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High Intensity Interval Training Induces Myostatin and Follistatin Expression in Fast- And Slow-Twitch Skeletal Muscles of Rats

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ABSTRACT

Background and Objectives: The aim of this study was to investigate effects of eight weeks of high intensity interval training (HIIT) on myostatin (MSTN) and follistatin (FLST) expression in fast and slow twitch muscles of Wistar rats.

Methods: First, 12 male 8-week old male Wistar rats were randomly divided into two groups of exercise (n=6) and control (n=6). The exercise group performed progressive HIIT, five times a week for eight weeks. The training protocol included alternating sprint running for 30 min/session, divided into three 4-min bouts (35-50 m/min, >90% VO2 max) separated by a 2-min active recovery period (30-50% VO2 max). The level of MSTN and FLST expression in soleus (SOL) and extensor digitorum longus (EDL) muscles were measured by real-time RT-PCR.

Results: Following HIIT, MSTN level decreased significantly in the SOL muscle and increased significantly in the EDL muscle ($P<0.001$). Moreover, FLST expression increased significantly both in the SOL and EDL muscles ($P<0.001$).

Conclusion: Based on the results, eight weeks of HIIT can significantly inhibit MSTN expression and increase FLST expression in fast and slow twitch muscles.

Keywords: Myostatin, Follistatin, Fast muscle, Slow muscle, High intensity interval training.

INTRODUCTION

The skeletal muscles' morphology and gene expression are highly influenced by external conditions. Skeletal muscle hypotrophy often occurs with increased mechanical load. Although this issue has been widely studied, its signaling mechanism is still unknown (1). Skeletal muscle hypertrophy in adults is dependent on the balance between negative and positive regulators of protein synthesis. Myostatin (MSTN) and follistatin (FLST) are among muscle growth regulators (2-5). It has been shown that inhibition of the *MSTN* gene can significantly increase mouse body weight due to increased muscle mass, which has been related to increased number of myofilaments (hyperplasia) and expanded cross section of muscular filaments (hypertrophy) (10). The MSTN signaling in skeletal muscles inhibits satellite cells activity through down regulation of myogenic factors, including myogenin, cycline and cyclic-dependent kinase 2 (11).

Follistatin is a glycoprotein that occupies the binding site of MSTN and inhibit its function (12), thus preventing muscle fatigue and increasing muscle mass (13). The ability of FLST to act as an MSTN antagonist is specified by simultaneous overexpression of both in the skeletal muscles. It has been shown that FLST and MSTN are directly in contact with each other in skeletal muscle but not in blood (15).

A previous study claimed that six-week injection of serum containing FLST could contribute to muscle growth (5). However, concerning the fact that gene doping is accompanied with irreversible side effects, recent investigations aim to stimulate the MSTN pathway using different training protocols to prevent muscular atrophy (16-18). Moreover, MSTN expression increases during inactivity of skeletal muscles (5), while MSTN inhibition increase strength and muscle mass (19), decrease adipose tissue (20) and increase bone density (21). Therefore, it seems that physical activity can lower MSTN expression. However, the results of a few studies on MSTN response to exercise have been controversial. In these studies, MSTN expression increased (22) and decreased (23) in response to a resistance training session.

Collectively, most studies show that MSTN expression decreases in response to long-term strength training and endurance training (17, 18, 24). In spite of the health benefits of such trainings, most adults do not have the time to participate in these activities. To overcome the time barrier, high intensity interval training (HIIT) with repeated bouts of high intensity effort followed by various recovery periods has been introduced as a time-efficient physical training that stimulates metabolic adaptations similar to traditional endurance training (25, 26). In this study, we evaluated effects of eight weeks of HIIT on MSTN and FLST expression in fast- and slow-twitch skeletal muscles of Wistar rats.

MATERIALS AND METHODS

Twelve male 8-week old male Wistar rats (mean weight: 210±20 g) were assigned into two groups of HIIT (n=6) and control (n=6). First, the rats were kept in standard cages at 22±3 °C and humidity of 40-60% on a 12h light–12h dark cycle for three days. The animals had free access to standard food and water ad libitum. The training group exercised for two weeks to become familiar with a motor-driven running treadmill. Animal care and experimental protocols were approved by the ethics committee of University of Tehran, Iran.

