

The Effect of Running in Shallow Water, Deep Water and Land on Serum Levels of Myostatin and Myogenin in Young Men

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Abstract

Introduction: The researchers believe that muscle growth and hypertrophy are influenced by different factors of myogenin and myostatin. The purpose of this study was to investigate the effect of running in shallow water, deep water and land on serum levels of myostatin and myogenin in young men.

Methods: In this quasi-experimental study, nine young men were selected and ran for 24 minutes in shallow water, deep water and land in three different days with one-week interval. Blood samples were taken in three steps, before, immediately after and 8 hours after each training session. To analyze the findings of the research, Kolmogorov-Smirnov tests, two-way analysis of variance along with Bonferroni's post hoc test were used. ($p \leq 0.05$).

Results: Immediately after running and eight hours after running, serum levels of myogenin increased and serum levels of myostatin decreased ($p \leq 0.05$). Nevertheless, myogenin and myostatin changes in different running conditions and at different periods were not significant ($p \geq 0.05$).

Conclusion: It seems that one session of running in shallow water, deep water and land can increase serum levels of myogenin and decrease serum levels of myostatin in young men. Also, running in shallow water, deep water and land has the same effects on the increase of myogenin and the reduction of myostatin.

Keywords: Myogenin, Myostatin, Running, Land, Water

Introduction

The skeletal muscle is the largest organ in the human body, accounting for 40% of the body mass, and is critical to maintaining and developing health. This dynamic limb shows different responses to adaptation and physiological stimuli and external factors (1, 2). Hence, the growth and increase of muscle mass is one of the adaptations resulting from physical activity. Researchers believe that muscle growth and hypertrophy are affected by various factors, including myogenic regulatory factors (MRFs) and beta (TGF- β) transforming growth factor. Hence, MRFs play a role in regulating the differentiation of the satellite cell and cause transcription of specific skeletal muscle genes such as keratin kinase,

light and heavy chains of myosin and troponin 1 (2) and TGF- β members, are the most important negative regulatory cytokines in the growth of skeletal muscle (3). Myostatin is a member of the TGF- β family that is expressed in skeletal muscle and after entering the blood, binds to its receptor (activin IIb) in muscle fibers and activates the myostatin-smad signaling pathway, which prevents myoblasts proliferation during myogenesis, increase the activity of satellite cells and increase the synthesis of protein in muscle cells, and ultimately contribute to regulating muscle mass growth (4). Myostatin deficiency is associated with increased myofibrile size (hypertrophy) and an increase in the number of myofibrils (hyperplasia) of the muscle (5). Researchers believe that the levels of

myostatin and myogenin are strongly influenced by the lifestyle and the level of activity of the individual, according to the role of muscles in maintaining and stabilizing the body and producing force, as well as performing metabolic functions and movements. In addition, the type of training and the energy system involved in activity through various changes in the regulatory factors of muscle growth can cause different muscle adaptations (6). Various studies have examined the effects of various types of physical activity on regulatory factors of muscle growth. For example, one-session (2), eight-week, high intensity (6) resistance training resulted in a significant increase in myogenin and decreased myostatin levels after exercise; in addition, resistance trainings in obese men and adolescents resulted in a significant increase in myogenin and decrease in myostatin levels (3 and 7- 9). Researchers stated that resistance training reduced beta transforming growth factor (TGF- β) in male rats (10); in conjunction with other sporting activities, the increasing and intermittent running on a special strip of rats caused increased myogenic expression in fast-twitch muscle of rats (1); however, low intensity resistance trainings had a significant effect on the increase in myogenin in inactive young women (6) and patients with kidney transplantation (11). Looking at previous studies, it can be concluded that most studies have investigated the effect of resistance trainings and high intensity trainings on regulatory factors of muscle growth, while high intensity exercises have always been associated with damage to muscle cells and increased inflammation (12). Thus, it is necessary to use exercises that, in addition to creating a sense of vitality, happiness, and socialization can make physiological adaptations without causing special damage. Therefore, the present study aimed to investigate the effect of running in shallow, deep water and land on serum levels of myostatin and myogenin in young men.

