Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

DETERMINING THE COMBINED EFFECTS OF THREE WEEKS OF CONSUMPTION OF HMB SUPPLEMENT AND ONE SESSION OF EXTREME ECCENTRIC RESISTANCE EXERCISE ON THE EXPRESSION RATES OF MYOG AND CD56 GENES IN SATELLITE CELLS

Elias Kowsari[1], Alireza Asgari[1,2], Hossein Shirvani[1]
Behzad Bazgir[1], Mostafa Rahimi[3]

ABSTRACT

The purpose of the present study was to determine the combined effects of consumption of HMB-FA supplement and one session of extreme Eccentric resistance training on the rate of activation of the satellite cells of mature male rats. To this end, once the preliminary studies were done, eighty Sprague Dowley male rats (Control group: 18 rats; HMB group: 19 rats; Exercise group: 19 rats; Exercise + HMB group: 19 rats) aged eight weeks and weighting 200 ± 20 grams were selected for the study. After the course of familiarization on ladders, the daily intake of supplement was started and sustained for three weeks. Once the rats were familiarized with the ladder and the supplement was taken in for three weeks, a session of Eccentric resistance training was administered. The protocol of the training included descending from a ladder with a slope of 80% while a 100-110% 1RM weight was attached to the rats' tails. Results of extraction of RNA and Immunohistochemistry showed that the entire three experimental interventions have resulted in an increase in the expression of the MyoG and CD56 genes in the triceps. The important point however, was that the most effective intervention was the exercise + HMB intervention. The amounts of CD56 and MyoG genes have been statistically significantly increased in the groups of HMB, Exercise and, Exercise + HMB (p= 0.001). According to the obtained results, consumption of HMB supplement in combination with effectuation of Eccentric exercises can play an effective role in the activation, propagation proliferation and differentiation of satellite cells. In fact, athletes and military men whom at some points get involved in heavy physical activities and use extreme Eccentric preparatory training in order to maximize their physical readiness can use HMB supplement simultaneously with their training and get even more efficient results.

Key words: Eccentric resistance exercise. Beta-hydroxy Beta-methyl Butyrate. Satellite cell.

1-Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2-Aerospace Medicine Research Center, Aja University of Medical Sciences, Tehran, Iran.
3-Department of Physical Education and Sport Sciences, Faculty of Humanities, Shahrekord University, Shahrekord, Iran.

RESUMO

Determinando os efeitos combinados de três semanas de consumo de suplemento de hormônio e uma sessão de exercício de resistência extrema nas taxas de expressão de genes myog e cd56 em células satélites

O objetivo do presente estudo foi determinar os efeitos combinados do consumo de suplemento HMB-FA e uma sessão de treinamento de resistência extrema sobre a taxa de ativação das células satélites de ratos machos maduros. Para este fim, uma vez realizados os estudos preliminares, oitenta ratos machos Sprague Dowley (grupo controle: 18 ratos; grupo HMB: 19 ratos; grupo exercício: 19 ratos; grupo exercício + HMB: 19 ratos) com oito semanas e peso de 200 ± 20 gramas foram selecionados para o estudo. Após o curso de familiarização em escadas, a ingestão diária de suplemento foi iniciada e mantida por três semanas. Uma vez que os ratos foram familiarizados com a escada e o suplemento foi recebido por três semanas, uma sessão de treinamento de resistência foi administrado. O protocolo do treinamento incluiu descer de uma escada com uma inclinação de 80% enquanto um peso de 100-110% 1RM foi anexado às caudas dos ratos. Os resultados da extração de RNA e imuno-histoquímica mostraram que as três intervenções experimentais resultaram em um aumento na expressão dos genes MyoG e CD56 no tríceps. O ponto importante, no entanto, foi que a intervenção mais efetiva foi a intervenção exercício + HMB. As quantidades de genes CD56 e MyoG foram estatisticamente significativamente aumentadas nos grupos de HMB, Exercício e Exercício + HMB (p = 0,001). De acordo com os resultados obtidos, o consumo de suplemento de HMB em combinação com a efetivação de exercícios excêntricos pode desempenhar um papel efetivo na ativação, proliferação de propagação e diferenciação de células satélites. De fato, atletas e militares que em alguns pontos se envolvem em atividades físicas pesadas e usam treinamento preparatório extremo para maximizar sua prontidão física podem usar suplemento HMB simultaneamente com seu treinamento e obter resultados ainda mais eficientes.

Palavras-chave: Exercício de resistência. Beta-hidrox beta-metil buritato. Célula satélite.

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

INTRODUCTION

It is currently well accepted that it in order to guarantee the efficiency of quality of life and overall human health, it is crucial to preserve the skeletal muscle mass. Any reduction in the content of contractile proteins and consequent reduction in the size of muscle fibers due to an illness, state of inactivity or, the passing of time would be followed by severe consequences including erosion of strength and also certain dangerous metabolic syndromes such as obesity, diabetes and, cardiovascular diseases. Participation in physical activities can help preserving both muscular mass and muscular performance.

The regeneration of the skeletal muscle in a response to an injury is crucially dependent on the number of satellite cells. An Eccentric contraction results in stretching of the muscle and hence causes damage. This damage initiates the process of proliferation of satellite cells as well as the synthesis of new fibers while also creating positive adaptations towards the development of the size and strength production capacity of the muscle fiber.

The response of satellite cells to physical activity has been widely stimulated through the infliction of Eccentric contractions on the skeletal muscles. McKay and collaborators (2010) reported a statistically significant increase in the number of satellite cells during the first 24 hours subsequent to effectuation of Exercise Training.