The HIIT was performed five times weekly for eight weeks at intensity close to VO_2 max, 35-45 m/min speed and zero incline. Initially, the HIIT group was trained at running speed of 35 m/min with three four-min bouts (>90% VO_2 max) separated by a two-min active recovery period (30% VO_2 max). Every two weeks, the running speed was increased progressively until the rats were running at 50 m/min with three four-min sprint running bouts (>90% VO_2 max) separated by a two-min active recovery period (50% VO_2 max) in the last two weeks. When including the warm-up and cool-down periods (12 min), the total duration of the exercise protocol was approximately 30 min/session. The control group remained sedentary in their cages in the study period.

Twenty-four hours after the last training session and after overnight fasting, all rats were sacrificed and sampling was performed. The soleus (SOL) and extensor digitorum longus (EDL) muscles were excised from the lower limbs, washed in physiological serum and weighed using a digital scale with precision of 0.0001 g. The collected samples were immediately frozen in liquid nitrogen and stored at -80 ^{o}C until analysis. isolated tissues were lyzed using 1 mmol of triazole solution and were homogenized using RNA extraction was performed using 50 mg of each EDL and SOL muscles.

The isolated tissues were lyzed using 1 mmol of triazole solution and were homogenized using a Polytron homogenizer (Ultra-Turrax T8, Ika labortechnik, Staufen, Germany). Homogenates were centrifuged at 12,000×g for 10 min at 4 °C to remove pellet. Next, chloroform (200 µl) was added to separate the sample into an aqueous and an organic phase. Then, 600 µl of isopropanol were added, and RNA was isolated according to the manufacturer's instructions. Concentration and purity of the extracted RNA was estimated by reading OD 260/280.

Synthesis of complementary DNA was performed with 1 µg RNA in a total reaction volume of 20 µl using random hexamer oligonucleotides. Reverse transcriptase reaction was performed according to the manufacturer's protocol. Quantitative real-time PCR experiments were performed using a 7300 Real-Time PCR machine (Applied Biosystems, Step One, Germany) and specific primers. The PCR reaction results were visualized using SYBR Green II and ROX as a reference dye. The amplification conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Gene expression was analyzed relative to the expression of the 18S housekeeping gene. The purity of each PCR product was established by drawing melting curves. Gene expression was assessed quantificationally using the 2-ΔΔct method (27).

Results are presented as mean ± standard deviation (SD). Normality of data was analyzed using the Shapiro-Wilk test. Independent t-test was performed to analyze intergroup differences. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) at significance level of ≤ 0.05.

RESULTS

The weight change of rats in the study groups are reported in table 1.

MSTN gene expression in the SOL muscle decreased significantly after the training program ($P<0.001$). Moreover, MSTN expression decreased 30% in EDL muscle of rats in the HIIT group compared to the control group ($P<0.001$, Figure 1). However, FLST expression in SOL muscle increased 31% in the HIIT group compared to the control group ($P<0.001$). Interestingly, FLST expression in EDL muscle increased one-fold in the HIIT group compared to the control group ($P<0.001$, Figure 2).

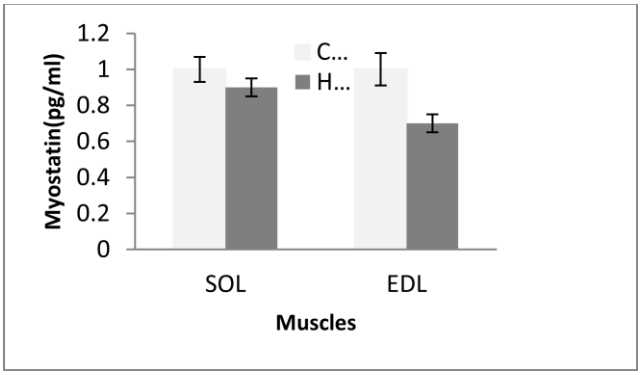

Figure 1. Myostatin level in EDL and SOL muscle of rats in the control (CON) and HIIT groups. Date are presented as mean ± SD.