Methods

In this quasi-experimental study, 9 young men with no history of disease, smoking, and special diet were selected as the subjects after completing the research consent questionnaire, medical information and physical activity status. The subjects lacked cardiovascular, musculoskeletal and diabetes mellitus and were not exposed to medical interventions affecting laboratory tests. In order to familiarize the subjects with how they run in water and maintain their water balance, a theoretical training session was held them a few days before starting the research. The study was conducted over a period of three weeks, so that the interval between each training session and the next session was one week. To eliminate the effects of training sessions on the research variables, all 9 subjects were categorized into three groups of 1, 2, and 3. Group 1 ran in the first, second and third weeks on land, shallow water and deep water for 24 minutes, respectively; group 2 ran in the first, second and third weeks, in shallow water, deep water and land for 24 minutes, and group 3 ran in the first, second and third weeks in deep water, land and shallow water for 24 minutes, respectively. The training protocol was the same for both trainings in water and land, because the main goal was to compare the effects of trainings in both water and land conditions. Each training session consisted of three steps: warming, main training, and cooling. At each training session, subjects performed warm-up exercises for joints and lower muscles of the trunk, such as hamstring, quadriceps and twins, for 5 to 7 minutes, and then, after the end of the main training, they did 5 to 7 minutes of cool training. Polar heart rate device was used to control heart rate during running. To calculate maximum heart rate, the maximum heart rate formula ($220 - \text{age}$) and to calculate the heart rate reserve (HRR) the Karvonen cardiovascular heart rate formula ($(\text{maximum heart rate} - \text{resting heart rate}) \times \text{exercise intensity} + \text{resting heart rate}$) was used. In Table 1, the training protocol on

land, shallow water and deep water has been reported. It is worth noting that blood sampling was performed in three steps, before running, immediately after running and 8 hours after running. At each time of blood collection, 10 ml of blood was taken from the brachial vein. To prevent hemolysis, blood samples were poured into EDTA-containing tubes and slowly mixed. The samples were then centrifuged at 3000 rpm for 15 minutes at 4 ° C for 15 minutes to separate the plasma. Myogenin and myostatin measurements were performed using an ELISA kit manufactured by Zellbio, Germany. To analyze the findings of the study, Kolmogorov-Smirnov test, two-way analysis of variance with repeated measurements along with Bonferroni's post hoc test were used ($p \leq 0.05$).

Results

The demographic characteristics of the subjects are presented in Table 2. Also, the levels of myostatin and myogenin before running, immediately after running and 8 hours after running on land, shallow water and deep water are presented in Tables 3 and 4, respectively. The results of two-way analysis of variance analysis with repeated measurements showed that there was no significant interaction between type of activity and myogenic measurement time ($F_{4,32} = 0.72$, sig = 0.58, Eta = 0.08). Also, there was no significant difference in the type of activity ($F_{2,16} = 1.008$, sig = 0.38, Eta = 0.11). However, there is a significant difference between different times of myogenin measurements ($F_{2,16} = 22.71$, sig = 0.001, Eta = 0.74), so that changes in myogenin before running time, immediately after running and 8 hours after running had significant differences (the main effect of measuring time was significant). The results of Bonferroni's post hoc test showed a significant increase in myogenic levels 8 hours after running on land compared to before running ($P = 0.01$); however, there was no significant difference in serum levels of myogenin in the time before

running and immediately after running ($P = 0.056$). Also, there was no significant difference in serum levels of myogenin at immediately after-running time and 8 hours after running ($P = 0.10$). The results of two-way variance analysis with repeated measurements showed that there was no significant interaction between the type of activity and the time of measurement of myostatin ($F_{4,32} = 1.26$, sig = 0.30, Eta = 0.13). Also, the main effect of type of activity ($F_{2,16} = 0.22$, sig = 0.80, Eta = 0.027) was not significant. However, the main effect of time variable ($F_{2,16} = 25.67$, sig = 0.001, Eta = 0.76) was significant so that the amount of myostatin in different conditions of running and at different times had no significant difference (based on non-significant interaction). Also, the amount of myostatin was not significantly different in three types of running activities on land, running in shallow water and running in deep water (the main effect of type of activity was not significant). However, the amount of myostatin before running, immediately after running and 8 hours after running had significant difference (the main effect of the measured time was significant). The results of Bonferroni's post hoc test showed that the amount of myostatin 8 hours after running had a significant difference compare to before running ($P = 0.01$); myostatin levels immediately after running ($P = 0.02$) and 8 hours after running ($P = 0.01$) had a significant decrease compared to before running. However, there was no significant difference between the amount of myostatin immediately after running and 8 hours after running ($P = 0.09$).

Discussion

The results of this study showed that immediately after running and 8 hours after it, serum levels of myogenin increased, and serum levels of myostatin decreased, however, myogenin and myostatin levels did not differ significantly in different conditions at different

Table 1. Running duration and intensity in four steps of running on land, shallow water and deep water

| Step | Duration of running | Intensity | |
|--------------------|---------------------|------------|-----------|
| One | 1 min. | 60-75% HRR | |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 3 min. active rest | | |
| | 1 min. | 60-75% HRR | |
| | 30 s. | 85-95 HRR | |
| | Two | 1 min. | 60-75 HRR |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |
| 3 min. active rest | | | |
| 1 min. | | 60-75% HRR | |
| 30 s. | | 85-95 HRR | |
| Three | | 1 min. | 60-75 HRR |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 1 min. | 60-75 HRR | |
| | 30 s. | 85-95 HRR | |
| | 3 min. active rest | | |
| | 1 min. | 60-75% HRR | |
| | 30 s. | 85-95 HRR | |
| | Four | 1 min. | 60-75 HRR |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |
| 1 min. | | 60-75 HRR | |
| 30 s. | | 85-95 HRR | |

