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RELATIONSHIP BETWEEN FUNCTION AND MORPHOLOGY AFTER HINDLIMB SUSPENSION: EFFECTS OF ELECTRICAL STIMULATION

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ABSTRACT

Purposes: To demonstrate the functional and morphological effects of electrical stimulation (ES) in preventing muscle atrophy and to examine the relationship between functional and morphological changes. **Design and Methods:** Adult male Wistar rats (age 10 weeks) were randomly divided into 3 groups: control (CON), hindlimb suspension (HS) and hindlimb suspension plus electrical stimulation (HS+ES). The rats in the HS+ES group were subjected to stimulation at 50 Hz every other day for 2 weeks. A functional testing was done using up and down stair-climbing, and a morphological analysis (muscle cross-sectional area; MCSA) was done using immunohistochemistry (IHC) staining methods. **Results and Conclusion:** ES at 50 Hz could suppress muscle atrophy as that seen in functional and morphological changes. All groups, except the HS group, were able to complete the task in both climbing up and down the stairs. Moreover, there was an observed positive relationship between functional and change in morphology of soleus (SOL) and tibialis anterior (TA) muscles for 2 weeks of Hindlimb unloaded (HU). For upstairs-climbing, the correlations were found to be 0.93 in the SOL muscle and 0.54 in the TA muscle. For downstairs-climbing, the correlations were found to be 0.82 in the SOL muscle and 0.48 in the TA muscle.

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KEYWORDS: Function, Morphology, Hindlimb suspension, Electrical stimulation

INTRODUCTION

Muscle atrophy occurs in a variety of pathophysiological settings. It results from inactivity, such as casting, immobilization, or prolonged bed rest,[1-13] and as a result of age-related loss of skeletal muscle,[14,15] as antigravity muscles may also experience a reduction in lower limb muscular performance. These can be in response to physical inactivity and involved in muscle function,[16] as observed by a decrease in walking, stair-climbing and standing up [6-8] as a result of altered muscle morphology.[2-5,9-11] Morphological changes can include muscle wasting,[17] a loss or reduction in muscle mass[2-5,18] and a decrease in cross-sectional area (CSA) of muscle.[2-4,9-11]

Methods such as ES have been used to stimulate movement and prevent muscle atrophy during periods of inactivity.[19-21] However, the progressive knowledge on the prevention or alleviation of muscle atrophy and maintenance of muscle function is limited because studies investigating both the functional and morphological changes induced by ES are rare. Further studies are needed to understand the effects of ES on the functional and morphological changes in muscle and how ES should be applied in various settings in the prevention or reduction of disused muscle atrophy.

The purposes of this study were 1) to demonstrate the efficacy of ES in preventing muscle atrophy, in maintaining functional and in inducing morphological changes, and 2) to examine the relationship between functional and morphological changes.

METHODS

Animal Groups

Fifteen adult male Wistar rats (age 10 weeks) were randomly divided into 3 groups: control (CON, n = 5), hindlimb suspension (HS, n = 5), and hindlimb suspension plus electrical stimulation (HS+ES, n = 5). The rats of each group were housed in an isolated and environmentally-controlled room (12-hour light-dark cycle) with a standard laboratory diet and tap water provided ad libitum.

Hindlimb Suspension

The rats in the HS and the HS+ES groups were treated by HU for 2 weeks according to the method defined by Morey et al. (1979). The forelimbs were allowed to maintain contact with the floor of the cage, and the rats were allowed to move freely to food and water while the hindlimbs were suspended to prevent the hindlimbs from bearing weight on the floor or sides of the cage.

Electrical Stimulation

The rats in the HS+ES group were given stimulation at a frequency of 50 Hz with an intensity of 10 mA for the duration of 15 minutes, twice a day, 3 days a week and every other day for 2 weeks using the electrical stimulator (SEN-3301, Nihon Kohden Corp., Tokyo, Japan). The stimulus was 10 mA, positive square wave with a duration pulse of 0.5 ms. An active electrode was placed on the skin at the closet point to the sciatic nerve on the gluteal area, and a ground electrode was placed on the foot surface. Each electrode was sized 12 × 12 mm.

