ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 14 (6): 267-273, 2015
ISSN 1682-296X

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/biotech.2015.267.273

Research on the Body Weight and Intramuscular MG29 Protein Expression Affected by Different Load Weights: A Treadmill Test of Old SPFSD Rat

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A R T I C L E I N F O
Article History:
Received: September 11, 2015
Accepted: November 02, 2015

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A B S T R A C T
In this study, by exploring the effect of different weights in treadmill training upon the body weight and intramuscular MG29 protein expression of old SPFSD rat. The experiment groups of rats were given with 12 weeks of treadmill training experiment with 20, 40 and 60% of max bearing weights, respectively and each experimental groups of rats will be weighed after 6 weeks of treadmill training for the first time and after 12 weeks of treadmill training for the second time and weighing results will be taken into analysis. After 12 weeks, rectus femoris of the rats in all groups were obtained and made into freezing microtome sections by regular production method, the we stained them with HE dye and observed the morphological characteristics of the skeletal muscles; In addition, we measured the levels of lipofuscin and calcium content of rectus femoris muscular homogenate and detected the MG29 protein expression level using Western Blot method. According to the two tests conducted respectively after 6 and 12 weeks of training, we can see the body weights of rats in all groups exhibits obvious changes, wherein, the body weights of rats in medium and heavy weight bearing groups exhibits greater decrease ($p<0.01$). The result shows that the weight bearing interval running training with 20 or 40% of max load weights can effectively enhance the expression level of MG29 protein in skeletal muscle of SPFSD rat and slow the aging process of old rat's skeletal muscle.

Key words: SPFSD rat, aging, skeletal muscle, weight bearing running, MG29 protein

INTRODUCTION

As people grow older, the structure and function of skeletal muscle will suffer a degenerative change as well as the ageing process of nervous system and cardiovascular system being accelerated (Wen, 2013). Therefore, the analysis on the aging mechanism of skeletal muscle is the key to defer organism aging, only when the skeletal muscle maintains a sound contraction coupling function can other organs perform their normal physiological functions. The function and structure of each organ and system of human body will be aging with the increase of age, that is, the mechanism of aging may exist in various organs and systems. With the rapid development of cell biology and molecular biology, the research on the mechanism of aging has made great achievements. Scientists have put forward many theories on the mechanism of aging (Huang *et al*., 2010).

It is important to note that the muscle is not only the movement of the animal *in vivo* but also an important metabolic organ, which is an important organ to maintain the energy balance of the body. Muscle activity will affect the total body energy metabolism, affecting the metabolism of the three major sources of energy for the lining, fat, protein will also affect to the changes in the body of certain hormones, which affect the bone metabolism (Stephen *et al*., 2013). In many parts of the body, muscle relaxation activity can squeeze the vein blood vessels, promote blood back to the heart and accelerate blood circulation, so as to promote the whole body of energy metabolism (Jin *et al*., 2011). In addition to this, the muscle in the maintenance of body posture and maintain the stability of the joints also plays an irreplaceable role. Many studies have demonstrated that a person's muscle strength is at the highest level of a lifetime in one year while the muscle strength will decrease significantly with age. A heart research institute in Copenhagen found that, generally from 50-70 years of age, muscle strength decreased by about 30%. A group of 80 year old people's knee strength is lower than the other group of 70 years old, 30%. In a group of forces test, it can be found that the force from the age of 80 began to decline significantly, only equals to half of the age of 47 (Li *et al*., 2009). Emanuele *et al*. (2013) research old women in 30, 50 and 70 years old, the maximum isometric contraction, the results showed that the three groups of the test muscle contraction reached its maximum force with age and the difference between the 50 and 30 years old group has reached a very significant level. Liu-Ambrose *et al*. (2012) tested older people in 19-93 years old and make regression analysis of the peak torque of muscle mass. The results showed that the muscle mass of the upper limb muscle mass was significantly higher than that of the lower limb and there was no significant difference between male and female. Gatta *et al*. (2014) test old people in 70 and 75 years respectively, the test content including knee flexor and extensor muscle strength of elbow flexion, extension strength and grip strength endurance. The results showed that the knee joint and elbow joint flexion and extension muscle strength were significantly decreased compared with those of the year of the year of the year.