By taking a look at the response of the satellite cells to various types of Exercise Training we can take notice of the significance of the effectiveness of Eccentric resistance exercise compared to other types of resistance exercises. It is a fact that among the resistance exercise, the highest priority is given to Eccentric resistance exercise. In a study conducted by Heinemeier and collaborators (2007), investigations were made regarding the effectiveness of various types of resistance exercise on the expression of Myostatin and IGF-1 isoforms in the muscles and tendons of mice. The interesting point in their research was that their results indicated that reduction in the expression rate of the Myostatin gene and increase in the expression rates of IGF-1 and MGF were more evident in response to Eccentric Resistance Exercise compared to isometric and concentric resistance Exercise.

The most suitable protocol for the increasing of the number of satellite cells is Eccentric Resistance Exercise which enforces its effects through the activation of the hormone system (IGF-1) and the immune system (IL-6) and growth factors (HGF and MGF) (Begue and collaborators, 2013; O'Reilly and collaborators, 2008).

It is believed that during extreme Eccentric Resistance Exercise, high mechanical tension is the primary incentive for activation of the satellite cells and the subsequent hypertrophy of the skeletal muscle; however, it may also result in tissue damages (MacDougall, 1986).

In this regard, alongside Exercise Training, nutrition is considered as an important tool that reduces fatigue and facilitates the recovery. Many athletes and people who are subjected to athletic, military and other heavy physical activity stresses, constantly seek several solutions for improvement of their athletic performance and physical conditions; consuming supplements being one nutritional solution (Maughan and Burke, 2011).

Beta-Hydroxy Beta-Methyl Butyrate (HMB) has been known as the main factor of improvement of strength level, body composition and also development of the skeletal muscle mass. HMB is endogenously produced through the metabolism of the leucine (Fitschen and collaborators, 2012).

Most researchers agree that the most suitable dosage of HMB for daily consumption is 3 grams per day. Hence, in order to obtain a proper dosage of HMB, it is necessary to consume supplements (Fitschen and collaborators, 2012).

Several mechanisms have been pointed out for the ergogenic and hypertrophy effects of HMB on skeletal muscles:

1) Increased Sarcolemma integrity
2) Increased metabolic efficiency
3) Increased IGF-1 expression
4) Proteolysis inhibition
5) Protein synthesis stimulation
6) Increased activation of satellite cells and myogenic factors (Lima-Soares and collaborators, 2018).

It has been observed in studies on extreme Exercise Training followed by insufficient recovery that HMB can either reduce the harmful effects of exercise training stress or improve the positive effects of exercise. Declination of strength, power and body mass of soldiers have been reported

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

during prolonged military operations. These stresses are usually followed by significant increases in the indices of inflammatory cytokines (Lieberman and collaborators, 2005). It has also been shown that three weeks of consuming HMB during heavy exercise training reduces the inflammatory response while maintaining muscular integrity (Hofffman and collaborators, 2016).

HMB supplement is commercially available in forms of calcium salt or free acid. HMB-CA is available in form of powders and capsules while the HMB-FA is only available in form of liquid containing capsules. Studies have shown that HMB-FA is absorbed faster and hence it can be used before Exercise. Although that HMB-FA has higher plasma levels compared to HMB-CA, there is no urinary excretion difference between them; showing that HMB-FA has a higher tissue absorption (Fuller and collaborators, 2015).

However, we still need more studies comparing these commercially available forms of HMB (Baxter and collaborators, 2011).

Considering the mentioned content and by taking into account the previous studies in this field, it is expected to observe that interacting with intense Eccentric resistance Exercise in addition to consumption of HMB supplement has a more effective role in activation of satellite cells. Hence, the purpose of the present study was to investigate the combined effects of three weeks of consuming HMB-FA supplement and one session of extreme Eccentric resistance Exercise on the rate of activation of satellite cells.

MATERIALS AND METHORDS

Eighty Sprague Dowley male rats aged eight weeks and weighting 200□20 grams were first procured and then, familiarized with ladders while also taking supplements. Five of the eighty rats could not tolerate the consumption of supplement and were hence left aside from the study. the remaining rats were randomly assigned to four groups: 1) control group (n= 18); 2) HMB group (n= 19); 3) Exercise group (n= 19); 4) Exercise + HMB group (n= 19).

Design of the Study

The rats were kept in a room provided with automatic temperature adjustment and standard lighting. They were kept in for-four fiberglass cages under normal nutritional conditions and 12/12 hours of light/darkness and an average temperature of 23 ± 2 degrees and a humidity of 40-60%. While keeping and working with the rats, the entire ethical considerations of working with lab animals were adhered to. Their food and water was supplied throughout the entire length of the study. Their cages were constantly being cleaned and air temperature and humidity were also controlled throughout the day-night cycle.

Firstly, the experimental groups of the study participated in three sessions of familiarization with ladders and also their 1Rm levels were measured. In order to obtain rats' 1RM, first of all a weight (50% of their body weight) was attached to their tales (Sukho and Roger, 2003).

If they could successfully descend from the ladders, the weight of the weights attached to their tales was increased. In every repetition after every successful descending, 10 grams were added to the weight of weights until the rats were exhausted and could no longer do so. The weight of the weights before reaching the exhaustion was considered as 110-130% of the 1RM. The length of resting time between each two reps was two minutes. The height of the ladder was one meter with adjustable slope.

Supplement Consumption Protocol

In previously conducted studies various different dosages and time lengths of consumption of HMB have been made use of. In most studies on human subjects, HMB is prescribed along with the breakfast, lunch and dinner meals irrespective of the time of effectuation of Exercise Training. In our study, the supplement was solved in 1 milliliter of distilled water and was then fed to rats on a daily basis for three weeks. In experimental groups, the dosage of 340 milligrams per each kilogram of bodyweight was taken into account.

