Research Article

Adaptive response of slow and fast skeletal muscle following mechanical hindlimb suspension in Wistar male rats

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Abstract

Mechanical hindlimb suspension of lower extremities leads to prompt atrophy in rats' skeletal muscles. The present research was designed to study cross-section area (CSA) and the expression level of the genes ATF4, P53, MST1, and atrogin-1 in slow and fast skeletal muscles following mechanical hindlimb suspension. 20 male Wistar rats were assigned randomly in to two groups: control (Con) and hind-limb suspension (HU) (10 rats per each group). In HU group, tail suspension was designed for 14 constitutive days; however, animals in the control group passed a normal life. The findings indicated that hind-limb suspension could relatively diminish CSA, myonuclei number per fiber and the weight of both soleus and EDL muscles. However, these reductions were not significant for EDL muscle. Furthermore, the expression level of the MST1, atrogin-1, ATF4, and p53 in soleus muscles elevated significantly. Moreover, the expression level of all four genes increased significantly in EDL muscle. Comparison of genes expression level between two soleus and EDL muscles showed that expression of MST1, ATF4, and p53 genes were higher in soleus than EDL, but it was not the case for atrogin-1 as its expression level was more in EDL compared to soleus. Our study provides novel evidence that immobilization of hind-limbs can induce a more powerful atrophic response in slow muscles in comparison to fast ones.

Key Words: **Hindlimb suspension, EDL muscle, Soleus muscle, Gene expression**

Introduction

Skeletal muscle is a changeable tissue that is adapted according to its level of activity through changes in muscular mass, muscular proteins and changes in the type of fibers from the contraction and metabolic views (Giger, Bodell, Zeng, Baldwin, & Haddad, 2009; Rodriguez et al., 2014). Maintaining the muscular mass and body function is necessary to be survived and healthy. Although the atrophy is a result of certain physiological processes (e.g., aging), it is considered as a major feature in most the diseases. Typically, injuries and diseases include a period of immobilization that may be extended as general (e.g., bed-rest) or local (e.g., casting a fractured organ). Maintaining the muscular mass and body function is necessary to be survived and healthy. Because of immobilization, changes in adaptation occur rapidly in mammal's skeletal muscles. Evidence show that suspension of lower extremities leads to prompt atrophy in rats' skeletal muscles, which it characterized by loss of muscular mass, decreasing fiber size (cross-sectional area, CSA), and turning from slow to fast fibers (Adams, Caiozzo, & Baldwin, 2003; Desplanches, Mayet, Sempore, & Flandrois, 1987).

The atrophy of skeletal muscles occurs in many of catabolic conditions including starvation, cancer, diabetes, and loss of mechanical load (Berg, Larsson, & Tesch, 1997; Ohira et al., 2002). Nonetheless, muscular atrophy is not similar in all muscle fibers. Skeletal muscles consist of fibers that have different metabolic and contraction properties. Evidence suggest that in comparison with glycolytic ones, the oxidative muscles are more resistant to atrophy. During starvation or exposure to glucocorticoids, diseases and cancer glycolytic (fast-contraction) muscles show more degree of atrophy than oxidative (slow-contraction) fibers (Acharyya et al., 2004; Tiao, Lieberman, Fischer& Hasselgren, 1997). On the contrary, during the suspension of posterior organ -a common animal model used to mimic the loss of mechanical load- slow-contraction muscles demonstrate more levels of atrophy comparing with fast-contraction muscles (Herbison, Jaweed, &

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Ditunno, 1979; Thomason & Booth, 1990). Nevertheless, our knowledge about molecular mechanisms (muscular atrophy) of fibers is very limited in the atrophic situations (suspension of the posterior organ). Several genes have involved in controlling the muscular mass in fast- and slow-contraction muscles. Although decomposition of cell proteins is often mediated through major pathways including calcium-dependent cysteine proteases (calpains), cysteine-aspartic acid protease (caspases), autophagolysosome and ubiquitin proteasome pathways; and many studies have shown that protein decomposition mediated by ubiquitin has a major role in muscular atrophy (Bialek et al., 2011). Researches have recognized two ligase ubiquitin proteins known as MuRF1 and atrogin-1/MAFbx. These two genes have been considered as enzymes that limit protein decomposition so that in the absence of them, the atrophy of muscle decreases significantly. Some atrophy stimulants including disuse/inactivity, starvation, and treatment with glucocorticoids motivate atrogin-1 and MuRF1 and are known as atrogenes (Verhees et al., 2011).