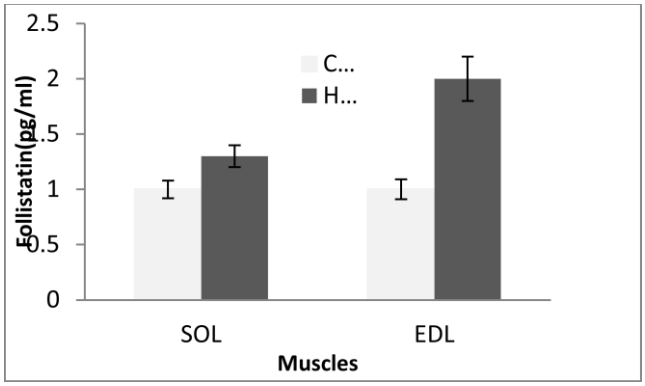

Figure 2. Follistatin level in EDL and SOL muscles of rats in the control (CON) and HIIT groups. Date are presented as mean ± SD

Table 1. Weight (mean ±SD) changes of rats in the study groups

Group	Baseline weight (g)	Post-training weight (g)	% change
Control	210.5±9.77	337.17±7.80	60.17
HIIT	212.5±9.25	305.83±11.46	44.25

DISCUSSION

The present study investigated the effects of eight weeks of HIIT on MSTN and FLST expression in EDL and SOL muscles of male Wistar rats. Following the training, MSTN expression decreased significantly in EDL and SOL muscles. In addition, FLST expression increased significantly in SOL EDL muscles compared to the control group. Previous studies also showed that exercise can significantly reduce MSTN expression in muscles (8, 17, 18). Schwarz et al. (2014) reported that resistance training at low intensity (50% of 1-repetition maximum; 1RM) and high intensity (80% of 1RM) significantly reduced MSTN expression (18). Ziaaldini et al. (2015) reported that aging leads to decreased FLST expression and increased MSTN expression, which can be reversed by exercise. They claimed that that the aging-related decrease in muscles could be due to the imbalance between down-regulation of anabolic and up-regulation of catabolic and pro-apoptotic factors, which can be corrected through aerobic exercise (8).

Contrary to our findings, some studies reported no change or an increase in MSTN expression after exercise (23, 28, 29). In another study, despite the increase in power and muscle mass, MSTN expression increased after 12 weeks of resistance training in elderly men (23). The inconsistency of findings might be due to difference in protocols, intensity and duration of trainings, gender and characteristics of subjects, measurement method or sampling time. For instance, in our study, biopsy was taken 24 hours after the last training session, while in a study by Willoughby et al. (2004), sampling was performed 15 minutes after resistance training (29).

It has been reported that MSTN will increase in response to one session of resistance training for 24 hours (30).

Similar to our findings, other studies also reported a significant increase in FLST expression following exercise (17,31,32). Hansen et al. (2011) demonstrated that plasma FLST level increases during exercise and exercise recovery period, which may be due to FLST expression in liver (31). It has been shown that FLST could act as a competitive inhibitor of MSTN by binding to activin IIb receptor (33).

Most previous studies investigated MSTN and FLST expression in skeletal muscles in response to resistance training (17, 18, 23), while few studies have studied the effect of other trainings on MSTN and FLST alterations. Hittel et al. (2010) reported a reduction in MSTN after endurance training (24). It is demonstrated that endurance training can lead to muscle hypertrophy by affecting oxidative metabolism (34). In another study, MSTN expression reduced 65% in gastrocnemius muscle, 49% in vastus lateralis and did not change in SOL muscle of trained rats (35).

To our knowledge, the present study is the first to investigate the effects of HIIT on MSTN and FLST expression. Based on the results, HIIT significantly decreased MSTN and increased FLST expression in EDL muscle of rats; however, it had minor effects on SOL muscle. The down-expression of MSTN in EDL muscles could be due to the muscle fiber type as fast twitch fibers are more involved in muscle growth. Since the weight of muscles did not differ significantly between the study groups, factors other than MSTN and FLST, such as i*nsulin-like growth factor 1* may be involved in the stimulation of muscle growth. In a study by Root et al. (2003), MSTN expression decreased in young and old participants following nine weeks of resistance training. In line with our findings, the mentioned study failed to observe a relationship between MSTN down-expression and muscle mass/strength (34). On the other hand, the overall weight(after exercise) differed between the study groups, while subjects in the training group experienced

less weight gain compared to the control group. It has been reported that MSTN and FLST expression changes might block adipogenesis (36).

Generally, in order to preserve the size of skeletal muscle fibers, there is a hemostatic balance between the main positive (such as IGF-1) and negative (such as MSTN) regulators of muscle growth, which is thought to be mediated through a complex negative circle feedback (37). Therefore, the down-expression of MSTN after HIIT could also be due to the imbalance between positive and negative regulators of muscle growth.

CONCLUSION

Based on the results, HIIT can efficiently inhibit MSTN expression and increase FLST expression in fast and slow twitch muscles.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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