Exercise Protocol

Once the rats were familiarized with the ladder and already fed with the supplement for three weeks, a session of extreme Eccentric Resistance Exercise including descending from a ladder with a slope of 80% while a 100-110% of 1RM weight being attached to the tails of the rats was set in motion. Each rat was placed on the top of the ladder by the researcher and then it had to

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

descend from the ladder for eight times. If the rat was able to complete the entire three sets of the experiment, the protocol was considered as perfectly complete while if the rats were exhausted before finishing the entire three sets and could not bear to complete the protocol even after being shocked for three times, the protocol was considered as incomplete. Each rat rested two minutes between the sets.

Immunohistochemistry

The rats were killed 24 hours after the finishing of the exercise protocol. Before mortification, the rats were sedated with a high dosage of Ketamine and Xylaseine and afterwards their triceps were removed from their arms. In addition a physiology special serum was used to wash the tissue until reaching tissue transparency. Additionally, for the purpose of postfix, the tissue was submerged in a 10% formalin solution with the fixative solution being changed 24 hours later. Afterwards, the phases of removal of tissue sections were completed in the following order:

Dehydration: for the purpose of dehydration of tissues, increasing percentages of alcohol were made use of. Afterwards, approximately 70-80 minutes after dehydration, the process of paraffin penetration process was executed. It is worthy of mentioning that the entire tissue preparation process mentioned here was performed using a Dide-Sabz-Iran DS-2080 tissue processor in 24 hours.

In the next phase, the samples were molded using paraffin and then the samples were kept in room temperature until the time of immunohistochemistry analysis. Once the samples were collected, the section of the muscle was serially cut in 6 micrometer slices using a microtome machine; slices of muscle were then placed on Plus+ lams and were kept in room temperature for one day.

After this phase, immunohistochemistry staining was done on the lams and then the tissue sections were both quantitatively and qualitatively scrutinized using an optical microscope. The paraffin containing lams were afterwards placed in an oven heated to 60 degrees for 45 minutes so that the paraffin content was melted. Afterwards, the immunohistochemistry test was performed in the following briefly presented steps:

-removal of paraffin by Xylol, increasing hydration by ethanol alcohol, inhibition of peroxidases in 3% methanol solution, two times of washing with PBS-T solution, Antigen retrieval using Dako solution, two times of washing with PBS-T solution, Blocking for one hour at 37 degrees using secondary antibody host serum (ab97245), Goat Anti Mouse (HRP), two times of washing with PBS-T solution. For the purpose of staining, the slices were incubated using the primary antibody for 24 hours at 4 degrees. These primary antibodies were primary monoclonal CD5 antibodies (ab6123, Abcam; 1:200) of mouse hosts (Abcam, Cambridge, UK).

Three times of washing with the PBS-T solution, conjugated goat anti mouse secondary antibody with dilution of 1:500 made by the American corporation of Abcam and known with the code of ab97245, two times of washing using the PBS-T solution, incubation using DAB, Hematoxylin for staining of muscular nucleuses, dehydration using 70-100% ethanol solution, washing with Xylo I and II in order to reach further tissue transparency, mounting the lams using an antelan, taking snapshots of the Lams using a Nikon optical microscope and a Nikon camera capable of 20X, 50X and 100X zooms.

Extraction of DNA and Real-time PCR

In order to investigate the expressions of the CD56 and MyoG genes in each group, the tissues were scrutinized through the application of the real-time PCR method. In this regard, the total RNA of the cellules collected in each group was extracted and then it was concerted to cDNA using the reverse copying enzyme. For the purpose of the genomic DNA, the yielded cDNA was treated with the DNase I enzyme and afterwards it was proliferated using the real-time PCR.

In order to execute molecular investigations on the level of gene expression, first of all the RNA extracted from the tissues of the entire groups of the study were investigated using the Germany made device of Kia-Gene. The purity of the extracted RNA can be investigated using spectrophotometry and the characteristic of absorption of light at the wavelength of 260 nanometers in addition to making use of the following relation:

$$C\ (\mu g/\mu l) = A260 \times \varepsilon \times d/1000$$

C: compactness

A260: light absorption at the wavelength of 260NM

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

ε: molar offset factor for RNA equals 40 and for DNA equals 50
d: dilution factor

The amount of impurity caused by presence of proteins or DNA in the RNA solution can be calculated through the calculation of the A260/A280 ratio. In pure RNA samples, this ratio is equal to 2±0.15 and for pure DNA samples it is equal to 1.8±0.15. If the calculated ratio was smaller than the standard value, then it could be concluded that the RNA is polluted with proteins.

Statistical Analyses

In previous studies, the numbers of stained satellite cells on the section of the muscle have mostly been calculated per muscular fibers (Nielsen and collaborators, 2012; Verdijk and collaborators, 2009; Wernbom and collaborators, 2013) [14-16], per each micrometer of the section of the muscular fiber (Nielsen and collaborators, 2012; Verdijk and collaborators, 2009) and, per percentage of changes of these cells (Verdijk and collaborators, 2009).

In order to calculate the percentage of changes of satellite cells against muscular nucleuses, the following formula has been proposed by Verdijk and collaborators (2009):

100 x (number of muscular nucleuses + number of SC+CD56) / number of SC+CD56 – Percentage of SC+CD56

Analysis of the results of q-PCR

The method of 2−ΔΔCT has been used for the investigation of relative/quantitative expression of MyoG and NCAM genes. The entire analyses have been separately run for each group.

Relative fold change in gene expression = 2-ΔΔCT

ΔCT = CT target gene – CT reference gene
ΔΔCT = ΔCT test sample – ΔCT Control sample

Statistical Methods

The first part of statistical methods is dedicated to the description of characteristics of the subjects and the raw data of the study which was carried out through the application of descriptive statistics. Afterwards, research hypotheses were tested using inferential statistics at the significance level of 0.05. in the first part, in order to discuss the normality of data distributions, the K-S test was used.

For the CD56 variable, variance means were homogenous and hence parametric tests were used in the next steps. In this regard for the purpose of inter group comparisons the one way ANOVA test was used and afterwards; in order to compare the differences between the groups, the Tukey post hoc test was used.