Mammalian ste20-like (MST) kinase one family expresses widely in different tissues and includes four members including MST1, MST2, MST3, and MST4. It has been shown that kinase MST1 increases in various stress conditions like oxidation stress (Brunet et al., 1999) and activation of caspases (Ura, Masuyama, Graves, & Gotoh, 2001). In addition, it has been shown that in nerveless conditions, MST1 causes atrophy in fast-contraction muscles. This increase in MST1 is associated with rising in atrogin-1 and LC3 gene expression through FOXO3a activation (Wei et al., 2013b). On the other hand, activating transcription factor 4 (ATF4) is a leucine-zipper transcription factor that mediates a wide spectrum of cell stress reactions (Baldwin, Haddad, Pandorf, Roy, & Edgerton, 2013). In skeletal muscles, immobilization of muscle increases ATF4 expression (Sacheck et al., 2007) and the rise in ATF4 expression in skeletal muscle causes atrophy in muscular fibers (Ebert et al., 2010). Furthermore, mice lacking ATF4 expression in their skeletal muscle fibers showed less atrophy in muscular fibers during organ's immobility; in other words, muscles having no ATF4 are not fully resistant against atrophy due to immobility (Ebert et al., 2012). Similar to ATF4, p53 tumor suppressor is a transcription factor resulted from stress that mediates the cell adaptions to stress (Horn & Vousden, 2007). In addition, there are evidence that p53 develops muscular atrophy. Evidence shows that combination of p53 and ATF4 expression caused more atrophy compared with each of them alone and decreased p53 expression, developed partial protection against atrophy caused by immobility and when a decrease in expression of both occur, and protected muscle almost significantly against atrophy resulted from immobility (Fox et al., 2014). Although atrophy is a result of certain physiological processes and is of interest as one of the major aspects of many diseases, it should be noted th-at muscular atrophy is different in various muscles. For example, low-tension fibers (type I) are more vulnerable and therefore show more protein reduction compared with fast-tension fibers (type II) (Thomason & Booth, 1990; Tsika, Herrick, & Baldwin, 1987; Zhang, Li, Ren, & Gao, 2015). Also, fast muscles (including gastrocnemius, EDL, and anterior tibialis) show a significant decrease in muscular mass after suspension of lower organ (Kyparos, Feeback, Layne, Martinez, & Clarke, 2005). Generally, according to muscle type, the type of muscular fibers, and the conditions of immobility, different results have been observed in various studies on rodents with lower organ suspension, and until now, there is no complete information about gene expression of specific muscular fibers in response to muscle immobility. Considering the above, the goal of this study was to investigate the change in above gene expression and to examine the expression level in two slow-tension soleus and fast-tension EDL after suspension of posterior organ and immobility.

Materials and Methods

Animals

In this study, twenty mature male Wistar rats, weighted 250±30 grams, were prepared from the animal breeding division of Razi Research Center and were transported to the animal laboratory of Lorestan University. All rats were kept in environmentally controlled conditions including a temperature of 23±3 °C, 12/12 light-dark cycle, relative humidity of 40%, and freely available water and rat-specific food. This study was performed according to the health guidelines for animal research and approved by Animal Care Committee of Lorestan University (under the code LUNS.REC.1395.170), according to the NIH Guidelines for Care and Use of Laboratory Animals (NIH publication, 1996). After that animals spent an environmental adaptation period of two weeks, the experimental protocol started. The rats were randomly assigned to two groups of with mechanical hindlimb suspension (HU) (n=10) and control (Parrini et al.) (n=10). Rats in the HU group were subjected to lower organ suspension for two weeks. In the control group, rats were kept in normal life conditions for two weeks and were sacrificed along with the HU group, simultaneously. All steps and tests were applied to the control group like the intervention one.