Muscle contraction requires energy consumption and the energy source is the energy metabolism of the muscle itself. Therefore, the motor function and the metabolic function of the muscles are inseparable. But from another point of view, the process of material metabolism and energy metabolism in muscle is important for the total energy metabolism. Numerous studies have shown that the amount of muscle mass will directly affect the basic metabolic rate of the organism, the more muscle mass, the higher the basic metabolic rate, the better the health status, the smaller the possibility of chronic metabolic syndrome (Hammar and Ostgren, 2013). This is a proof of the importance of the effect of muscle on the metabolism of the organism. The World Health Organization and the United States sports medicine have integrated many research results, the old man's muscle function has a very strong training, old man also can improve muscle function through appropriate resistance training and then improve their life self-care ability and health status (Wen, 2013).

Pasini *et al*. (2012) make analysis of 60 older people, people were randomly divided into three groups, namely as control group, low intensity exercise group and high intensity training group. The low intensity group was trained with the intensity of the intensity of the intensity of the repeated training of high intensity training group used both to repeat. The two group of bench press and supine pedal lifting exercises, training three times a week, a total training week. Results compared with control group, the biggest strength training in two groups of subjects have obvious growth, comprehensive strength and bench press and supine leg lift average test shows, big load exercise group and small load strength group respectively, an increase of 17 and 16%. DeRuisseau *et al*. (2013) make 58 healthy aged men (65-78 years) were divided into three groups. They were divided into three groups: low intensity, medium intensity and high strength. The results were 6 times a week.Vasques *et al*. (2012) confirms this point from the other side. A comparative study of the fine structure and metabolic function of the young and old aged rats was carried out. After 8 weeks of extreme speed, the limit of the old group increased by 140% and the young group only increased by 92%.

Researches show that the MG29 protein and contraction coupling function of skeletal muscle are significantly correlated with the organism aging process (Li *et al*., 2009). Some researches have proved the trainability of the sarcopenia muscle, even though the mechanism within remained unknown (Qu and Zhang, 2010). Since the intramuscular MG29 protein of rats is approximately the same with that of human beings in terms of the muscle structure, in this experiments, the rats were given with weight-bearing interval running training in different amounts of exercises, so as to explore the correlation of intramuscular MG29 protein expression between old people and rats and thus providing new thought for sports-caused anti-aging research.

MATERIALS AND METHODS

Tested rats and grouping: This study was conducted from January, 2014 to September, 2014, we purchased rats from Shanghai Slyke Laboratory Animal Co., LTD at ZhongXin Road, Jiuting Town, Songjiang District, the tested animals are 32 male SPFSD rats aged 20 months with 180.0-200 g weight in health and clean condition. There rats are fed in isolated cages, in free condition of getting food and drink. Results in Table 1 show that after one week of adaptive feed, all rats are divided into four groups, each of which contains 8 rats. For the control group, the average weight is 190.3 g, standard deviation is 5.5, difference to sample ensemble average weight is 1.1, for the less weight bearing group (20% of max weight), the average weight is 199.6 g, standard deviation is 6.1, difference to sample ensemble average weight is 0.4, for the medium weight bearing group (40% of max weight), the average weight is 189.1 g, standard deviation is 5.2, difference to sample ensemble average weight is -0.1, For the heavy weight bearing group (60% of max weight), the average weight is 188.0 g, standard deviation is 5.0, difference to sample ensemble average weight is -1.2, given that the pre-experimental data for all groups: average weight is 189.2 g, standard deviation is 5.3, F value is 0.242, p-value is

Table 1: Comparison of rat's weight data of all groups after one week of experiment (n = 32)

Index	Average value	Standard deviation	Difference	F-value	p-value
Control groups	190.3	5.5	1.1	0.242	0.867
less weight bearing group (20% of max weight)	189.6	6.1	0.4		
medium weight bearing group (40% of max weight)	189.1	5.2	-0.1		
heavy weight bearing group (60% of max weight)	188.0	5.0	-1.2		
Total	189.2	5.3	0.0		

0.867 (close to 1>0.05), all of these indicate the grouping arrangement is very reasonable and there is no statistical significance in the analysis of rat's weight difference (p>0.05), which provides research basis for the experiment.