Up and Down Stair-Climbing

The functional of the rats was tested using up and down stair-climbing (10 steps, each step 5-cm high and 5-cm wide), as adapted from Shaffer et al. (2000) and Ichihara et al. (2002). The time of the functional testing was recorded using a digital video camera. Each rat climbed up and down of its own accord. They were timed as they climbed up, were turned around on the top platform and continued the round-trip climb down. The times were recorded with an accuracy of 0.1 s. During an exploratory test session, the suspension apparatus were removed from the rats in the HS and HS+ES groups. It was made sure that the rats could climb up and down the stairs. One week before the experimental period, each rat was observed during a once-per-day stair-climbing training session, which consisted of exposure to the testing room and climbing up and down the stairs one time.

Preparation of Muscle Samples

After the 2-week experimental period, the rats were weighed and then anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg. The soleus (SOL) and the tibialis anterior (TA) muscles were cut from the right hindlimb of each rat, and then fixed in 10% formalin for morphological analysis.

Morphological Analysis

The SOL and the TA morphological changes were quantitated on immunohistochemical (IHC)[24] stained cross-sections (5-μm thick). The sections were examined under a light microscope to determine the CSA of the muscle. The muscle cross-sectional area (MCSA) was calculated using image software (Scion image, Scion Corp., Maryland, USA).

Statistical Analysis

Group differences were compared with an analysis of variance (ANOVA), with Tukey's post-hoc analysis. The relationship between MCSA and stair-climbing time were examined by linear regression and correlation analysis. All data were expressed as mean $\pm$ standard deviation (SD). Values were considered statistically significant at $p < 0.05$.

RESULTS

Up and Down Stair-Climbing

All groups, except the HS group, were able to complete the task in both climbing up and down the stairs. The stair-climbing times were lower ($p < 0.05$) for the CON than for the HS+ES groups (Table 1). The relationship between mean MCSA and mean stair-climbing time after the 2-week experimental period and the Pearson product moment correlations between mean MCSA and mean stair-climbing time are presented in Table 2. For the upstairs-climbing, the mean MCSA of the SOL and the TA muscles were correlated with mean stair-climbing time: 0.93 in the SOL muscle and 0.54 in the TA muscle. For the downstairs-climbing, the mean MCSA of the SOL and the TA muscles were correlated with the mean stair-climbing time: 0.82 in the SOL muscle and 0.48 in the TA muscle. A comparison of the SOL and the TA muscles data showed that the mean MCSA of the SOL muscle was highly correlated with mean upstairs-climbing ($r = 0.93$) and mean downstairs-climbing ($r = 0.82$).

TABLE 1 The functional test using up and down stair-climbing

Time (s)	Group		
	CON (n = 5)	HS (n = 5)	HS+ES (n = 5)
Up stair-climbing	3.95 $\pm$ 0.03	Non	7.68 $\pm$ 0.06[a]
Down stair-climbing	5.59 $\pm$ 0.06	Non	11.43 $\pm$ 0.05[a]

Non, non-functional.

[a]Significant difference from CON, $p < 0.05$.

TABLE 2 Relationship between mean muscle cross-sectional area and mean stair-climbing time

MCSA	Time (s)	
	Up stair-climbing	Down stair-climbing
SOL (μm^2)	0.93[a]	0.82[a]
TA (μm^2)	0.54[a]	0.48[a]

MCSA, muscle cross-sectional area.

[a]Correlation is significant at the 0.05 level (1-tailed).

Muscle Cross-Sectional Area

In the SOL muscle, there was a significant decrease in CSA after the 2-week experimental period in the HS group (54.24%) compared with the CON group ($p < 0.05$). There was no apparent change ($p > 0.05$) in the HS+ES group. In the TA muscle, there was a significant decrease in CSA after the 2-week experimental period in the HS group (21.71%) compared with the CON group ($p < 0.05$). There was no apparent change (p

> 0.05) in the HS+ES groups. For the SOL and the TA muscles, the reduction in CSA was highly expressed in the SOL muscle (Table 3).