Mechanical hindlimb suspension

To unload the lower organ mechanically, we applied the method of lower organ suspension (Zhang et al., 2010). Briefly, distal one- third of rats' tails were tied to metal bars top of the cage using orthopedic adhesives and metal rings so that, the suspension height was at a level that the animal's lower organ did not touch any supportive surface (about 30º). Animals' hands were in touch with cage bottom to make it freely move and access food and water; animals were in this position for two weeks. In th-

-e HU group, only a single rat was kept in each cage. During the study period, rats were displaced and manipulated by one person.

Tissue extraction

Fourteen days after suspension, rats were anesthetized via intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) and were immediately weighted. Then, in a fully sterile condition, an incision was made to a posterior section of animals' legs, and the soleus and EDL muscles were extracted thru cutting proximal and distal tendons. Subsequently, the extracted muscles were weighted by a lab scale, were immediately frozen in liquid nitrogen, and were reserved in -80ºC freezer until experiments.

Soleus and EDL removing and weighting

Soleus and EDL muscles from the left leg were dissected from the bone quantitatively, immediately weighed, and frozen at 20°C. At a later date, these muscles were lyophilized and weighed for dry weight. After excision of muscles the left tibia was removed and freed from connective tissue, and the maximal length was measured. Yin et al. showed that expressing muscle mass per unit of tibial length is a valid way to normalize mass when body weight differs between experimental groups.

Extracting DNA and synthesizing cDNA

To extract total DNA from soleus and EDL muscles, these muscles were homogenized in QIAzol Lysis Reagent a fraction of 1:10. To remove protein components, the product was centrifuged in 12000g, at four ºC, for 10 minutes. Then, the product was mixed with chloroform (1:0.5) and was shaken rigorously for 15 seconds. The mineral and aqueous parts of the product were separated in 4 °C with 12000g centrifuge during 15 minutes; the RNA containing part was removed and was mixed with isopropanol (1:0.5), kept in room temperature for 10 minutes and was centrifuged in 4°C, 12000g for 10 minutes. The pellet containing RNA was washed with ethanol and then was solved in 20µL RNAS-free Water. RNA concentration was measured (using Eppendorf, Germany) and the ratio of 260 to 280 between 1.8 to 2 was defined as desired purification level. Synthesis of cDNA was completed using 1 µg RNA and cDNA synthesis kit for fermentase and enzyme Reverse Mulv transcriptase.

Table 1. Sequencing of Primers Used in Current Study

Gene	Primer sequencing	Gene bank
ATF4	F= 5'GCAATAGCTCAGTCCTTCA3' R= 5'CTCGGTGTATATCTCACTCT3'	NM 024352.1
P53	F=5'CATCAGGAGAAGTGGATCTATGT3' R= 5'GCTTCCCCCAAAGTGCAGTA3'	NM 133521.1
Atrogin-1	F=5'CATCAGGAGAAGTGGATCTATGT3' R= 5'GCTTCCCCCAAAGTGCAGTA3'	NM 133521.1
MST1	F= 5'GCAATAGCTCAGTCCTTCA3' R= 5'CTCGGTGTATATCTCACTCT3	NM 024352.1
GAPDH	F=5'GACATGCCGCCTGGAGAAAC3' R=5'AGCCCAGGATGCCTTTAGT3'	NM 017008.4

Real-time PCR

We used Real-time PCR by Premix SYBR green II (Applied Biosystems, USA) for determining the levels of expression of genes ATF4, P53, MST1, and ertrogin -1. The Final volume of reaction mixture was 20 µL, and each test was completed duplicately. Macrogen Company (Macrogen Inc., Seoul, Korea) designed the primers according to data relating to genes ATF4, P53, MST1, origin-1, and GAPGH from NCBI (table 1). The sequencing of primers is listed in Table 1. The GAPDH was used as a control gene. Temperature plan for Rela-time PCR was as follows: 95 °C for 10 minutes, 95 °C for 15 seconds, 60 °C for 1 minute (repetition for 40 cycles). The gene expression of the studied genes was measured by the $2^{-\Delta\Delta CT}$ method. Ten rats in each group were used for this analysis.

Immunohistochemical Analysis

Fascia of soleus and EDL muscles was removed, then muscles were frozen in nitrogen-chilled isopentane and kept at -800C until use. 10 mm-thick cryosections were prepared for immunolabelings. Then, cryosections were labeled with antibody against laminin (L9393) overnight at 40C and labeling against Cy3 (Jackson Immunoresearch Inc) was performed as a second antibody for 2 h at 370C. Hoechst staining (1:1000, B2261, Sigma-Aldrich) was used to visualize nuclei and sections were mounted using Fluoromount G medium.

