. Using this special training type is favored by many coaches and sport scientists (Dellal).

On the other hand, football is a game based on explosive movements such ad kicking, jumping, and quick changes in directions. Players walk a distance about 10 km and they need to fast and frequent movement in regular intervals while playing. The previous researches have shown that the speed of running can be improved by various training such as speed training without external resistance, high speed, and special plyometric drills. Also, the ability of jumping is one of the important factors of football performance which greatly depends on the muscle fiber type and maximum power (Hoff). Therefore, one of the most important parts of the players' training programs must be football-specific strength improvement which is defined as a player's ability to use muscle power and strength in football-specific activities effectively and constantly (Santos, 1997). Wong et al.(2009) have also studied the effect of 6-week combined training (resistance-plyometric) on physical performance of 27 boys under 14 years old and showed that this combined training method has had a considerable effect on the physical performance of young athletes (Wong, 2009). As such, since it has been shown that each of resistance and plyometric training, alone, can improve physical performance, and based on the recent research, it can be said that the combination of these two types of training lead to more improvements in physical performance (Wong P.L., 2010).

Regarding the importance of the muscle hypotrophy through the mentioned growth factors in athletes, particularly young football players, and assuming that there is a homeostatic balance between negative and positive regulators of the growth of skeletal muscle while football-specific training which results in increasing the muscle mass, the current study aims to determine the effect of the two training methods on Small-Sided Game (SSG) on the growth factors of the young football player.

METHODOLOGY

In this research, based on the comparison method, the effect of two football-specific training method and resistance-plyometric method on the changes of some growth factors of young football players was compared. The plan of research is so that two protocols of football-specific training and resistance-plyometric training on the serum level of myostatin GASP-1 and IGF-I are compared after young football players' 8 weeks training. The required information to measure dependent variables was gathered using pre-test and post-test and the effect of independent variables on the dependent variables were studied in the experimental subjects. The studied group included 30 young football players who were continuously participating in training of Giti Pasand football team and they were selected to perform two training protocols of football-specific and resistance-plyometric. Regarding the participant, technic, and methodology i.e. having two experimental and control group, pre-test and post-test, and providing an independent variable to the experimental group, this research is semi-experimental survey, and regarding the use of obtained result, it is practical.

Samples

The research participants include 50 young football players from Giti Pasand club. According to the arrangement made with the coach of the team, 30 players who have been continuously participating in trainings (at least for the last year), and in full physical health who did not have any history of acute illness or severe injuries were selected for available and targeted sampling. Then, these people divided randomly into three groups including experimental group 1 (football-specific training group 8 people), experimental group 2 (resistance-plyometric training group 8 people), and control group (11 people). The selection method was done firstly by primary arrangements made with the managers and coaches of Giti Pasand team and distributing medical history questionnaire between selected players of Giti Pasand football team. Compliance with certain conditions of entry such as no history of illness or following diet, and not using cigarette, alcohol, caffeine, and drugs was highlighted in the questionnaire. Then, 30 people who were qualified based on the conditions and were participating continuously in training were selected for performing the two training protocols. It should be noted that the players entered randomly into the groups and all the participants announced that they had full physical health and no history of illness or special infection. Programming of the research was done after making required arrangements with the club manager and receiving written consent from the parents and players. The central index and dispersion index related to general characteristics of the participants such as age, height, weight; body mass and VO2max are provided in table 1.

Research protocol

The participants were asked to come to the club to measure primary anthropometric indexes like height, weight, and body composition. Height variable was measured per centimeter using tape meter and weight variable was measured per kilogram using Seca model scale while people wore the least cloth and no shoes. Moreover, body mass index was measured using evaluating device of body composite model In Body2, in the club office. After measuring these indexes, 12-minute run test was done to measure the maximum oxygen consumption. To measure the participants' maximum oxygen consumption, 12-minute walk and run test was used, so that the participants ran around the field and the taken distant was measured by each participant and then, their VO2max was measured using the related formula (Earle RW, 2004).
SSG training program was done in 8 weeks which was divided into two 4-week stages to adjust the intensity of work. The first stage included 4 weeks SSG training with two 11-minute sets and 120 seconds rest between the sets which was done in 4 versus 4-method and in a field of 20 meters at 40 meters. In the second 4-week, SSG protocol included 3 7-minute sets and 60 second rest, done by 8 players in 2 versus 2-method and in a field of 20 meters at 15 meters. This program was already used by Hill-Hass et al. (2009) and it proved to be effective (Hill-Hass S.C., 2009). These drills were done two times a week along with the other ordinary drills of football.
The second training protocol (resistance-plyometric training) which was done by 11 players included 8 weeks of combined training (Hedrick, 1993). The participants did the resistance-plyometric drills twice a week and along with the ordinary drills of football. The intensity of drills was lower in the first 2 weeks, and it was medium in the next 6 weeks. The resistance drills included front thigh, plover, back thigh, bench press, leg press, and let, and the plyometric drills included movements with box and medicine ball. 11 other players of the participants in the control group will also do their ordinary football drills under the supervision of their coaches within 8 weeks.

