

Effect of low-intensity eccentric resistance training with blood flow restriction on the activation and proliferation indicators of satellite cells

Azam Mosavian¹ [Abbas Ali Gaeini](#)² Mohammad Hemmatinafar³ Mohammad Reza Kordi² Reza Nori¹

¹ Department of Exercise Physiology, Kish International Camps, Tehran University, Kish Island, Iran.

² Department of Exercise Physiology, Faculty of Sport Sciences, Tehran University, Tehran, Iran.

³ Department of Sport Sciences, Faculty of Education & Psychology, Shiraz University, Shiraz, Iran.

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Abstract

Introduction: The objective of the present research was to determine the effect of low-intensity eccentric training with blood flow restriction (BFR) on Hepatocyte Growth Factor (HGF) and myogenic factor 5 (Myf5), in non-athletes young men.

Methods: In this study, 20 non-athlete young men were allocated to two eccentric training and eccentric training with BFR groups. Eccentric training program includes 75 knee extension repetitions in severity of 30% IRM within three sets by using isokinetic device. BFR was made by using air pressure meter and a muscular biopsy was taken from the vastus lateralis muscle of active leg, 48 hours before and 24 hours after the training program. The data were analyzed using independent and paired t-tests.

Results: The results of analysis showed that HGF serum levels and mRNA Myf5 expression in both eccentric resistance training and without BFR groups had a significant increase compared to pre-test levels ($P \leq 0.05$). Moreover, it was observed that HGF serum levels and mRNA Myf5 expression in training group with BFR had a significant increase of 6.39% and 4.05%, respectively, compared to training group without BFR ($P \leq 0.05$).

Conclusion: The results of this research showed that eccentric training with and without BFR may improve the activation and proliferation indicators of satellite cells. But, in spite of this matter, it seems that eccentric resistance training with BFR may have a more significant effect on activation and reproduction of satellite cells.

Key words: Satellite Cells, Hepatocyte Growth Factor, Myogenic Regulatory Factor 5

Correspondence:

Abbas Ali Gaeini, PhD.

Faculty of Sport Sciences,
University of Tehran.

Tehran, Iran

Tel: +98 9123351872

Email:

aagaieini@yahoo.com

Orcid iD:

0000-0003-0028-2688

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Orcid iD: *0000-0002-4307-1007

Introduction:

Maintaining or increasing muscle mass is of special importance in many conditions such as **sarcopenia**, diseases related to muscular atrophy (eg. cancer, AIDS, and diabetes) and particularly, important for athletes (1). Mature skeletal muscle in mammals is a stable tissue in natural conditions (2). But, it has been observed that skeletal muscles have a significant ability in adaptation to physiological stressful factors resulted from growth, exercise activities, hurt,

and disease (3). Hence, researchers have conducted widespread studies to reveal cellular and molecular mechanisms on hypertrophy, atrophy and reconstruction of skeletal muscles. They found that growth and regeneration of muscles are initiated by activating Mesenchymal Progenitor Cells (MPCs), also called satellite cells. These cells are activated in response to different stimuli such as physical stresses, oxidative stresses, and endocrine or inflammatory factors (4). The most important role of satellite cells after

the birth is generating multi-nuclei myofibers aiming to grow skeletal muscles and to increase the number of muscle fibers. In a mature muscle, the role of these cells is changed and without increasing the number of muscle fibers, the number of nuclei increased and then, led to muscle hypertrophy (5). When satellite cells are activated, they cause cell organelles hypertrophy, cytoplasm spreading and cell deformability (5, 6). Myogenic process is tuned by Myogenic Regulator Factor (MRF). MRF controls the development processes of satellite cells in turn from immobility to proliferation, differentiation and separation or self-regeneration. Myof5, MyoD and Proliferating Cell Nuclear Antigen (PCNA) and Desmin are regarded as indices related to satellite cells proliferation, surviving inactively in silenced satellite cells. The studies show that introducing satellite cells to myogenic processes depends on predomination of Myf5 and MyoD. Particularly, Myof5 promoter activity was observed in silenced satellite cells (1, 2, 5).

Although, Myof5 protein expression has not been reported in silenced satellite cells, Myof5 is definitely a proliferation index of satellite cells and its expression reduced in differentiation phase of satellite cells (1). Moreover, research suggests that Hepatocyte Growth Factor (HGF) is a key activator of satellite cells, playing a very important role in initial repair and rebuilding phases. As a result, HGF acts in tuned autocrine and paracrine manners, so that it could activate satellite cells (2, 6). Hence, after stimulating a muscle, tuned and complex mechanisms will operate to repair and rebuild the muscle, so that increased HGF serum levels and Myof5 expression are the key indications of getting activated and proliferation of satellite cells (3, 4).