TABLE 3 Muscle cross-sectional area for the SOL and the TA muscles

MCSA	Group		
	CON (n = 5)	HS (n = 5)	HS+ES (n = 5)
SOL (μm^2)	4,832.00 $\pm$ 135.61	2,211.00 $\pm$ 72.03^{ab}	4,723.00 $\pm$ 130.02
TA (μm^2)	4,777.00 $\pm$ 113.24	3,740.00 $\pm$ 68.50^{ab}	4,752.00 $\pm$ 104.89

MCSA, muscle cross-sectional area.

[a]Significant difference from CON, $p < 0.05$.

[b]Significant difference from HS+ES, $p < 0.05$.

DISCUSSION

Effectiveness of Electrical Stimulation to Prevent Muscle Atrophy

The study used parameters for ES at a frequency of 50 Hz, twice a day, 3 days a week, duration 15 minutes and intensity 10 mA. In the SOL muscle, the results of the study showed that HS decreased CSA in the HS group, while this change was maintained in the HS+ES group (Table 3). In the TA muscle, a similar pattern was observed in that HS decreased CSA in the HS group, while this change was maintained in the HS+ES group (Table 3). Remarkable are the morphological characteristic changes associated with muscle atrophy of the SOL[2-4,21,25-28] and the TA muscles, and the results of this study confirm and extend the findings previously reported. Data also suggest that ES can prevent changes in morphology in the atrophy muscle. These may be induced muscle hypertrophy. Therefore, the muscle hypertrophy that occurred might be related to the muscle adaptation in respond to ES.

Relationship Between Functional and Morphological

The results of the study showed that following 2 weeks of HU caused a greater decrease in CSA of the SOL than of the TA muscles (Table 3). Another similar difference in atrophy between these muscles has been reported after disuse atrophy induced by inactivity.[1,3,9,21] The higher atrophic response of the SOL muscle than that of the TA muscle has been attributed to the SOL muscle's greater function as an antigravity muscle, whether it be postural or locomotor.[2,9,29-30] Whatever the mechanism is, the greater atrophy in the SOL muscle after mechanical unloading of the muscle appears to be of functional significance.[6,11,31] Another similar response occurs during prolonged bed rest of effects of physical inactivity in humans; the rate of muscle atrophy is, in fact, extremely fast in 4 h during periods of bed rest, and the antigravity muscles (extensor muscles) are more affected than the non-antigravity muscles (flexor muscles).[18,32] Importantly, the study found a relationship between mean MCSA and mean stair-climbing time following 2 weeks of HU of the SOL and the TA muscles (Table 2). Additionally, the relationship between the mean MCSA of the SOL muscle to find a high correlation with mean upstairs-climbing (r = 0.93) and mean downstairs-climbing (r = 0.82). A positive correlation was observed between morphology (MCSA) and function (up and down stair-climbing), noting that changes in morphology may influence the functional.

CONCLUSION

The results suggest that muscle atrophy as that seen in functional and morphological changes could be suppressed by ES. Importantly, the study showed the relationship between functional and morphological changes of SOL and TA muscles.

REFERENCES

1. Boonyarom O, Inui K. Atrophy and hypertrophy of skeletal muscles: structural and functional aspects. Acta Physiol 2006;188:77-89
2. Thomason DB, Booth FW. Atrophy of the soleus muscle by hindlimb unweighting. J Appl Physiol 1990;68:1-12
3. Ohira Y, Jiang B, Roy RR, et al. Rat soleus muscle fiber responses to 14 days of spaceflight and hindlimb suspension. J Appl Physiol 1992;73:51S-57S
4. Riley DA, Slocum GR, Bain JLW, Sedlak FR, Sowa TE, Mellender JW. Rat hindlimb unloading: soleus histochemistry, ultrastructure, and electromyography. J Appl Physiol 1990;69:58-66
5. Bloomfield SA. Changes in musculoskeletal structure and function with prolonged bed rest. Med Sci Sports Exerc 1997;29:197-206
6. Shaffer MA, Okereke E, Esterhai JL Jr, et al. Effects of immobilization on plantar-flexion torque, fatigue resistance, and functional ability following an ankle fracture. Phys Ther 2000;80:769-780
7. Fitts RH. Effects of regular exercise training on skeletal muscle contractile function. Am J Phys Med Rehabil 2003;82:320-331
8. Snyder-Mackler L, Ladin Z, Schepsis AA, et al. Electrical stimulation of the thigh muscles after reconstruction of the anterior cruciate ligament. J Bone Joint Surg Am 1991;73:1025-1036
9. *Stevens JE, Walter GA, Okereke E, et al.* Muscle adaptations with immobilization and rehabilitation after ankle fracture. Med Sci Sports Exerc *2004;*36:1695-1701
10. *Berry P, Berry I, Manelfe C.* Magnetic resonance imaging evaluation of lower limb muscles during bed rest: a microgravity simulation model. Aviat Space Environ Med 1993;64:212-218
11. Vandenborne K, Elliott MA, Walter GA, et al. Longitudinal study of skeletal muscle adaptations during immobilization and rehabilitation. Muscle Nerve 1998;21:1006-1012
12. LeBlanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, Spector E. Regional changes in muscle mass following 17 weeks of bed rest. J Appl Physiol 1992;73:2172-2178