However since in the variables of MyoG and NCAM, the variance averages were heterogeneous, non-parametric tests were used afterwards. For these variables, the Kruskal-Wallis test was used for the purpose of evaluation of inter-group differences while the Mann-Whitney test was used for the investigation of intra-group differences. The entire statistical operations have been completed using the SPSS v.23.0 and the entire diagrams have been drawn using the EXCEL 2010 software.

Immunohistochemistry Analysis

Results of the one way ANOVA test showed that the groups have statistically significant differences in terms of CD56 (P= 0.001) (diagram 1). In this regard, the Tukey test's results have shown that compared to the control group, the level of CD56 was significantly higher in the groups of exercise, HMB and, supplement + HMB (P= 0.001). In addition compared to the HMB group, the level of CD56 was significantly higher in exercise and exercise + HMB groups (P= 0.001). Nevertheless, a difference has been observed in the level of CD56 between the exercise and exercise + HMB groups (P= 0.001). According to these results, the entire mentioned experimental interventions have resulted in a statistically significant increase in the level of CD56. The other point worthy of mentioning is that it seems that consuming HMB supplement along with participation in Exercise Training has been the most effective (figure 1).

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

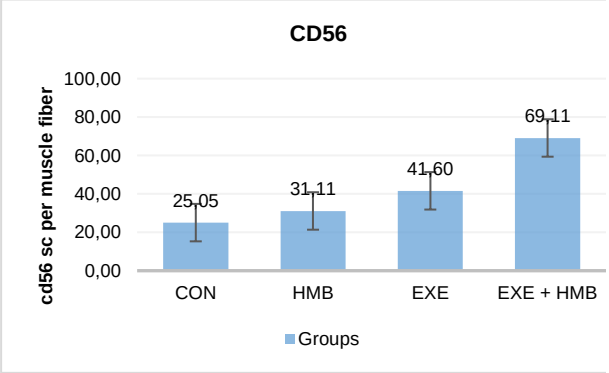
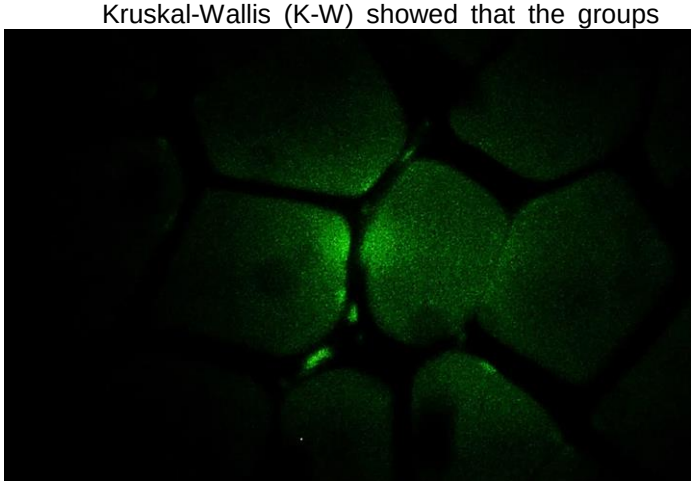


Diagram 1 - Changes of CD56 in groups; significant difference with the control group; significance difference with the Exercise group, Con: control group; HMB: supplement group, EXE: exercise group, EXE+HMB: exercise + HMB group.

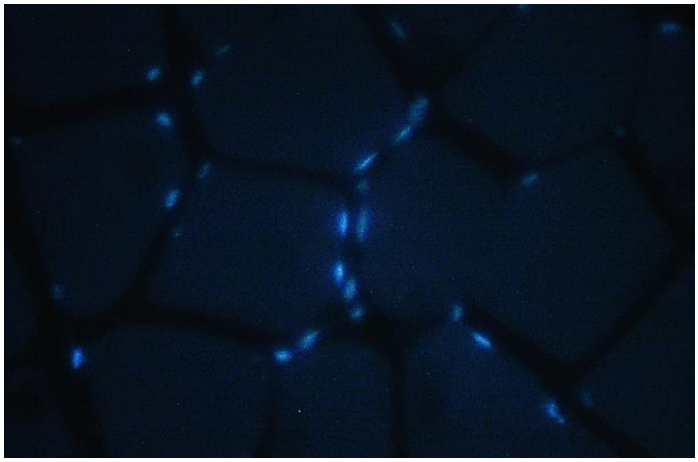
mRNA Expression in the Skeletal Muscle

Results of the non-parametric test of Kruskal-Wallis (K-W) showed that the groups has different levels of MyoG expression in the triceps (P= 0.001) (diagram 2). In the paired comparisons of the groups using the Mann-Whitney test it was revealed that compared to the control group, the expression of the MyoG gene in the triceps tissue was statistically significantly higher in the exercise, HMB, and exercise + HMB groups (P= 0.001).

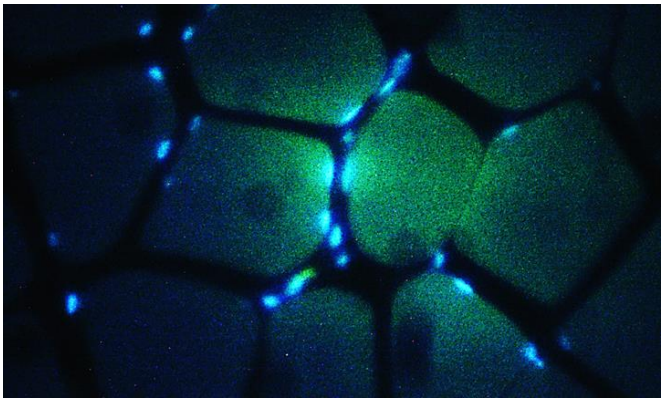
However, no statistically significant difference was observed between the exercise and exercise + HMB groups in terms of MyoG gene expression (P= 0.354). on the other hand, while comparing the HMB and exercise + HMB groups (P= 0.001) and the exercise and exercise + HMB groups (P= 0.023), no statistically significant differences were recorded. Overall, the results show that the entire experimental interventions have caused increases in the expression of the MyoG gene in the triceps tissue while it seems that the exercise + HMB intervention has been the most effective.