Resistance training are considered as the most effective training interventions to improve the muscular volume and power administered to prevent damages, rehabilitate skeleton muscles, reduce risk of falling, and increase performance abilities (7). To achieve these goals, American College of Sport Medicine (ACSM) recommends 70%-80% training intensity of *one-repetition maximum* (1RM). But, this amount of work is out of all individuals' power and in some cases it may cause severe muscular damages (8). Therefore, researchers address resistance exercise activities

at 20%-50% training intensity of 1RM together with Blood Flow Restriction (BFR) as a new training method to be replaced with traditional resistance trainings (9). In addition, Roig et al, showed that eccentric resistance training to be more effective in promoting increases in muscle mass compared to concentric resistance training (10). Furthermore, eccentric resistance training also showed a trend towards increased muscle cross-sectional area (11). Nielsen et al (2012), also revealed that 3 weeks of low-intensity eccentric resistance training with partial blood flow restriction induces increases in maximal muscle strength accompanied by highly marked gains in muscle fibre size (12).

However, the physiological mechanisms underlying the gain in muscle strength and muscle mass observed with blood flow restricted low-intensity eccentric resistance training haven't been identified yet and more studies need to be done in this regard. Therefore, the objective of the present research was to study the effect of low-intensity eccentric resistance training with BFR on HFG serum levels and Myf5 mRNA expression in non-athlete young men.

Methods:

The present study was a semi-experimental research conducted in two eccentric training groups (with BFR and without BFR) with pre-test and post-test. The statistical population of the study consisted of young non-athletic men aged 20 to 30 years in Tehran. The process of performing this research was approved by ethnic ethics committee No. 43 from Baqiyatallah University of medical sciences, No. 6770/3/340/sin 1/11/2014. Then, 20 volunteers were selected by random sampling and randomly divided into two groups: including low- intensity eccentric training with BFR (n=10) and low- intensity eccentric training without BFR (N=10).

Phases of carrying out the training program

Before carrying out the training program, a detailed explanation and implementation phases of the research purposes with informed consent form was given to the subjects. Then, the subjects were taken anthropometric *variables*, body

composition and one-repetition maximum (1RM) test (Table 1).

In order to obtain 1RM, the subjects were asked to select a sub-maximal weight (that the number of its repetition to be less than 10) after warming up for 10 min through static and dynamic stretching movements and to repeat knee extension activity to exhaustion. Next, the

rate of 1RM for each subject was identified and recorded by inserting the number of repetition and the weight of weight in the following formula (11-13):

$$1RM = \frac{\text{displaced weight}}{[1.0278 - (\text{number of repetition to exhaustion} \times 0.0278)]}$$

Table 1. Mean and standard deviation of individual subjects personal characteristics

Variable	Mean	Standard deviation	Minimum	Maximum
age (year)	24.34	1.98	23.3	25.9
height (cm)	173	2.8	169	185
weight (kg)	76.3	5	75.7	86.8
Body Mass Index (kg/m ²)	20.2	1.2	18.4	23.3
1RM	46.34	3.32	42.76	49.56

Table 2. Sequences of target and reference gene primers

Gene	Direct primer sequence	Reverse primer sequence	Length (bp)
Myf5	TGTACCAGGACCAACCCCAAC	ATAGTAGTTCTCCACCTGCTCTCTC	192
GAPDH	CCAGGTGGTCTCTCTGACTTCAACAG	AGGGTCTCTCTCTCTTGTGCTCT	223

In this study, a low-intensity eccentric resistance training session was conducted as follows. The training program included three sets of 25 repetitive knee extension movements at 30% intensity of 1RM with a 30-second relaxation between each set and speed of 120 degree/s that performed on the isokinetic device (12-14). In resistance training group with BFR, before carrying out the training program, blood flow restriction was set up using pneumatic barometer with a 5 cmband and pressure 100 mmHg in the initial part of subjects' thighs (13).

Muscle biopsy

Muscle biopsy was conducted after local anesthesia by a specialist physician through injecting lidocaine 1% using a 5 mm needle from vastus lateralis muscle (15 cm upper than patella). In pre-test phase, in order to control the intervention effect on the research results, a muscle biopsy was taken from the subjects' non-training legs (15, 16) in 48 hours before training program (17). In the present research, a muscle biopsy was taken from the muscles of the subjects' training legs in 24 hours after performing training program (18, 19). Then, the samples were frozen at temperature -80°C and delivered to the laboratory for testing mRNA Myf5 levels.