Immunohistochemistry quantification

For IHC, images were captured at ×10 magnification using a Carl Zeiss AxioImager fluorescent microscope (Carl Zeiss, Jena, Germany). Muscle fiber cross-sectional area was analyzed using Open-CSAM software. This reliable and highly sensitive muscle analysis software has been validated against manual human counts and is both accurate and reliable. Laminin stained muscles were also used to quantify myonuclear number per fiber. Nuclei that clearly resided within the laminin border of the muscle fiber were scored as myonuclei using Image J software. All manual counting was performed by a blinded, well-experienced technician.

Statistical analysis

We used distribution indicators including standard deviation, average, and graph. In inferential statistics part, Kolmogorov–Smirnov Test (K-S test) was applied for determining the normality of data. Furthermore, the consistency of variances was measured by the Levene Test. We applied one-way analysis of variance (A-

NOVA) to determine the significance of difference between variables. For analyzing data, we used SPSS ver. 20.

Results

Effects of mechanical hindlimb suspension on muscle weight

To determine the effect of mechanical hindlimb suspension on body and muscle weight, these variables were measured. At the beginning of the study, the mean body weight in two groups was not different (P>0.05). In addition, there was no difference in body weight after fourteen days of mechanical hindlimb suspension in two groups (P>0.05) (Figure 1A). The results of this study suggested that fourteen days after suspending lower organ, the relative weights of both soleus (Figure 1B) and EDL (Figure 1C) muscle were decreased (p=0.001 and 0.7, respectively), but this reduction was not significant in EDL muscle (Figure 1).

Effects of mechanical hindlimb suspension on muscle size and myonuclear number

Muscular atrophy is a consequence of muscle disuse, leading to CSA decline. We assessed myofiber atrophy after fourteen days of mechanical hindlimb suspension in both the soleus and EDL muscles of male Wistar rats. We next assessed the magnitude of myonuclear number per fiber. Average muscle fiber size (CSA) in response to mechanical hindlimb suspension is shown in Figure 2. The CSA decreased significantly in soleus (Figure 2B), but not in EDL (Figure 2C) muscles of HU rats compared to the Con group (p<0.001 and p>0.05; respectively). The number of myonuclei followed the decrease of CSA in HU rats. The analysis of the data from muscle nuclei counts showed that the number of muscle nuclei in HU group was significantly lower in soleus (Figure 2D) (p<0.001), while there was no significant difference for EDL (Figure 2E) between two groups (p >0.05) (Figure 2).

Effects of mechanical hindlimb suspension on gene expression of the genes involved in muscle mass control

Given that muscular atrophy is highly associated with gene expression machinery of skeletal muscle, we next investigated if fourteen days of mechanical hindlimb suspension can change gene expression pattern of the genes involved in muscle mass control. ATF4, P53, MST1, and atrogin-1 gene expression (p=0.001 for all) had significant elevations in soleus muscle (Figure 3A). Furthermore, ATF4, P53, MST1, and atrogin-1 (p=0.001 for all) showed significant elevations in EDL muscle (Figure 3B). ATF4, P53, and MST1 expression were higher in soleus muscle compared to EDL one, but EDL muscle showed higher increases in atrogin-1 relative to soleus muscle. In addition, MST1 gene expression in soleus muscle was higher (~6 times) compared to EDL planters (~2.3 times); atrogin-1 gene expression in soleus muscle was lower (~4.3 times) vs. EDL muscle (~6 times). At the same time, ATF4 gene expression in soleus muscle was higher (~8.3 times) than that in EDL muscle (~3.1 times), and P53 gene expression in soleus muscle was lower (~7 times) in comparison to EDL muscle (~3 times) in male Wistar rats after suspending the lower organ for fourteen days (Figure 3).