Training method: In the first stage, we gave one week of adaptive training: all experiment groups of rats will receive 2 weeks of adaptive training without bearing any weight to be specific, rats were put on treadmill with running speed settings of 8 m min^{-1} and 30 min day^{-1} and slope of 0°, the bearing weights for all experiment groups should not exceed 10% of max weight and adaptive training should be given 6 times a day, after that, one another week of adaptive training without bearing any weight will be given to the experiment groups of rats. In the second stage, formal weight-bearing running training will be given: With strip-type of weight tied on the back , the rats of experiment groups were put on the treadmill with running speed settings of 8 m min^{-1} and 30 min day^{-1} and slope of 0°, the bearing weight for 3 experiment groups were set as respectively 20, 40 and 60% of max weight, to be specific: the rats of each experiment group were put on treadmill with weight bearing and running settings was 13 m min^{-1} and slope of 0°, a 3 min of rest will be allowed for every two minutes and 6 running trainings a day. The weight-bearing capacities of low, medium and heavy weight bearing groups were respective 20, 40 and 60% of max weights set in pre-experiment. The above formal weight bearing running training will last for 12 weeks. No weight bearing running training was assigned to rats of control group.

Sample collection: After 12 weeks of formal weight bearing running training, the rats were kept off food or water for 12 h, after measurement of body weight, the rats were dissected under anaesthesia. We selected the midpiece of rectus femoris on the left, removed adipose tissue and cleaned it using normal saline, then freeze-dry it with liquid nitrogen before storing in the freezer at ultra low temperature -80 for later test.

Index inspection: Feed (average value): 5-6 g/100 g body weight/d, Water (average value): 9-11 mL 100 g body weight/d:

- Body weight and other normal indexes: Body weight measurement was conducted once every 3 weeks and we recorded the total feeding amount for each groups of rats, observed the variation situation of normal indexes such as mental state, coat color and behavior
- Skeletal muscle morphology observation: select proper amount of skeletal muscle tissue and make it into freezing microtome sections by normal processing method, stain it with HE dye and put it under optical microscope with amplifications of 100× and 500×, respectively for observation and shooting a photo
- Measurement of lipofuscin in rectus femoris muscular homogenate: select 400 mg of skeletal muscle tissue from rat body of each experiment group and make it into muscular homogenate before conducting fluorescent colorimetry and use the fluorospectro photometer to measure the lipofuscin content in sample of each experiment group
- Measurement of calcium content in rectus femoris muscular homogenate: By adopting the methylthymol blue colorimetric method, we can measured the calcium content in rectus femoris muscular homogenate using ultraviolet spectrophotometer under the instructions prescribed on the kit
- Measurement of MG29 protein expression: the specific process sequence are extraction of protein, measurement of protein concentration, Western Blot detection method; We conducted the gray analysis for the skeletal muscle tissue strips using BANDSCAN software and calculated the gray value using Image J software; ratio of target protein grey value and β-actin grey value was adopted for error correction, so as to obtained the relative amount of target protein.

Statistical analysis: The SPSS16.0 statistical software was adopted, all research data were expressed by mean number and standard deviation, single factor variance analysis was conducted, where the verification level was set as $\alpha = 0.05$ or $\alpha = 0.01$.