Blood sampling

In the present research, 5 cc blood samples were taken from subjects' brachial veins (medial) in two phases, before training and immediately after training. At first, blood samples were kept in room temperature for 20-30 min, then, centrifuged for 15 min and stored at temperature -80° C. To measure the serum level of HGF hormone, ELISA kit specific for human HGF made by Immunodiagnostic System company (Germany, catalogue No. CK-E10164) was applied and HGF serum levels were tested using ELISA method.

RT-PCR reaction

In the present research, to study mRNA Myf5 expression, RT-PCR method was applied. In this method, RNA was initially extracted from vastus lateralis muscle tissue (RNeasy Mini kit, made by Qiagen com., Germany, catalogue No. 74124) and after synthesizing cDNA (cDNA synthesis kit, made by Clontech com., U.S.A., catalogue No. 634925) from Real-Time SYBR Mix (made by Clontech com., U.S.A., catalogue No. RR037A), it was applied to perform RT-PCR method by using Rotor Gene™ 6000 device. In the present research, $\Delta\Delta\text{Ct}$ was utilized to normalize RT-PCR results from GAPDH reference gene as well as to analyze RT-PCR data quantitatively (20). In Tab.

2, direct and inverse sequences of target and reference gene primers used in this research were given.

Statistical analysis

IBM SPSS statistics 19 for windows (IBM Corp, Armonk, NY, USA) was used for all statistical analyses. Quantitative variables were expressed as mean±standard deviation. Independent and paired t tests were used to examine the intragroup and intergroup changes. Alpha was set at 0.05.

Results:

Figure 1, 2 show the changes of HGF serum levels and mRNA Myf5 expression in response to low-intensity eccentric resistance training with and without BFR. As shown in Figure 1, HGF serum levels have significant increases of 5.83% and 1.73% in both eccentric resistance training with BFR and eccentric resistance training without BFR groups when compared to pre-test rates, respectively ($P \leq 0.05$). Moreover, it was observed that HGF serum levels in training group with BFR had a significant increase of 6.39% compared to training group without BFR ($P \leq 0.05$).

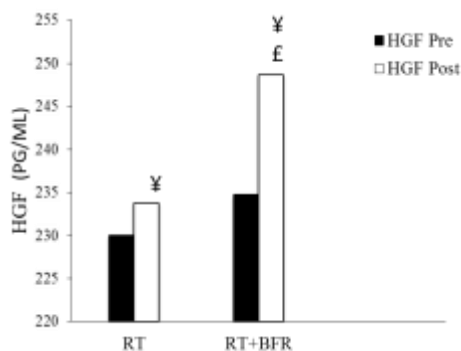


Figure 1. Changes of HGF serum levels in response to low-intensity eccentric resistance training with BFR and without BFR. (RT: resistance training group; RT+BFR: resistance training with BFR; ¥: significant difference with pre-test rates; £: significant difference between two groups in post-test rates; HGF Pre: pre-test HGF levels; HGF Post: post-test HGF levels)

As seen in Figure 2, mRNA Myf5 expression have significant increases of 3.7% and 5.22% in both of eccentric resistance training with BFR and with no BFR groups when compared to pre-test rates, respectively ($P \leq 0.05$). As a result, it is observed that mRNA Myf5 expression have significant increases of 4.05% in training group

with BFR when compared to training group without BFR, respectively ($P \leq 0.05$).

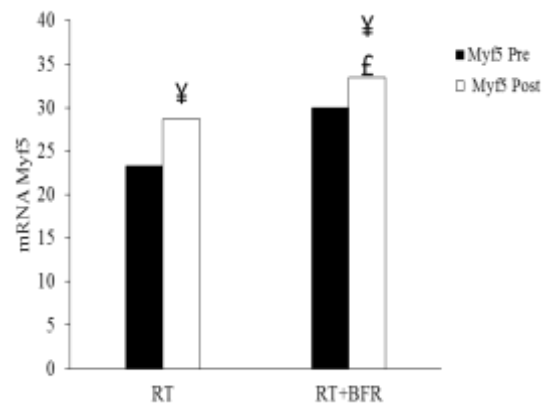


Figure 2. Changes of mRNA Myf5 expression in response to low-intensity eccentric resistance training with BFR and without FR. (RT: resistance training group; RT+BFR: resistance training with BFR; ¥: significant difference with pre-test rates; £: significant difference between two groups in post-test rates)

Conclusion:

In the present research, low-intensity eccentric resistance training with BFR and without BFR led to a significant increase in HGF serum levels and mRNA Myf5 expression. These results show that low-intensity eccentric resistance training without imposing BFR can increase the activation and proliferation of satellite cells. But, considering the mean of two groups showed that resistance training with BFR has a more significant effect on HGF serum levels and mRNA Myf5 expression compared to resistance training without BFR.