A: Nuclei stained by DAPI



B: Primary antibody to CD56



C: Merge A&B

Figure 1 - Identification of the nucleus and satellite cells of the skeletal muscle.

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

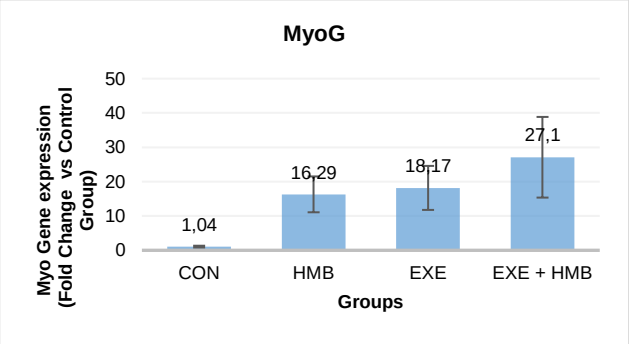


Diagram 2 - Changes in the expression of the MyoG gene in the triceps tissue; significant difference with the control group; significant difference with the EXE+HMB group, Con: control group; HMB: supplement group, EXE: exercise group, EXE+HMB: exercise + HMB group.

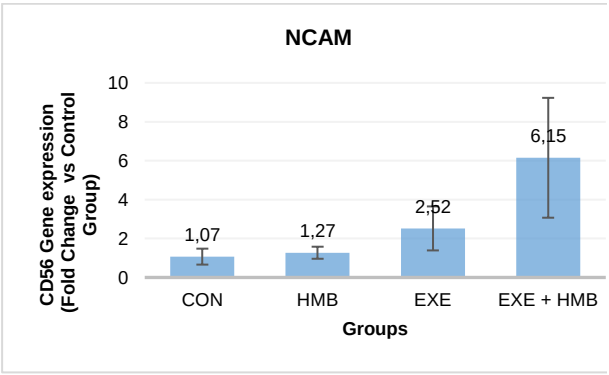


Diagram 3 - Changes in the expression of the NCAM gene in the triceps tissue; significant difference with the control group; significant difference with the EXE+HMB group, significant difference with the EXE group, Con: control group; HMB: supplement group, EXE: exercise group, EXE+HMB: exercise + HMB group.

Results of the non-parametric test of Kruskal-Wallis showed that the groups of the study were statistically significantly different in terms of expression of NCAM gene in the triceps (P= 0.001) (diagram 3). Paired comparisons showed that compared to the control group, the expression of the NCAM gene in the triceps of the entire groups of exercise, HMB, and exercise + HMB was higher; however the difference was not statistically significant (P= 0.081). On the other hand, exercise and exercise + HMB groups have shown a statistically significant increase in the expression of the NCAM gene. Nevertheless, a statistically significant differences was observed between the HMB and exercise groups (P= 0.001).

In addition, compared to the HMB and exercise groups, the exercise + HMB group was found to have a statistically significantly higher NCAM gene expression (P= 0.001). Overall, the results have shown that resistance Exercise and resistance Exercise in addition to consumption of HMB supplement increase the expression of the NCAM gene.

DISCUSSION AND CONCLUSIONS

In a specific part of the present study, we tried to analyze the effects of effectuation of extreme Eccentric Resistance Exercise on the activity of satellite cells. Results have shown that the former Exercise can have a statistically significant and positive effect on the rate of activation and increase of satellite cells. In this regard, there are certain consistent and inconsistent studies which will be investigated in the following. The reason behind the inconsistency of the results can be traced back to adoption of different Exercise modalities, different Exercise intensity and volume and also the sampled muscles.

In a study conducted by Nederveen and collaborators (2015), three different modalities of resistance exercise, high intensity interval exercise and, aerobic exercise were scrutinized among elderly subjects. The all pointed to increased number of satellite cells while stating that the most effective protocol was the resistance exercise and compared to the aerobic exercise, it was stated that the high intensity interval exercise were more effective.

It is correct that even traditional aerobic exercises can induce increased satellite cells proliferation, but this response is observed to be weaker compared to the response triggered by resistance exercises and high intensity interval exercises. The reason behind the fact that resistance exercise triggers a faster response is that there is an anabolic response caused by reduction of expression of Myostatin and SMAD3, which has only been observed in subjects that participated in resistance Exercise protocols.

This might be a reflection of the higher recruitment of the fast-twitch fibers (type II) of skeletal muscles. It might also seem important in the sense that in our study, the muscle under investigation was the triceps that has a higher ratio of fast-twitch muscle fibers

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

compared to the muscles investigated by other studies.

Nevertheless, increased expressions of Myostatin and SMAD3 have inhibitory effects on MyoG. It is true that majority of exercise protocols are able to limit the reduction of Myostatin expression in time, but it is the resistance exercises that have the highest temporal effects; showing the necessity of providing a rapid response to the processes of repairing, adaptability and regeneration after effectuation of resistance exercises (Nederveen and collaborators, 2015).

In addition, previous studies show that after effectuation of resistance activities, the number of satellite cells increase in the type II fibers (Verney and collaborators, 2008). Such a response has not been observed in the fast-twitch fibers when traditional aerobic exercises were effectuated (Snijders and collaborators, 2011); however there have been developments in the slow-twitch fibers (Fry and collaborators, 2014). The compatibility of the fibers during various Exercise Training can cause differences in the responses of the pools of satellite cells.