RESULTS

Body weight variations after 6 and 12 weeks of training: After 6 and 12 weeks of training, the body weights of rats in all groups (including control group, less, medium and heavy weight bearing groups) are obviously lower than that in pre-training period (p = 0.003, p = 0.001<0.01), wherein, the most significant decrease of body weight existed in heavy weight bearing group and the weight variation was of statistical significance (p<0.01). In the early stage of running training, the food-intake of rats in experiment groups (less, medium and heavy weight bearing groups) were less than

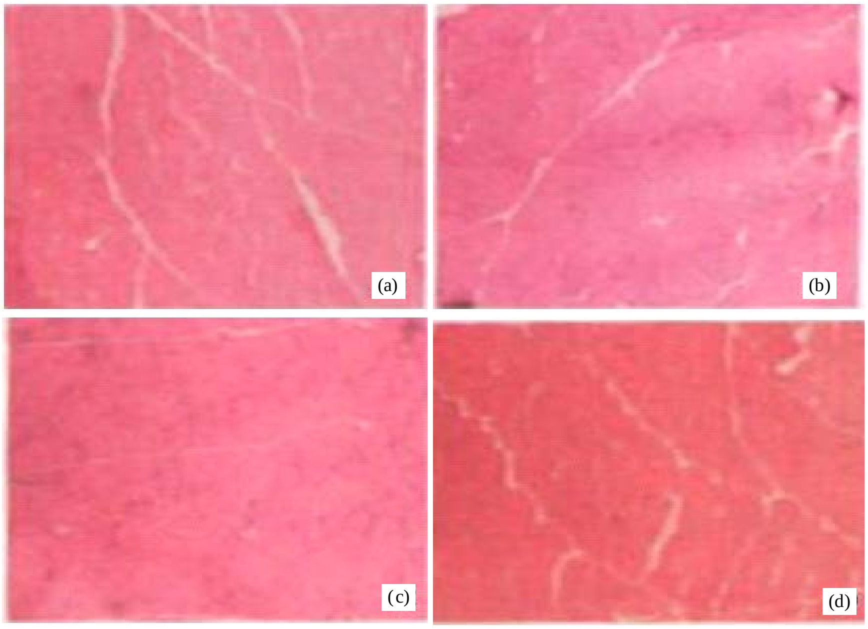

Fig. 1(a-d): Skeletal muscle tissue sections (100×)

Table 2: Comparison of body weights after 6 weeks of training and body weights after 12 weeks of training (n=32)

Index	Average value	Standard deviation	Difference	F-value	p-value
After 6 weeks of training					
Control group	190.5	5.7	5.0	5.929	0.003
Less weight bearing group (20% of max weight)	185.5	5.6	4.1		
Medium weight bearing group (40% of max weight)	181.4	6.2	4.4		
Heavy weight bearing group (60% of max weight)	177.0	8.7	-6.6		
Total	183.6	8.1	0.0		
After 12 weeks of training					
Less weight bearing group (20% of max weight)	192.5	9.8	5.9	7.060	0.001
Medium weight bearing group (40% of max weight)	183.6	3.5	3.7		
Heavy weight bearing group (60% of max weight)	179.9	5.7	3.3		
Total	176.6	8.5	-6.6		
Less weight bearing group (20% of max weight)	183.2	9.2	0.0		

that of control group while with the time extending, the food-intake of rats in experiment groups were significantly larger. The rats in medium and heavy weight bearing groups exhibited some fatigue conditions such as unwillingness to exercise and tachypnea during training intervals and after training and this situation was more significant in heavy weight bearing group. After training, the rats in experiments groups exhibited superior performances over that of control group in terms of mental state and response sensitivity, the result was shown in Table 2.

Change of Skeletal muscle morphology: It can observe among the four groups that different levels of muscle fiber disorderly arrangement, various sizes of muscle cells gradually becoming into round shapes, cell gap increase and white tissues existing in the middle parts of the muscle fiber, wherein, the most significant variation existed in control group followed by heavy weight bearing group, The tightness of muscle fiber arrangements of medium and less weight bearing groups are obviously higher than that of control group and less weight bearing group, the diameters of some cells in medium and heavy weight bearing groups are relatively bigger. (Fig. 1-2).

Measurements of lipofuscin concentration and calcium contents in rectus femoris muscular homogenate: For the rats in control group and heavy weight bearing group, there was no significant change of the lipofuscin concentration in rectus femoris muscular homogenate, so was that for less and medium weight bearing group; the lipofuscin concentrations in control group and heavy weight bearing group were significantly higher than that in less and medium weight bearing group and the variation was of statistical significance ($p<0.05$). The calcium contents in rectus femoris muscular homogenate of three experiment groups were higher than that of control group, especially in the less weight bearing group, the calcium content increased most.