In this regard, Burd et al.(2006), Wilborne et al. (2009) and Cladow et al. (2015) found that mRNA Myf5 expression are increased in response to various intensities and volumes of resistance trainings in turn 24, 2,3 hours after performing resistance trainings to more than 3 times of relaxation rates. Moreover, they reported that resistance training with high intensity and low volume didn't lead to a significant change in Myf5 levels. Therefore, they concluded that low-intensity resistance training with high volume had a more significant effect on anabolic activity of skeleton muscle compared with high-intensity resistance training with low volume (17, 20, 21).

Since, in the present research low-intensity resistance training with high volume has been applied, it seems that low-intensity eccentric

resistance training is a suitable driver to increase the proliferation of satellite cells (increased of mRNA Myf5 expression) and consequently, muscle hypertrophy in line with the results of the above research. From the perspective of physiological mechanism, it may be demonstrated that muscle damage resulted from eccentric resistance training causes a wide stimulation of myogenic process in skeleton muscles. This process causes satellite cells to move toward mature muscle fibers through developing a balance between proliferation and differentiation of satellite cells (2, 22, 23). Hence, increased mRNA Myf5 expression is largely resulted from mechanisms like cell - cell balance, cell - matrix interaction as well as extracellular matrix secreted factors (24, 25). As Myf5 has a vital role in proliferation of satellite cells and myogenic process and with respect to the research results and in comparison with the results of previous research, it may be demonstrated that one of the cellular and molecular mechanisms resulted from low-intensity eccentric resistance training may be the increased expression of Myf5, mRNA and consequently, muscle hypertrophy.

Takata et al, o'Reilly et al, and Snijders et al, in their works concerning the activation of satellite cells reported the increased HGF serum levels after resistance training with BFR and without BFR (14, 18, 26, 27). In addition, Reeve et al, reported that HGF serum levels are significantly increased following resistance training together with vascular obstruction compared with traditional resistance training (23). In this regard, the results of Takara et al's research demonstrate that low-intensity resistance training with BFR results in muscle hypertrophy and increased muscle power in athletes in addition to increased muscle strength (14). The results of o'Reilly et al's research also indicates that HGF serum levels were increased significantly from the beginning of training to 4 hours after the training (18).

Regarding the results of the present research and its comparison with the results of the above research, it seems that another possible mechanism resulted from resistance training might be the increased serum levels of HGF, that in turn is accompanied with the activation of satellite cells. The studies recently conducted also

justify the increased HGF serum levels through different mechanisms including: HGF local and systemic responses to the damage resulted from long-term eccentric muscle constriction (28) increased number of granulocytes within an inflammatory response to muscle fibers damage resulted from eccentric resistance training causing HGF transmission to the damaged location (29) and damage of muscle fibers members within introducing the proteins to the blood circulation and consequently, increased HGF serum levels (30). Therefore, with respect to the above-mentioned mechanisms, it seems in the present research that eccentric resistance training leads to HGF local and systemic responses through developing muscle damage and finally, causes its increased serum levels.

Meanwhile, HGF is necessary for muscle growth and hypertrophy by activated satellite cells mediation. HGF also is stored as inactivated and locally in the muscle during relaxation in order to facilitate a rapid response to damaged muscles and their repair (2, 22). After resistance training with BFR, HGF expression is increased in relation to the rate of damage and activated form of HGF released from extracellular matrix and then, released HGFs cause the activation of satellite cells by linking to Receptor c-Met at the surface of silenced satellite cells (22, 23).

Therefore, according to the present research, increased HGF serum levels can activate satellite cells and consequently, stimulate myogenic process by connecting to its receptors at the surface of silenced satellite cells.

On the other hand, research indicates that blood flow restriction (with no exercise activity) may cause increased cellular inflammation by its own and this process is felt by sensors sensitive to the blood volume and then, leads to the activation of mTOR message route (30).

Researchers also reported that using BFR together with resistance training may cause increased intracellular calcium level resulting in increased activation of satellite cells (26, 28, 29, 31). As the results of this research demonstrated that resistance training with BFR resulted in a significant increase of activation and proliferation indices of satellite cells when compared to resistance training with no BFR, it seems that blood flow restriction together with resistance

training, using mechanisms associated with increasing intracellular calcium level cause the increased HGF serum levels and mRNA Myf5 (32). The main limitation in the present study was the lack of measurement of other indicators affecting the activation and proliferation of satellite cells due to lack of financial resources, which could result in more accurate conclusions in this regard.