Laboratory methods

To study biochemical variables, blood sample was taken after 12-14 hour fasting and in two stages (before starting the trainings and after 8 weeks trainings). For taking blood in the first stage, the participants were asked not to do any sport activity from two days before the test. Then, the participants presented in a medical laboratory. The temperature and time of the test was recorded to maintain the same condition for the next stage. 7 milliliters blood clot was taken from the vein of each participant's left hand while they were sitting and resting and serums were immediately separated. The obtained serum was kept in the laboratory in -80 centigrade degree in order to be used in due time. After this stage, the participants started special training for 8 weeks and after passing this period and 24 hours after the last training session, the participants were again invited to the laboratory and they were taken blood similarly to the first stage. Serum levels of myostatin were examined by ELISA method and byproducts include ALPCO kit and Bender Med Systems kit made in America and eBioscience kit made in Germany respectively.

Statistical analysis

All the measured variables of this research were examined to control outliers. Regarding the report of the significance level $P \geq 0.05$ of Kolmogorov-Smirnov Test, all variables of bodily, physiological, and biochemical characteristics have a natural distribution. Descriptive statistics, average, variance, standard deviation, and paired T-test (to evaluate the changes of each index from pre-test to post-test) were used to analyze the required research data, and One-Way ANOVA test (to compare changes of indexes during the implementation of various protocols between the differences of pre-test and post-test of different groups) was used to compare the results of similar series of the samples between the groups, and if it was considered significant, post hoc LSD test was used for which SPSS software, version 17, was applied for analysis. Significance level in this study is ($p \leq 0.05$).

RESULTS

The result showed that myostatin (p = 0.001) decreased significantly in resistance-plyometric group. This variable was insignificant in the control group and SSG group (p = 0.812 and p = 0.067 respectively). A SSG training period and a resistance-plyometric training program increased the insulin-like growth factor-1 significantly in this research (p = 0.003 and p = 0.01 respectively) but this difference was not significant in the control group (p = 0.601). Also, a SSG training period and a resistance-plyometric training program increased GASP-1 level significantly in this research (p = 0.001 and p = 0.003 respectively), but the difference was not significant in the control group (p = 0.571). Data associated with the comparison between the groups show that there is a significant difference between the mean differences of 3 groups in the post-test. Post hoc test (Scheffe) was used to determine differences between groups. The results of the post hoc test showed that there is a significant difference in all three above factors between SSG and control groups and also SSG and resistance-plyometric groups.
Table 1 shows the participants' physiologic and anthropometric characteristics and table 2 shows the changes of variables within eight weeks of training.

Table1. Participants' physiologic and anthropometric characteristics

Statistics / Group	No.	Age	Height (cm)	Weight (kg)	Body Mass Index (kg/m2)	VO2MAX (ml/kg)
SSG Group	8	14.29± 1.10	1.76±/7.89	49.59± 11.7	23.66± 2.14	39.22± 2.84
Resistance-Plyometric Group	11	14.55± 0.90	1.77± 7.86	51.93± 14.6	21.74± 2.95	40.54± 3.04
Control Group	11	14.97± 0.60	1.74± 9.31	52.19± 8.7	21.31± 2.11	40.89± 2.76

Table 2. Descriptive changes of data

Statistics / elbairaV	Group	Pre-test	Post-test	Intergroup P	Between group P
Myostatin (ng/ml)	SSG	119.3±7.1	120.1±4.9	0.812	*0.005
	Resistance-Plyometric	116.2±4.5	104.2±3.7	*0.001	
	Control	114.9±6.3	113.8±5.7	0.067	
GASP-1 (ng/ml)	SSG	109.2±4.3	127.5±3.7	*0/001	*0/009
	Resistance-Plyometric	113.6±3.2	121.4±6.5	*0/003	
	Control	110.1±8.7	108.5±9.6	0/571	
IGF-I (ng/ml)	SSG	485.2±26.3	516.7±7.1	*0.01	*0/031
	Resistance-Plyometric	491.1±28.2	554.6±57.9	*0.003	
	Control	511.3±16.7	498.5±34.1	0.601	