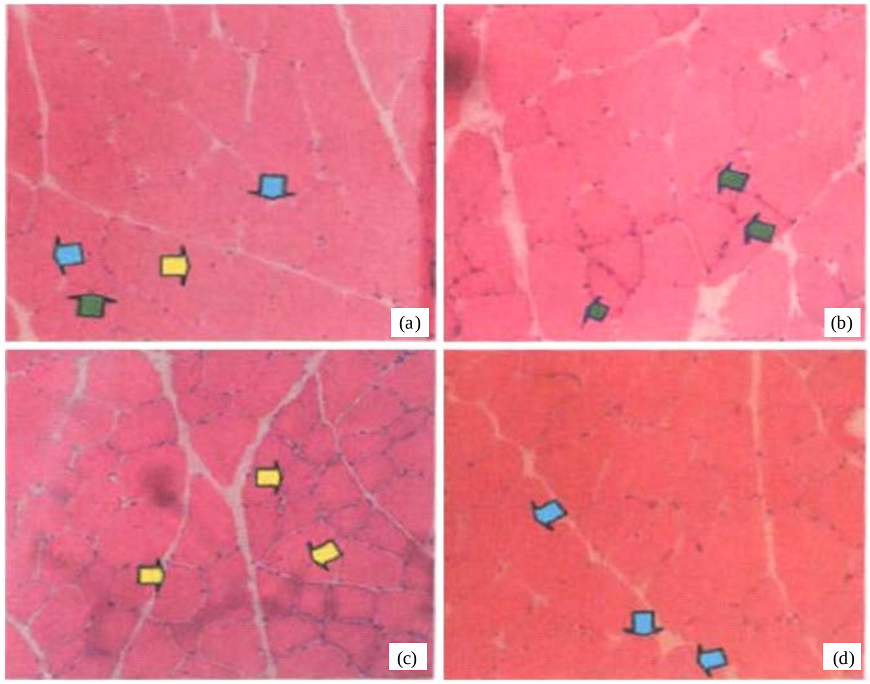

Fig. 2(a-d): Skeletal muscle tissue sections (400 ×), (a) Control group, (b) Less weight bearing group (20% of max weight), (c) Medium weight bearing group (40% of max weight) and (d) Heavy weight bearing group (60% of max weight)

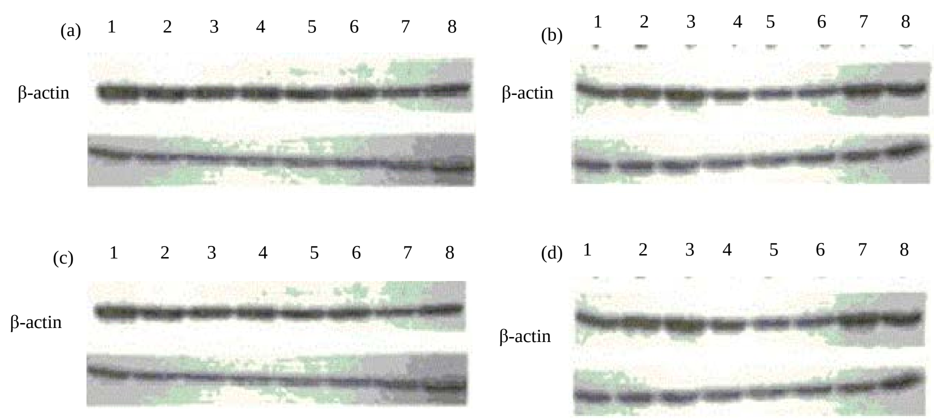

Fig. 3(a-d): Electrophoretogram of the expression level of rectus femoris MG29 protein, (a) Control group, (b) Less weight bearing group, (c) Medium weight bearing group and (d) Heavy weight bearing group

Measurements of expression of rectus femoris MG29 protein: Using Western Blot detection method, we can measure the expression level of rectus femoris MG29 protein and measurement results of all samples were shown in Fig. 3. We can see that there was no significant variation of expression levels of rectus femoris MG29 protein in less and medium weight bearing group, so was that for the control group and heavy weight bearing group. While the rectus femoris MG29 protein expression level in less and medium weight bearing groups were higher than that of control group and heavy weight bearing group and the variation was of statistical significance ($p<0.05$).