In conclusion, the results of the present research showed that eccentric resistance training with and without vascular obstruction may cause the increased activation and proliferation indices of satellite cells.

Nevertheless, it seems that eccentric resistance training with BFR has a more significant effect on activation and proliferation indices of satellite cells. Hence, it seems that resistance training with BFR would be a suitable technique for the patients or hurt people who are subject to muscle atrophy and intend to keep or increase their muscle mass.

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تأثیر تمرین مقاومتی برون‌گرای کم شدت همراه با انسداد جریان خون بر شاخص‌های فعال‌سازی و تکثیر سلول‌های ماهواره‌ای

اعظم موسویان^۱ عباسعلی گائینی^۲ محمد همتی‌نفر^۳ محمدرضا کردی^۴ رضا نوری^۱

^۱ گروه فیزیولوژی ورزشی، پردیس بین‌الملل کیش، دانشگاه تهران، کیش، ایران.

^۲ گروه فیزیولوژی ورزشی، دانشکده علوم ورزشی، دانشگاه تهران، تهران، ایران.

^۳ گروه علوم ورزشی، دانشکده علوم تربیتی و روانشناسی، دانشگاه شیراز، شیراز، ایران.

مجله پزشکی هرمزگان سال بیست و دوم شماره اول ۹۷ صفحات ۹۵-۱۰۲

چکیده

مقدمه: هدف از پژوهش حاضر، تعیین تأثیر تمرین مقاومتی برون‌گرای کم شدت همراه با انسداد جریان خون (BFR) بر عامل رشد هیپاتوسیتی (HGF) و عامل میوژنیک ۵ (Myf5) در مردان جوان غیرورزشکار بود.

روش کار: به این منظور، ۲۰ مرد جوان غیرورزشکار (سن: ۲۴/۳۴±۱/۹۸، شاخص توده بدنی: ۲۰/۲±۱/۲) به صورت تصادفی در دو گروه تمرین مقاومتی برون‌گرا و تمرین مقاومتی برون‌گرا با BFR قرار گرفتند. برنامه تمرین مقاومتی برون‌گرا با استفاده از دستگاه ایزوکتیک، شامل ۷۵ تکرار باز کردن زانو با شدت ۳۰ درصد 1RM در سه ست بود و BFR با استفاده از دستگاه فشارسنج بادی ایجاد گردید و ۴۸ ساعت قبل و ۲۴ ساعت پس از برنامه تمرینی از عضله پهن جانبی پای فعال، بیوپسی عضلانی صورت گرفت. سپس، داده توسط آزمون‌های آماری تی وابسته و مستقل تجزیه و تحلیل شدند.

نتایج: نتایج تجزیه و تحلیل داده‌ها نشان داد، مقادیر سرمی HGF و بیان mRNA Myf5 در هر دو گروه تمرین مقاومتی برون‌گرا با و بدون BFR در مقایسه با مقادیر پیش آزمون افزایش معنی‌داری داشته‌اند ($P \leq 0.05$). همچنین، مشاهده شد مقادیر سرمی HGF و بیان mRNA Myf5 در گروه تمرینی با BFR در مقایسه با گروه تمرینی بدون BFR به ترتیب افزایش معنی‌دار ۶/۳۹ و ۴/۰۵ درصدی داشته‌اند ($P \leq 0.05$).

نتیجه‌گیری: نتایج پژوهش حاضر نشان داد تمرین مقاومتی برون‌گرا با و بدون BFR می‌تواند به افزایش شاخص‌های فعال‌سازی و تکثیر سلول‌های ماهواره‌ای منجر شود، اما با وجود این، به نظر می‌رسد تمرین مقاومتی برون‌گرا با BFR تأثیر بارزتری بر شاخص‌های فعال‌سازی و تکثیر سلول‌های ماهواره‌ای دارد.

کلیدواژه‌ها: سلول‌های ماهواره‌ای، عامل رشد هیپاتوسیتی، عامل میوژنیک ۵

نویسنده مسئول:
دکتر عباسعلی گائینی
دانشکده علوم ورزشی، دانشگاه تهران
تهران-ایران
تلفن: +۹۸ ۹۱۲۳۳۵۱۸۷۲
پست الکترونیکی:
aagaieini@yahoo.com

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