Discussion

There is no similarity between muscular atrophy in all kinds of muscular fibers. Skeletal muscles are constituted from some types of fibers with different metabolic and contraction properties. However, our knowledge about molecular mechanisms (muscular atrophy) of fibers is highly unknown (Acharyya et al., 2004; Tiao, Lieberman, Fischer, & asselgren, 1997). Hence, in t-

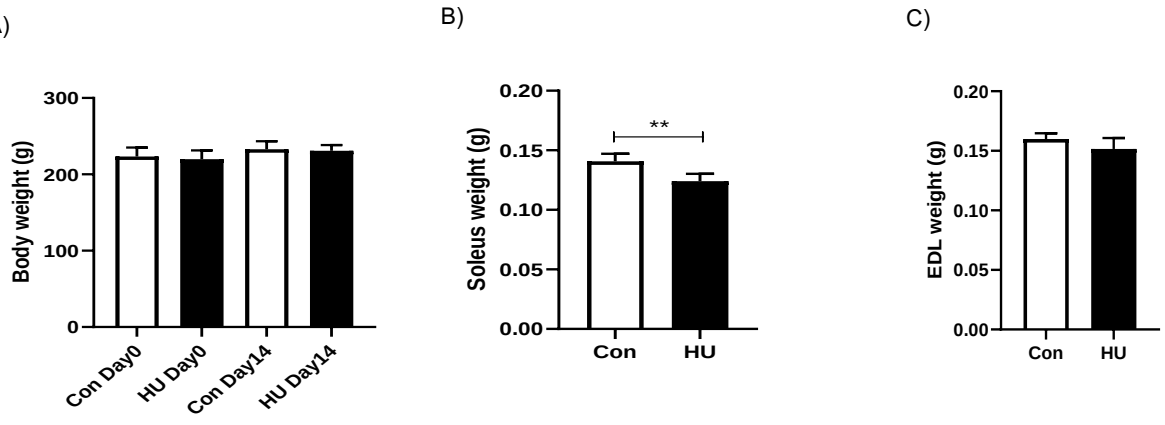


Figure 1. Descriptive values of body and muscle weight. (A) Body weight in day0 and day14. (B) Soleus weight after fourteen days after mechanical hindlimb suspension. (C) EDL weight after fourteen days after mechanical hindlimb suspension. Values are expressed as mean ± S.E.M. **p < 0.01. Con: control rats; HU: mechanical hindlimb suspension rats. Ten rats in each group were used for this analysis.