Table 2. Descriptive statistics of the characteristics of the subjects tested

| Age (Year) | Height (cm) | Weight (Kg) | BMI (Kg/m ²) |
|------------|-------------|-------------|--------------------------|
| 22.41±2.12 | 178.17±4.08 | 72.94±8.88 | 22.97±2.71 |

Table 3. Results of ANOVA test with repeated measurement to study myogenin changes

| Type of Activity | 8 hours after running | Immediately after running | Before running | ANOVA |
|-------------------------------|---|--|--|--|
| Land | 5.61 ± 2.354 | †2.52 ± 1.336 | 3.43 ± 6.320 | F= 13.517, sig = 0.148, Eta = 0.212 |
| Shallow Water | 1.86 ± 8.379 | 46.82 ± 2.361 | 4.24 ± 8.328 | F= 2.993, sig = 0.079, Eta = 0.272 |
| Deep Water | 9.84 ± 8.419 | 6.64 ± 7.381 | 7.29 ± 3.339 | F= 11.138, sig = 0.001*, Eta = 0.582 |
| Repeated measure ANOVA | F = 1.144, sig = 0.343, Eta = 0.125 | F= 0.748, sig = 0.466, Eta = 0.086 | F= 0.595, sig = 0.563, Eta = 0.069 | |

† Significant difference with pretest (P <0.05)

* Significant difference between three measurement times (P <0.05)

Table 4. Results of ANOVA test with repeated measurements to evaluate myostatin changes

| Type of Activity | 8 hours after running | Immediately after running | Before running | Repeated measure ANOVA |
|-------------------------------|--|--|---|--|
| Land | 0.23 ± 7.174 † | 1.46 ± 4.216 † | 2.72 ± 7.270 | F= 13.517, sig = 0.000*, Eta = 0.628 |
| Shallow Water | 9.67 ± 0.206 † | 6.71 ± 0.239 | 3.73 ± 8.254 | F= 5.530, sig = 0.015*, Eta = 0.409 |
| Deep Water | 8.59 ± 8.218 | 6.73 ± 1.242 | 54.79 ± 2.266 | F= 3.325, sig = 0.062, Eta = 0.294 |
| Repeated measure ANOVA | F= 1.157, sig = 0.339, Eta = 0.126 | F= 0.316, sig = 0.734, Eta = 0.038 | F = 0.076, sig = 0.927, Eta = 0.009 | |

† Significant difference with pretest (P <0.05)

* Significant difference between three measurement times (P <0.05)

times. Muscle cell differentiation seems to be controlled by MRFs. MRFs are interacting with E protein to regulate gene expression during myogenesis. MRFs include MyoD, Myf5, and MEF4 myogenins, which are part of the family of transcription factors of bHLH in vertebrates (13). MyoD acts at the onset of

the differentiation and activation of the contractile protein gene. Myogenin function and MRF4 are cell differentiation regulators (14). Myogenin and MRF4 independently activate muscle differentiation, in accordance with their delayed regulatory functions in the process of muscle differentiation, muscle

reconstruction, and the specificity of the type of muscle fiber. These MRFs regulate contractile proteins and other specific muscle genes (15). Probably the levels of myostatin in the physical activity can be related to the involvement of more muscles, higher workload, and the use of both types of warp, in particular, slow oxidative strains and increased blood flow to active muscle (2). Therefore, sports exercises are likely to increase muscle hypertrophy by mechanical stimulation and increased muscle growth factors. Nevertheless, some researchers believe that with the migration of C-met and CD34 receptors in response to severe muscle damage caused by intense and resistant activity, the satellite cells begin to proliferate, and MyoD and Myf5 are activated, and then myogenin and MRF4 and other differentiated genes are activated (16). The satellite cells express Myf5 at low levels to maintain their myogenic properties (17). Endothelial and blood tissues can also help the ancestral cells to repair muscle. Although the origin of these cells is unknown, it may establish cellular-cellular connections between the ancestors of the muscle and other cells and cause myogenic cells (13). The myogenic bHLH agent is also disabled by the beta-growth transforming factor. But it has the ability to maintain DNA binding under these conditions, which indicates that an essential myogenic cofactor may be the target of negative setting of the TGF- β message pathway (18). The reducing mechanism of physical activity on plasma myostatin is not completely clear. It seems logical that the decrease in myostatin plasma may reduce the production and accumulation of myostatin protein by reducing its secretion into the bloodstream. There are two possibilities for this. The first possibility is that the decrease in myostatin may be due to a reduction in its stability. Increasing the distribution of myostatin throughout the blood circulation increases the extraction of this protein and its related compounds, which is attributed to exercise (2). On the other hand, the hormones