13. Bodine SC. Disuse-induced muscle wasting. 2013;45:2200-2208
14. 14. Morley JE. Sarcopenia in the elderly. Family Practice 2012;29 Suppl 1:i44-i48
15. Glass D, Roubenoff R. Recent advances in the biology and therapy of muscle wasting. Ann N Y Acad Sci 2010;1211:25-36
16. Russell RP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function [online]. 2010 [cited 2014 Jan 29]. Available from: http://jp.physoc.org/content/early/recent
17. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models [online]. 2011 [cited 2014 Jan 29]. Available from: http://www.skeletalmusclejournal.com/content/1/1/4
18. Boonyarom O. Rehabilitation: Atrophy of skeletal muscles. Naresuan University Journal 2009;17:85-90
19. Petrofsky JS. Electrical stimulation: neurophysiological basis and application. Basic Appl Myol 2004;14:205-213
20. Qin L, Appell HJ, Chan KM, Maffulli N. Electrical stimulation prevents immobilization atrophy in skeletal muscle of rabbits. Arch Phys Med Rehabil 1997;78:512-517
21. Boonyarom O, Kozuka N, Matsuyama K, Murakami S. Effect of electrical stimulation to prevent muscle atrophy on morphologic and histologic properties of hindlimb suspended rat hindlimb muscles. Am J Phys Med Rehabil 2009;88:719-726
22. Morey E, Sabelman E, Turner R, Baylink D. A new rat model simulating some aspects of space flight. Physiologist 1979;22:S23-S24
23. Ichihara S, Murakami S, Fujito Y. The mechanism of behavioral and brain recovery after unilateral cortical lesion in rats. The society for the study of medical basis of physical therapy 2002;5:9-15
24. Dubowitz V, Sewry CA. Muscle Biopsy: A Practical Approach, ed 3. Saunders, London, 2007
25. Ansved T. Effects of immobilization on the rat soleus muscle in relation to age. Acta Physiol Scand 1995;154:291-302
26. Qin L, Appell HJ, Chan KM, Maffulli N. Electrical stimulation prevents immobilization atrophy in skeletal muscle of rabbits. Arch Phys Med Rehabil 1997;78:512-517
27. Leterme D, Falempin M. Compensatory effects of chronic electrostimulation on unweighted rat soleus muscle. Pflugers Arch 1994;426:155-160
28. Maxwell LC, Moody MR, Enwemeka CS. Muscle atrophy continues after early cast removal following tendon repair. Anat Rec 1992;233:376-386
29. Herrera NM Jr, Zimmerman AN, Dykstra DD, et al. Clenbuterol in the prevention of muscle atrophy: a study of hindlimb-unweighted rats. Arch Phys Med Rehabil 2001;82:930-934

30. Thompson LV, Johnson SA, Shoeman JA. Single soleus muscle fiber function after hindlimb unweighting in adult and aged rats. J Appl Physiol 1998;84:1937-1942
31. Ohira M, Hanada H, Kawano F, et al. Regulation of the properties of rat hind limb muscles following gravitational unloading. Jpn J Physiol 2002;52:235-245
32. Kasper CE, Talbot LA, Gaines JM. Skeletal muscle damage and recovery. AACN Clin Issues 2002;13:237-247