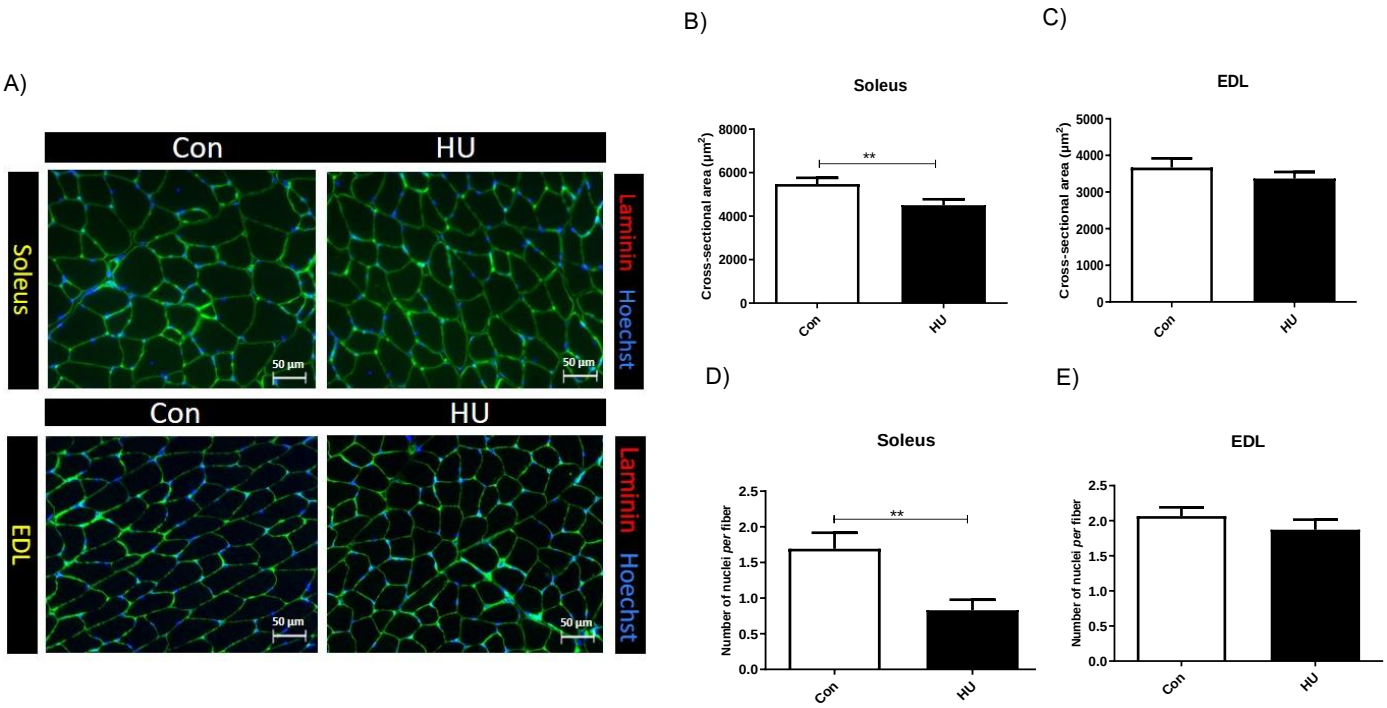


Figure 2. Muscle fiber cross-sectional area (CSA) in different groups. (A) Sections of soleus and EDL muscles immunolabeled for Laminin (red) and Hoechst (blue). Average muscle fiber CSA and myonuclei numbers in soleus (B) and EDL (C) after fourteen days after mechanical hindlimb suspension. Average myonuclei number per fiber in soleus (D) and EDL (E) after fourteen days after mechanical hindlimb suspension.

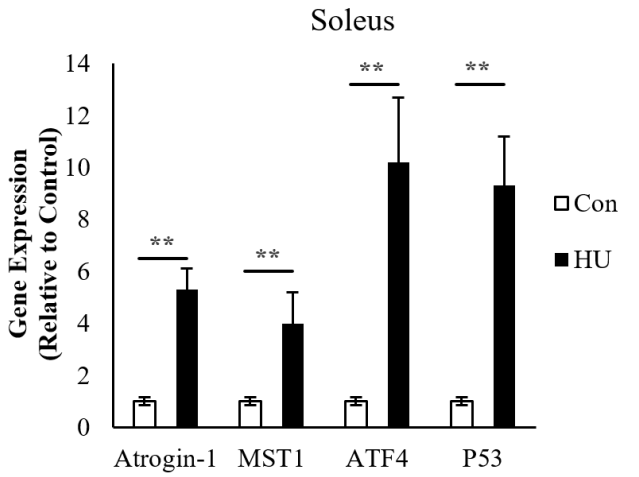
-his study, to investigate the degree of gene expression to muscular atrophy in no-use conditions within two kinds of muscles (fast and slow), we decided to apply the model of posterior organ suspension. Herein, we showed data suggesting the differences in atrogin-1, MST1, ATF1, and P53 gene expression in response to atrophy resulted from the immobility of slow and fast muscles. In general, the results of this study illustrated that fourteen days after lower organ's suspension, the CSA and myonuclear number per fiber of soleus and EDL muscles had reduced, but these decrease was not significant in EDL. Furthermore, atrogin-1, MST1, ATF1, and P53 gene expressions significantly increased in soleus and EDL muscles; although, MST1, ATF1, and P53 gene expressions in soleus muscle was higher than in EDL muscle. However, atrogin-1 gene expression showed higher increases in EDL muscle. Therefore, according to results of our study, we can state that atrogin-1, MST1, AFT4, and P53 may be considered as one of the major objectives to control atrophy in no mechanical load situations including hospitalization and space voyages.

Evidence show that two atrophic muscle-specific E3 ubiquitin ligases atrogin-1, (MuRF1) are regulated in several models of atrophic muscle and are responsible for protein decomposition through the ubiquitin-proteasome system (Sandri et al., 2004). Expression of these genes, currently, serves as an early indicator for skeletal muscles atrophy and thus, these genes are consider-