Regarding simultaneous Exercise Training, a study conducted by Babcock and collaborators (2012) investigated the effect of simultaneous resistance and aerobic exercises and their results have shown that the response of the satellite cells to the resistance exercise is suppressed if the aerobic exercises are effectuated right afterwards. The reason was found to be that aerobic exercises probably change the conditions of the physiological environment in a way that the importance of the muscular nucleus for the growth of fibers of skeletal muscles and the subsequent strength production capacity is underestimated.

The most well-known model used for infliction of structural damages and further determination of the roles of satellite cells in the restoration of muscular fibers, is effectuation of Eccentric exercises. In humans' skeletal muscle, several positive changes have been recorded in the number of satellite cells during the first days after effectuation of one session of Eccentric exercise [22-24]. It has been shown that Eccentric muscular contractions are powerful stimulants for activity of satellite cells.

Cermac and collaborators (2013) ran 300 knee-extension machine Eccentric contractions on their subjects and the results showed a statistically significant increase in the number of satellite cells after the effectuation of the mentioned Eccentric exercise; which is consistent with the results of the present study. However, they reported a 73 percent increase in the content of satellite cells of the fast-twitch fibers while we observed a 40 percent increase in the content of the satellite cells. This difference can be due to the higher volume of prescribed exercise in their study.

On the other hand, Hyldahl and collaborators (2014) reported that after the effectuation of resistance exercises, the proliferation of satellite cells was halted for a short time in the first 24 hours after the training. They ran the knee extension exercise for both the concentric and Eccentric groups separately. However, they stated that the increase in the rate of satellite cells was made between 24-72 hours after the effectuation of the Eccentric protocol. The interesting point in their study was that they did not record any increase in the content of satellite cells in the Concentric exercise group.

Hyldahl and collaborators (2014) also did not report any differences between the responses of any of the types of the muscle fibers regarding satellite cells. The highly important issue here is that it seems that low intensity Eccentric exercise may not be able to trigger a response as large as the one triggered by intense and extreme Eccentric resistance exercise.

The debate of importance of intensity and volume of sports protocols for suitability of effectiveness on the activity of satellite cells has become very bold during the past few years. In the study conducted by Parise and collaborators (2008), it was observed that with maximal exercise until exhaustion on treadmills, the amounts of satellite cells were increased between the 48-96 hours after the exercise.

On the contrary, in the study conducted by Smith and collaborators (2001) no such response was observed; comparing the results of these two studies while also considering for their adopted exercise protocols shows that there is a high importance given to the intensity and time length of Exercise Training.

Kurosoka and collaborators (2012) divided rats into three groups and subjected them to exercise protocols for ten weeks (running on treadmill at high intensity but for a short time, at low intensity for a long time and, at low intensity and for a short time and). They showed that compared to time length, the

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

increase in the amount of satellite cells is more dependent on the intensity and that the development of the satellite cell pool may require a threshold intensity in order to be able to stimulate the type II fibers.

On the other hand, some other researchers have also pointed to the high importance of volume of prescribed resistance exercises and maintain that high-volume resistance exercises with low intensity is more effective on the development of the satellite cell pools compared to intense and low intensity resistance exercises (Burd and collaborators, 2010).

In this regard, Burd and collaborators (2010) investigated the effects of effectuation of resistance exercises under three different intensities of 30% 1RM until exhaustion, 90% 1RM until exhaustion and 30% 1RM with a working load equal to the 90% 1RM. 24 hours after the effectuation of the exercises, it was revealed that the group that participated in 30% 1RM until exhaustion protocol has higher factors of differentiation of satellite cells compared to the other two groups.

Although in the present study the administered intensity was higher but considering the number of sets of the exercise protocol, 8 reps of the total protocol is still considered as a medium volume and hence it seems that in order to better stimulate the satellite cells, both the intensity and volume factors must be desirably set. Another important factor to investigate can be the age of the subjects. In this regard many studies have reported that as age goes up, the number of satellite cells decreases (Kadi and collaborators, 2004). There also some other studies that have not reported any decrease in the latter due to the former (Dreyer and collaborators, 2006).

Newer studies have pointed to the mitigation of satellite cells in type II fibers. The process of aging not only is accompanied by mitigated satellite cells pool, but also due to aging the responsiveness of the satellite cells pool deteriorates after one session of exercise training (McKay and collaborators, 2012).

Snijders and collaborators (2014) carried out a study and showed that in elders the response of the satellite cells to one session of exercise training is delayed while the levels of factors of differentiation and proliferation of satellite cells are higher in youths compared to elderlies. On the other hand, they have also pointed out that increased adjustment of the Myostatin produced through exercise training in elders remains high for a longer length of time; showing that for having a better hypertrophy, the elderly requires longer recovery times between their exercise sessions.

In the present study, first of all it should be reminded that the test subjects were not humans; in addition the rats were young and one reason for observing the activation of satellite cells can lie in the age of the test subjects.

Another purpose of the present study was to investigate the effects of three weeks of consumption of HMB-FA supplement on the activation of satellite cells. Results showed that HMB alone can trigger increased activation, differentiation and proliferation in satellite cells. Several studies have investigated the potential effects of HMB on satellite cells, myoblast and myo-tubes (Always and collaborators, 2013; Aversa and collaborators, 2012; Kornasio and collaborators, 2009; Vallejo and collaborators, 2016).

These studies have pointed to the potential effects of HMB on the direct stimulation of satellite cells of the muscles. The results of the present study in this sense are consistent with the majority of previously conducted studies; however, the results are inconsistent with the results of the study conducted by Munroe and collaborators (2016).

Monroe and collaborators (2016) investigated the effects of consumption of 450mg/kg of HMB for 5.5 weeks on the muscular strength, neurogenesis and cognition of young and old rats. At the end it was revealed that no difference was made in the indices of number of satellite cells and hence, it was concluded that either the effect of the supplement on the satellite cells is selective, or it just would not happen in 5.5 weeks.