DISCUSSION

Researches have proved the beneficial influence of intermittent movement to the myocardium and skeletal muscle, in this research, considering that the old rats are already at a decreased function of cardiovascular system, so the training plan of running for 2 min and rest for 3 min. In addition, through the preliminary experiment, we determined the optimal treadmill parameter settings, times and sets of training, similar with previous studies (Stephen *et al*., 2013). The results show that for experimental groups, the rats exhibit such excellent performances as good appetite, energetic mental state and excellent reaction capacity after training and the less weight bearing group exhibits most significant variation, which indicates the weight bearing running (especially the less weight bearing running) can effectively defer the aging process of rats and old people and enhance the function of nerve and muscle system.

During normal aging process, the cell membrane as an important barrier is the first one to react against the external stimulation factors such as pathogens infection, free radical and injury (Jin *et al*., 2011; Yan *et al*., 2009). In this research, we found a significant increase of round-shape muscle fiber in the skeletal muscle sections of old rats and old people, which may be resulted from the cell membrane's reaction against external simulation. We can see that most muscle fiber changing into round shape in control group while in the less and medium weight bearing group, there are least of muscle fiber changing into round shape as well as relative tighter arrangement of muscle fiber (Pasini *et al*., 2012), which indicates that less and medium weight bearing intermittent training can stimulate to maintain the motor function of most muscle fiber, effectively deferring the aging process of muscle cells. However, there is a considerable amount of muscle fiber changing into round shape in heavy weight bearing group, which may probably be resulted from the injured cell caused by over intensive exercises.

During the natural aging process, the organism needs to keep defensive against the attacks from external free radicals, which leads to the formation of lipofuscin in unsaturated lipids during this defending period (Emanuele *et al*., 2013), therefore, the lipofuscin is a vital marker for judge a organism old or not (Wei *et al*., 2009). In this study, the rectus femoris lipofuscin concentrations in less and medium weight bearing groups are significantly lower than that in control group and heavy weight bearing group, which indicates that the less and medium weight bearing running can effectively decrease the formation of free radicals of the old rats and ole people and enhance the elimination of free radicals, so as to decrease the concentration of free radicals in the muscle tissue and defer the aging process. Moreover in this research, the calcium contents in experiment groups are significantly higher than that of control group and the reason may be that when exercising, the skeletal muscle needs more allocation of calcium ions and more calcium ions are mobilized within (DeRuisseau *et al*., 2013). On the other hand, the calcium content in less weight bearing group is significantly higher than that in medium and heavy weight bearing groups, which indicates the proper amount of exercises can effectively maintain the muscle's motor function.

With the function of regulating external calcium intake, the MG29 protein plays a vital role in maintaining the normal physiological function of the skeletal muscle (Huang *et al*., 2010). In this study, the expression levels of MG29 protein in less and medium weight bearing group are obviously higher than that of the other two groups, which indicates the less and medium weight bearing exercise can effective improve the expression level of skeletal muscle MG29 protein for old people while the expression level of MG29 protein in heavy weight bearing group is significantly lower than that of control group and the reason may be that the over-load exercise negatively affects the mechanism of skeletal muscle contraction, thus decreasing the expression of MG29 protein.

CONCLUSION

In sum, the weight bearing interval running (with 20 and 40% of max weights) can effectively defer the aging process of skeletal muscle for old rats and old people and improve the expression level of skeletal muscle MG29 protein, so as to maintain the normal skeletal muscle contraction, decrease the free radical content in muscle cell and defer the muscle atrophy caused by natural aging process.

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