of testosterone and growth, as well as insulin-like growth factor, by activating various signal paths, especially the b5 activating signaling pathway, activate a series of highly complex cellular cascading pathways, regulate the negative expression of myostatin expression from muscle cells and subsequently reduce its secretion to the blood (19). It is argued that myostatin changes in response to interfering factors, such as exercise, are associated with changes in the number and activity of receptors in the skeletal muscle. Changes in the number and activity of myostatin receptors in the skeletal muscle are due to an increase or decrease in some of the factors contributing to the number and activity of myostatin receptors and their binding to these receptors. It has been seen that after exercise, the increase in the superiority of the function of increasing regulators, the number of kinase serine/threonine receptors of myostatin activin α II and β II (especially the activin receptor β II) and its binding to these receptors on the function of reducing regulators, cause further increase in the binding of myostatin to these intramuscular receptors, and ultimately, decrease the amount of myostatin in plasma (20). In confirmation of the findings of the present study, 13 and 23 weeks of weightlifting training led to a decrease in serum levels of myostatin in men weightlifters (21); among the possible reasons for the alignment of the results of this study with the present study, we can point to the same mechanism of the effect of sports activities on reduction of myostatin, 18 days of resistance training reduced the expression of myostatin gene in men (22). This study was similar to the present study due to the duration of the study, so this factor could be a reason for the consistency of these two studies; six months of aerobic training with moderate intensity reduced muscular and plasma myostatin in middle-aged men (23), eight weeks, three sessions per week of high intensity resistance training led to increased myogenin and decreased myostatin levels in inactive young

women (6). Among the reasons for coherence of these studies with the present study, one can refer to the similarity of the statistical population and the mechanical pressure on the skeletal muscle to stimulate the reduction of myostatin and the increase of myogenin; one session of resistance activity with a 55% intensity of one maximum repeat (1RM) in three periods, and for each period, 15 repetitions and two minutes of rest between each period significantly increased myogenin during the exercise, one hour after the exercise and 24 hours after the exercise (2). Also, performing a session of resistance activity had a significant effect on the increase of myostatin during and after an hour of exercise; however, 24 hours after resistance exercise, the levels of myostatin significantly decreased compared to immediately after exercise and one hour after sport activity (2). Eight weeks, four sessions a week, increasing and intermittent running exercises on the special treadmill of rats increased myogenin expression in the fast-twitch muscle of rats (1); 10 weeks, three sessions a week resistance training resulted in a significant decrease in myostatin levels in obese men and adolescents (3, 7). One of the possible reasons for the compatibility of these studies with the present study is the similarity in the statistical population. Six months of aerobic training and weight loss significantly decreased serum levels of myostatin in elderly men (8); eight weeks, three sessions and four sessions of resistance training resulted in a significant decrease in myostatin levels, however, four sessions per week exercise had a greater effect on the reduction of myostatin in non-athlete men (9); on the other hand, eight weeks of resistance training did not have a significant effect on myogenin expression in renal transplant patients (11); eight weeks, three sessions a week, low intensity resistance trainings had no significant effect on myogenin of inactive young women (6). Among the probable reasons for the inconsistency of these studies with the present

study, we can mention the differences in the statistical population, as well as the differences in the type and intensity of exercise. Eight weeks of combined training did not have a significant effect on myostatin (24), which this inconsistency can be due to differences in the duration of the training period, as well as the type of exercise. Seven sessions of high intense extrinsic resistance training with one leg and intrinsic as knee isokenetic opening moves had no effect on myostatin mRNA in 20 young women (25). This study was also inconsistent with the present study. For reasons of incongruity, the differences in the subjects' gender, the number of training sessions and the types of exercises can be pointed out. Regarding the remarkable effect of sex hormones in the expression of myogenin and reduction of myostatin levels, the lack of measurement of these factors is one of the limitations of the present study. Therefore, it is suggested that in future studies, the relationship between regulatory factors of muscle growth and sex hormones following sport activities in the studies analogous to the present study should be considered. Also, the present study failed to report changes in body composition following present sport activities. Thus, in future studies, it is suggested that changes in the subjects' protein content should be reported by measuring the body composition of the subjects.

Conclusion

According to the results of this study, running on land and running in shallow water and deep water can increase serum levels of myogenin and decrease serum levels of myostatin in young men. Also, running on land and running in shallow water and deep water have the same effects on the increase of myogenin and the reduction of myostatin.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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