In addition, they stated that in the absence of an external incentive, HMB cannot have any effects on the hypertrophy response and muscle weight of rats (Kim and collaborators, 2012).

This is while the present study has showed that three weeks of consumption of HMB alone can also increase the number of satellite cells. These studies do not differ much in terms of prescribed HMB dosage since both studies have selected a medium dosage of between 350 and 450 milligrams per each kilogram of body weight. It is worthy of mentioning that unlike the present study, the type of the HMB used in this study was the

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

calcium type. As it was mentioned earlier, these two types of HMB are different in terms of bioavailability and solubility and hence they may trigger different anabolic and anti-catabolic responses.

Another important issue regarding the consumption of the HMB supplement is time of consumption. The recommended time length is above two weeks which has been cared for in most of the previously conducted studies. In addition to the consumption time, the daily consumption schedule is also an effective variable. In the present study, the supplement was consumed twenty minutes before the effectuation of the one-session Eccentric resistance exercise; while some other studies have failed to consider for this issue and have prescribed the dosage in varying times.

Kornasio and collaborators (2009) carried out a study and concluded that at least in the cell culture environment, HMB is able to enter the offset satellite cells into the cellular cycle irrespective of the age. By increasing the expression of IGF-1, consumption of HMB increases the expression of MRFs. Consequently, Interleukin-4 and interleukin-13 are expressed and hence cell fusion is improved.

Overall, the effects of HMB on the muscular strength and performance can have protecting role on satellite cells in cases such as muscular damage. Alway and collaborators (2013) investigated the effect of HMB on the activation and proliferation and differentiation of satellite cells after HLS. Their results showed that satellite cells play significant roles in atrophied muscles in terms of restoration of strength and performance of the muscles. In this regard, HMB supplement with its effects on the activity of satellite cells can be effective and beneficial in this context. However it has also been pointed out by them that the positive effects of HMB were only tangible in addition to load (Always and collaborators, 2013).

Ultimately, in the present study, by the consumption of HMB in combination with effectuation of extreme Eccentric resistance exercises we have witnessed a higher increase in the level of satellite cells. This shows the effective role of selected supplement in the response of satellite cells to Eccentric exercises. No previous study has investigated the effect of short-term consumption of HMB on Eccentric exercises and satellite cells and the majority of the studies have considered longer time lengths such as 8 and 10 weeks.

Kim and collaborators (2010) investigated the effects of HMB on skeletal muscles of aged female rats during ten weeks of extreme resistance training. In spite of the significant increase in the expression of the IGF-1 in the HMB group and the significant increases in the MyoG and MGF expressions in both HMB and non-HMB groups compared to the control group, no statistically significant difference was observed between the HMB and non-HMB groups in terms of the number of satellite cells. They concluded that intensity of exercise protocol was enough for the activation of satellite cells and covered for the potential effects of the supplement.

Still, this study has made use of resistance training while the present study has emphasized on Eccentric resistance training. According to previous reports, Eccentric resistance training is a more robust incentive for stimulation of activity of satellite cells. On the other hand, the lengths of running the protocol and the types of consumed HMB were different between these studies and it seems that first of all, it is the type of exercise training that plays a very crucial role in observing a response from satellite cells (Kim and collaborators, 2012), and second, the consumption of HMB supplement is better to be in HMB-FA form which is more rapidly absorbed and lasts longer. Another point that can be investigated in future studies is to answer whether consumption of HMB in combination with Exercise Training with Concentric, Eccentric and other natures will result in different responses or not.

REFERENCES

1-Alway, S.E.; and collaborators. β-Hydroxy-β-methylbutyrate (HMB) enhances the proliferation of satellite cells in fast muscles of aged rats during recovery from disuse atrophy. Experimental gerontology. Vol. 48. Num. 9. p. 973-984. 2013.

2-Aversa, Z.; and collaborators. β-Hydroxy-β-methylbutyrate (HMB) prevents dexamethasone-induced myotube atrophy. Biochemical and biophysical research communications. Vol. 423. Num. 4. p. 739-743. 2012.

3-Babcock, L.; and collaborators. Concurrent aerobic exercise interferes with the satellite cell response to acute resistance exercise. American Journal of Physiology-Regulatory,

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

Integrative and Comparative Physiology. Vol. 302. Num. 12. p. R1458-R1465. 2012.

4-Baxter, J.H.; and collaborators. Direct determination of β-hydroxy-β-methylbutyrate (HMB) in liquid nutritional products. Food Analytical Methods. Vol. 4. Num. 3. p. 341-346. 2011.

5-Begue, G.; and collaborators. Early Activation of Rat Skeletal Muscle IL-6/STAT1/STAT3 Dependent Gene Expression in Resistance Exercise Linked to Hypertrophy. PloS one. Vol. 8. Num. 2. p. e57141. 2013.

6-Burd, N.A.; and collaborators. Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. PloS one. Vol. 5. Num. 8. p. e12033. 2010.

7-Cermak, N.M.; and collaborators. Eccentric exercise increases satellite cell content in type II muscle fibers. Med Sci Sports Exerc. Vol. 45. Num. 2. p. 230-237. 2013.

8-Crameri, R.M.; and collaborators. Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. The Journal of physiology. Vol. 558. Num. 1. p. 333-340. 2004.

9-Dreyer, H.C.; and collaborators. Satellite cell numbers in young and older men 24 hours after eccentric exercise. Muscle & nerve. Vol. 33. Num. 2. p. 242-253. 2006.

10Fitschen, P.J.; and collaborators. Efficacy of β-hydroxy-β-methylbutyrate supplementation in elderly and clinical populations. Nutrition, 2012.

11-Fry, C.S.; and collaborators. Fibre type-specific satellite cell response to aerobic training in sedentary adults. The Journal of physiology. Vol. 592. Num. 12. p. 2625-2635. 2014.