-ed as major regulating factors for muscular atrophy. The expression of MAFbx/atrogin-1 and MuRF1 is regulated by FOXO transcription factors (Sandri et al., 2004; Stitt et al., 2004). FOXOs consist of three isoforms that include FOXO1, FOXOa3, and FOXO4. Phosphorylation of FOXO causes it to exit from nucleus to cytoplasm. Moving and activity of FOXO are necessary for up-regulations of MAFbx/Atrogin-1 and MuRF1. FOXO3 induces MAFbx/Atrogin-1 expression and muscular atrophy (Sandri et al., 2004). These two muscle-specific ubiquitin ligases have been widely studied, and it has been suggested that they play major roles in atrophy of skeletal muscles (Bodine & Baehr, 2014; Foletta, White, Larsen, Leger, & Russell, 2011). Evidence show that decreased or limited use of skeletal muscles is one of the major factors contributing to muscular atrophy and it may occur in several conditions among unhealthy or healthy persons (Evans, 2010). For example, many common diseases including diabetes (Bonaldo & Sandri, 2013), cancer (Stephens et al., 2010), renal failure (Gordon, Kelleher, & Kimball, 2013), genetic muscular diseases (Sandri, 2010), and neurologic disorders (Verdijk et al., 2012) cause significant reduction in muscular mass. On the other hand, in healthy people, muscular atrophy may occur because of conditions including space flights, bed rest, reduction in steps, and immobility. In addition, aging is accompanied by muscle loss (Hughes et al., 2016; Keller & Engelhardt, 2013).

Our results revealed that transcription of gene MST1 and atrogin increases within both slow- and fast-tension muscles, but this increase in MST1 was higher in slow-tension soleus muscle compared to EDL one (6 vs. 2.3 times). It suggests that MST1 controls the muscular atrophy program in a fiber-dependent manner. Moreover, researchers suggested that in various atrophic conditions including denervation, MST1 follows a pattern dependent to the type muscular fiber (Wei et al., 2013a).

A)



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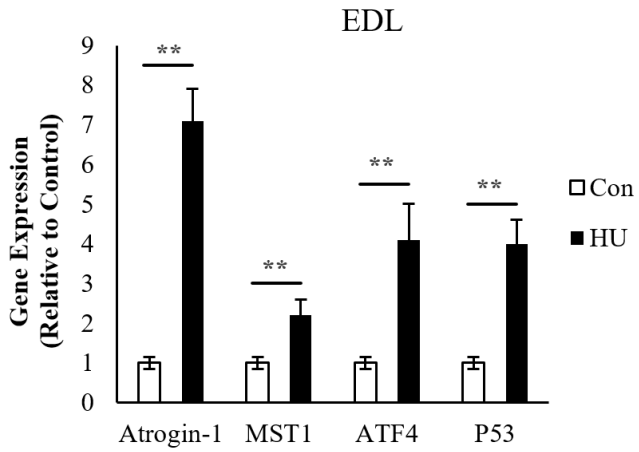

Figure 3. Gene expression levels in muscles in response to mechanical hindlimb suspension. (A) Gene expression pattern of Soleus after fourteen days after mechanical hindlimb suspension. (B) Gene expression pattern of EDL after fourteen days after mechanical hindlimb suspension. **P<0.005. Con: control rats; HU: mechanical hindlimb suspension rats. Five rats in each group were used for this analysis. Moreover, the analysis was performed in triplicate.

According to these findings, our study suggested that smaller atrophy in EDL muscle is associated with lesser MST1 gene expression. Although, we demonstrated that MST1 expression and its atrophic role is higher in fast-contraction muscles under suspension conditions. Accordingly, it has been shown that the reduction in activity of absolute EMG in EDL muscle (~90 mV/h) compared to soleus muscle (~473 mV/h) is lower under suspension condition (Ohira et al., 2006; Ohira et al., 2002).

One of the interesting results of this study was that despite higher increase of MST1 in soleus muscles against EDL ones, atrogin-1 expression was higher in EDL compared to soleus muscles. We observed that EDL muscles had higher expression in atrogin-1 than soleus muscle. Some evidence suggests that fast-contraction muscles in most catabolic conditions are more sensitive to atrophy than that slow-contraction muscles are. These conditions include starvation, cancer, and chronic heart failure all of which trigger the expression of MAFbx/atrogin-1 and MuRF1 (Gomes, Lecker, Jagoe, Navon, & Goldberg, 2001; Yu et al., 2008). Thus, atrogin-1 may be more sensitive to skeletal muscle atrophy in models due to immobility of fast muscular fibers when compared to slow muscular fibers. These findings suggest that it is possible that other pathways are responsible for the loss of muscle mass in soleus muscle; this requires more researches.