*Meaningful Change

DISCUSSION AND CONCLUSION

The result of the research showed that eight weeks of resistance-plyometric training brought about a significant decrease of myostatin and a significant increase of GASP-1 and IGF-I. Moreover, eight weeks of SSG training led to a significant increase of GASP-1 and IGF-I. In recent years, many attempts have been made to clarify the issue of cellular and molecular mechanisms of muscle hypotrophy and atrophy (Gharekhanloo, R., 2009). In this regard, McPherron et al. (1997) detected the unique inhibitor factor of muscle growth called myostatin (McPherron, 1997). The role of myostatin has been well proven in decreased muscle mass in different conditions such as microgravity (Toigo, 2006), AIDs and cancer (Joulia, 2007), and aging (Walsh, 2005). As such, in some studies, it has been assumed that change in myostatin may effect on the muscle adaptation to exercise (wehling, 2000). For the first time, Roth et al. (2003) reported that mRNA expression in young and old men and women decreases after 9 weeks of resistance training (Sale D. G., 2003), while Willoghby et al. (2004) showed that in spite of the increase of participants' muscle strength and mass, mRNA expression increased after 12 weeks of resistance training (Willoghby, 2004). These scholars believe that myostatin has probably no role in adaptations of the resistance training. The inconsistency of findings is perhaps due to differences in time and method of sampling and intensity and period of training. For example, in Roth et al.'s study, the time of biopsy was 48 to 72 hours after the last training session, while in Willoghby et al.'s research blood sampling was done 15 minutes after the last resistance training session. Therefore, sampling time was adjusted 24 hours after the last session to measure the rest level of myostatin in this research. Since myostatin protein has a series of post-translational modifications after synthesis, myostatin mRNA cannot precisely present blood circulation levels and active form of myostatin (McMahon, 2003). So, in some studies, in spite of myostatin mRNA increase, muscle strength and even mass have also shown a significant increase (Willoghby, 2004). The result of this research is consistent with Roth et al.'s research, and it indicates a decrease in serum level of myostatin in response to the resistance training.

As a strong inhibitor, GASP-1 is effective in reducing myostatin signaling (process, activation, and binding to cell receptor) (Hill, 2003). The findings of Gharekhanloo et al.'s research showed for the first time that after eight weeks of resistance training, GASP-1 serum levels increased while myostatin decreased, and this might have led to additional suppression of myostatin signaling (Gharekhanloo, R., 2009). Myostatin and consequently GASP-1 changes indicate a kind of scheduling in these complicated processes in different training methods and more investigations are needed.

In this research, IGF-I serum level showed a significant increase in two groups of SSG and resistance-plyometric training. This result is consistent with the result of Nemet et al.'s research (2010) which was done on the wrestlers for 5 weeks (Nemet, 2002). Also, Eliakim et al. (2006) showed that ten weeks stationary bike exercise above anaerobic threshold leads to IGF-I decrease (Eliakim, 2001) and this is consistent with the results of our research. In Meckle et al.'s research (2009), IGF-I level, in comparison to the control group, showed a significant increase which is not consistent with our research (229). The difference in the IGF-I in two researches can be due to the type of training method, participants' base level of insulin-like growth factor-1 in different ages, training time length, and also pro-inflammatory cytokines. High levels of pro-inflammatory cytokines can suppress growth factors such as the growth hormone axis and insulin-like growth factor-1 as a result of long-term training. This depends on the participants' compatibility level with the training methods. By increasing compatibility, secretion of inflammatory cytokines decreases and consequently, suppression of growth factors decreases as well. The participants in Meckel et al.'s research were 17 to 20 years old handball players who had higher IGF-I basic level in comparison to our research (Meckel, 2009).

In Nemet et al.'s research (2002), the effect of one competitive season of training and wrestling matches was studied in young wrestlers for 6 weeks. The result of this research about IGF-I is consistent with the result of our research. This similarity can be due to high level of cytokines in the participants (Nemet, 2002). In their research, Timoothey et al. (2009) studied the effect of endurance training on adolescents with early and late maturity for 5 weeks. IGF-I had a significant decrease in this research too which is consistent with the present study (Timoothy, 2002).

The insulin-like growth factor-1 is a single-chain polypeptide which regulates the somatic growth (Meckel, 2009). Liver secretion of IGF-I is triggered by the growth hormone; yet, there are evidences which show IGF-I can be secreted from different fibers like skeletal muscle and bone (Meckel, 2009). IGF-I has both autocrin and paracrine effects and there is a strong relationship between IGF-I levels and bone density (Meckel, 2009), muscle mass (231) and the risk of cancer (231). It has been proven that IGF-I levels in circulation are influenced by exercise and IGF-I levels in circulation have a positive correlation with aerobic fitness (Meckel, 2009), muscle strength (Roth, 1993), and physical activity, and it has a negative correlation with body fat (Sutton, 1997). Exercise-induced responses in IGF-I system have a fundamental role in triggering compatibility with training. The researches have shown that the length (Roth, 1993) and intensity of drills can effect on IGF-I response to training.

All in all, our findings show that myostatin serum level decreased and IGF-I and GASP-1 showed a significant increase in response to eight weeks resistance-plyometric training. Also, in SSG group, a significant increase was seen in IGF-I and GASP-1 and these changes indicate the efficiency of these two training methods for young players in this sport. More studies of the interchange and relationship of these variables with growth biochemical mediators in plasma can show us a clearer image of the role of these changes.

ACKNOWLEDGEMENT

We need to thank the Physical Education Faculty of Kish International Campus of Tehran University and the players and club staff of Giti Pasand in Isfahan who helped us to carry out the project.

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