12-Fuller, J.C.; and collaborators. Comparison of availability and plasma clearance rates of β-hydroxy-β-methylbutyrate delivery in the free acid and calcium salt forms. British Journal of Nutrition. Vol. 114. Num. 9. p. 1403-1409. 2015.

13-Heinemeier, K.M.; and collaborators. Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. Journal of Applied Physiology. Vol. 102. Num. 2. p. 573-581. 2007.

14-Hoffman, J.R.; and collaborators. β-Hydroxy-β-methylbutyrate attenuates cytokine response during sustained military training. Nutrition research. Vol. 36. Num. 6. p. 553-563. 2016.

15-Hyldahl, R.D.; and collaborators. Satellite Cell Response to a Repeated Bout of Eccentric Contractions in Human Skeletal Muscle: 2386 Board# 91 May 30, 9. Medicine & Science in Sports & Exercise. Vol. 46. Num. 5S. p. 640. 2014.

16-Kadi, F.; and collaborators. The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. The Journal of physiology. Vol. 558. Num. 3. p. 1005-1012. 2004.

17-Kim, J.-S.; and collaborators. β-hydroxy-β-methylbutyrate did not enhance high intensity resistance training-induced improvements in myofiber dimensions and myogenic capacity in aged female rats. Molecules and cells. Vol. 34. Num. 5. p. 439-448. 2012.

18-Kornasio, R.; and collaborators. β-hydroxy-β-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. Vol. 1793. Num. 5. p. p. 755-763. 2009.

19-Kurosaka, M.; and collaborators. Satellite cell pool enhancement in rat plantaris muscle by endurance training depends on intensity rather than duration. Acta Physiologica. Vol. 205. Num. 1. p. 159-166. 2012.

20-Lieberman, H.R.; and collaborators. Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. Biological psychiatry. Vol. 57. Num. 4. p. 422-429. 2005.

21-Lima-Soares, F.; and collaborators. HMB Supplementation: Clinical and Performance-Related Effects and Mechanisms of Action, in Sustained Energy for Enhanced Human Functions and Activity, Elsevier. p. 363-381. 2018.

Revista Brasileira de Nutrição Esportiva
ISSN 1981-9927 ***versão eletrônica***

Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício

w w w . i b p e f e x . c o m . b r / w w w . r b n e . c o m . b r

22-MacDougall, D., Morphological changes in human skeletal muscle following strength training and immobilization. Human Muscle and Power. 1986.

23-Maughan, R.J.; Burke, L. M.; Practical nutritional recommendations for the athlete. Nestle Nutr Inst Workshop Ser. Vol. 69. p. 131-49. 2011.

24-McKay, B.R.; and collaborators. Satellite cell number and cell cycle kinetics in response to acute myotrauma in humans: immunohistochemistry versus flow cytometry. The Journal of physiology. Vol. 588. Num. 17. p. 3307-3320. 2010.

25-McKay, B.R.; and collaborators. Myostatin is associated with age-related human muscle stem cell dysfunction. The FASEB Journal. Vol. 26. Num. 6. p. 2509-2521. 2012.

26-Munroe, M.; and collaborators. Impact of β-hydroxy β-methylbutyrate (HMB) on age-related functional deficits in mice. Experimental gerontology. Vol. 87. p. 57-66. 2017.

27-Nederveen, J.; and collaborators. The effect of exercise mode on the acute response of satellite cells in old men. Acta Physiologica. Vol. 215. Num. 4. p. 177-190. 2015.

28-Nielsen, J.L.; and collaborators. Proliferation of myogenic stem cells in human skeletal muscle in response to low load resistance training with blood flow restriction. The Journal of physiology. Vol. 590. Num. 17. p. 4351-4361. 2012.

29-O'Reilly, C.; and collaborators. Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans. Muscle & nerve. Vol. 38. Num. 5. p. 1434-1442. 2008.

30-Parise, G., McKinnell, I.W.; Rudnicki, M. A. Muscle satellite cell and atypical myogenic progenitor response following exercise. Muscle & nerve. Vol. 37. Num. 5. p. 611-619. 2008.

31-Smith, H.K.; and collaborators. Exercise-enhanced satellite cell proliferation and new myonuclear accretion in rat skeletal muscle. Journal of Applied Physiology. Vol. 90. Num. 4. p. 1407-1414. 2001.

32-Snijders, T.; and collaborators. Continuous endurance-type exercise training does not modulate satellite cell content in obese type 2 diabetes patients. Muscle & nerve. Vol. 43. Num. 3. p. 393-401. 2011.

33-Snijders, T.; and collaborators. The skeletal muscle satellite cell response to a single bout of resistance-type exercise is delayed with aging in men. Age. Vol. 36. Num. 4. p. 9699. 2014.

34-Sukho, L.; Roger, F. Resistance training, muscle mass and function in the rats. Journal of Exercise Physiology. Vol. 62. p. 80-87. 2003.

35-Vallejo, J.; and collaborators. Cellular and physiological effects of dietary supplementation with β-hydroxy-β-methylbutyrate (HMB) and β-alanine in late middle-aged mice. PloS one. Vol. 11. Num. 3. p. e0150066. 2016.

36-Verdijk, L.B.; and collaborators. Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type specific increase in satellite cell content in elderly men. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. Vol. 64. Num. 3. p. 332-339. 2009.

37-Verney, J.; and collaborators. Effects of combined lower body endurance and upper body resistance training on the satellite cell pool in elderly subjects. Muscle & nerve. Vol. 38. Num. 3. p. 1147-1154. 2008.

38-Wernbom, M.A.; and collaborators. Acute low-load resistance exercise with and without blood flow restriction increased protein signalling and number of satellite cells in human skeletal muscle. European journal of applied physiology. Vol. 113. Num. 12. p. 2953-2965. 2013.

E-mails:
elias.k91@gmail.com

Received for publish in 09/16/2018
Accept in 01/05/2019