In addition, evidence suggest that some key factors like P53, activation of transcription factor 4 (ATF4), increase under conditions like inactivation and immobility. It has been shown that upregulation of ATF4 causes atrophy of muscles in healthy male rats (Ebert et al., 2010). Additionally, it has been reported that suppressing ATF4 gene inhibits -to some degrees- atrophy due to immobilization and starvation (Fox et al., 2014). Our study's results showed that transcription of ATF4 gene increases 14 days after lower organ suspension in both slow- and fast-tension muscles but, this increase was higher in soleus muscles than in EDL ones. This suggests that ATF4 controls the muscular atrophy program in a muscular fiber-dependent manner. This change in ATF4 expression was consistent with an observed alteration in soleus and EDL muscular mass. The mechanisms that increase ATF4 are not completely known, but it has been shown that kinase elf2a and transcription factor foxo3 may involve in ATF4 expression. For example, it has been suggested that inhibition of elf2 decreases ATF4 expression as well as muscular atrophy during starvation (Ebert et al., 2010). Recent studies suggest that foxo3 has an interaction with ATF4 in atrophic stimulation conditions and rats lacking foxo3 in skeletal muscles demonstrate diminished ATF4 expression and reduced muscular atrophy (Milan et al., 2015). Furthermore, researchers suggested that through phosphorylation of serin7, MST1 could cause an increase in foxo3 and nuclear transportation of it within skeletal muscles (Li et al., 2007). Therefore, the increase of MST1 can involve in ATF4 gene expression through upregulation

of foxo3 and nuclear transportation of it. Interestingly, in the current research, MST1 and ATF4 demonstrated a similar expression pattern in both soleus and EDL muscles in response to the two-week suspension of the lower organ.

This study also suggested that ATF4 and P53 expressions are dependent on muscular fiber type so that their expression is higher in soleus compared to EDL muscles. Similar to ATF4, it was shown that P53 is upregulated in both types of muscles because of lower organ suspension, although its expression was higher within soleus muscles. It has been suggested that p53 mediates the cell adaptations to stress (Horn & Vousden, 2007). Likewise, there vidence that show activation of P53 causes atrophy in muscular fibers (Schwarzkopf, Coletti, Marazzi, & Sassoon, 2008). Additionally, increased expression of P53 has been detected in various atrophic stimulations (Edwards et al., 2007; Ehrnhoefer et al., 2013). Previous studies demonstrated that P53 involves atrophy independently from ATF4, but simultaneously increased expression of them applies an excessive impact on muscular masses and causes more intensive atrophy (Fox et al., 2014). Therefore, according to this evidence, we can conclude that stronger activation of P53 and ATF4 expression in soleus muscle in this study was probably one of the reasons for more reduction in muscular mass.

Conclusion

Our study suggests that ATf4, P53, MST1, and atrogin-1 gene expression in soleus and EDL muscles under immobility due to the suspension of posterior organ increases by various levels. Considering the responses of different types of muscles to atrophy, it seems that we should seek for various treatment strategies to prevent muscular atrophy. Future researches should be done to identify these genes in slow and fast muscles because this information is necessary for the development of new therapies for muscular atrophy.

What is already known on this subject?

Mechanical hindlimb suspension of lower extremities leads to prompt atrophy in rats' skeletal muscles.

What this study adds?

This study suggests that ATf4, P53, MST1, and atrogin-1 gene expression in soleus and EDL muscles under immobility due to the suspension of posterior organ increases by various levels.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The study protocol was approved by the Ethics Committee of Lorestan Azad University (Code number: LUNS.REC.1395.170).

Informed consent Animal study.

Author contributions

Conceptualization: M.R., A.K.; Methodology: A.K., L.Z.; Software: M.A.M., M.R.; Validation: M.R.; Formal analysis: A.K.; Investigation: L.Z., M.A.M.; Resources: M.R.; Data curation: M.A.M.; Writing - original draft: M.R., A.K.; Writing - review & editing: L.Z.; Visualization: M.A.M., L.Z.; Supervision: A.K.; Project administration: M.R.; Funding acquisition: A.K.

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