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● *Original Contribution*

LOW-INTENSITY PULSED ULTRASOUND PROMOTES EXERCISE-INDUCED MUSCLE HYPERTROPHY

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Abstract—The purpose of this study was to investigate whether low-intensity pulsed ultrasound (LIPUS) promotes exercise-induced muscle hypertrophy. Twenty-four adult Sprague-Dawley (SD) rats were randomly assigned to three groups (n = 8 per group): normal control group (NC), treadmill exercise group (TE) and treadmill exercise + LIPUS group (TE + LIPUS). The TE + LIPUS group received a LIPUS treatment (1 MHz, 30 mW/cm^2) at the gastrocnemius for 20 min/d after treadmill exercise. The TE group was sham-treated. Eight weeks of treadmill training successfully established the exercise-induced muscle hypertrophy model. Muscle strength, muscle mass and muscle fiber cross-sectional area were significantly increased in the TE + LIPUS group compared with the TE group. Moreover, LIPUS treatment significantly upregulated the expression of Akt, mTOR, p-Akt and p-mTOR and significantly downregulated the expression of MSTN, ActRIIB, FoxO1 and its phosphorylation. The results indicated that LIPUS promotes exercise-induced muscle hypertrophy by facilitating protein synthesis and inhibiting the protein catabolism pathway. (E-mail: sunlijun@snnu.edu.cn) **© 2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Low-intensity pulsed ultrasound, Treadmill exercise, Skeletal muscle hypertrophy, Protein catabolism, Protein synthesis.**

INTRODUCTION

Skeletal muscle hypertrophy is an increase in skeletal muscle cross-sectional area, but not in the number of muscle fibers (Kasashima et al. 2002). Many indicators can be used to evaluate skeletal muscle hypertrophy, including cross-sectional area of muscle fiber, muscle mass, muscle mass index weight, muscle strength and protein content. Among these, cross-sectional area of muscle fiber, muscle mass and muscle strength are easier to use and accurately reflect muscle hypertrophy (Brook et al. 2015; Fry et al. 2014; Schoenfeld et al. 2017). Many studies have reported that exercise training promotes an increase in skeletal muscle cross-sectional area and results in skeletal muscle physiologic hypertrophy (Harber et al. 2012; Moraska et al. 2000; Nalbandian et al. 2013). Muscle mass is the result of a dynamic balance between protein synthesis and degradation (Ahtiainen et al. 2015; Damas et al. 2015; Hansen et al. 2012). The balance is coordinately regulated by two major branches of the protein kinase B (Akt) signaling pathway: the Akt/mammalian target of rapamycin (mTOR) pathway controls protein synthesis, and the Akt/forkhead box protein O1 (FoxO1) pathway controls protein degradation (Kazior et al. 2016; Tang et al. 2014a). Myostatin (MSTN), a member of the transforming growth factor-β superfamily, is a potent negative regulator of skeletal muscle growth. Exercise training has been reported to induce skeletal muscle hypertrophy by inhibiting MSTN expression (de Souza et al. 2014; Santos et al. 2015).

In past decades, low-intensity pulsed ultrasound (LIPUS) has been used in sports medicine (Warden 2003). The effect of LIPUS on muscle contusion and bone fracture healing has been reported (Chongsatientam and Yimlamai 2016; Chung et al. 2011; Hu et al. 2014; Matsumoto et al.

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2014) Subsequently, growing evidence has indicated that LIPUS also plays an important role in skeletal muscle cells. For instance, LIPUS therapy has been reported to stimulate myoblast proliferation and differentiation and enhance the regeneration of myofibers, with better physiologic performance in injured mice muscles after laceration (Chan et al. 2010). Other findings also have indicated that therapeutic ultrasound can stimulate the differentiation of skeletal muscle cells *in vitro* (Abrunhosa et al. 2014; Ikeda et al. 2006). However, whether LIPUS promotes exercise-induced skeletal muscle hypertrophy has not been investigated previously.

Accordingly, we examined the effects of LIPUS on exercise-induced skeletal muscle hypertrophy by evaluating muscle strength, mass and fiber diameter. Furthermore, possible molecular mechanisms were also explored through analysis of gene and protein expression of Akt, mTOR, FoxO1, MSTN and its receptor activin receptor type IIB (ActRIIB).

METHODS

Animals

Healthy Sprague-Dawley rats (200–220 g) were purchased from the Laboratory Animal Breeding and Research Center of Xi'an Jiaotong University (Xi'an, China) and were housed in a temperature- and humidity-controlled room (22 ± 2°C, 60 ± 5% humidity and 12-h light/dark cycle). After 5 d of acclimation, rats were randomly assigned to three groups (n = 8 in each group): normal control group (NC), treadmill exercise group (TE) and treadmill exercise + LIPUS group (TE + LIPUS). All experiments were conducted with approval of the ethics committee of Shaanxi Normal University and in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Exercise protocol

Exercise training was achieved by treadmill exercise; the slope was 5°. Rats in the TE and TE + LIPUS groups ran on the treadmill at 28 m/min, 60 min/d, 6 d/wk for 8 wk.

Grip test

In this study, grip tests were performed on forelegs with a grip strength meter (YLS-13 A, Anhui Zhenghua Bioinstrumentation, Huaibei, Anhui, China). Rats were tested three times in succession without rest. The results of three tests were averaged for each rat (Fig. 1).

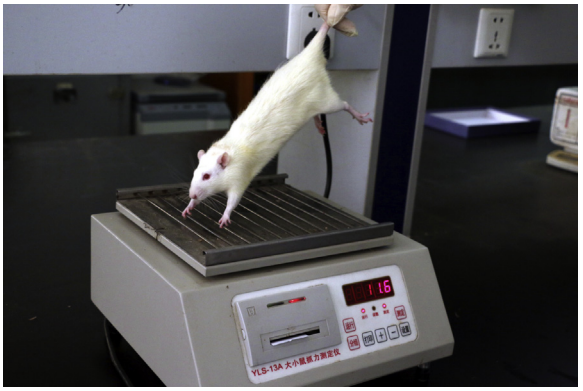

Fig. 1. Grip test. Rats were tested three times in succession without rest. The results of three tests were averaged for each rat.

Ultrasound treatment

The LIPUS device (Shanghai Acoustics Laboratory, Chinese Academy of Sciences, Shanghai, China) produced a 2000-μs burst of 1-MHz acoustic sine waves repeating at 100 Hz with a spatial-averaged temporal-averaged (SATA) intensity of 30 mW/cm^2. For the TE + LIPUS group, LIPUS was applied to the hindlimb after shaving at the pulsed mode (2 ms on, 8 ms off) for 20 min/d after treadmill exercise 6 d/wk for 8 wk. The TE group was treated similarly to the TE + LIPUS group, except the power was not turned on. Ultrasound coupling agent (Chengxin Medical Auxiliary Material Factory, Tianjin, China) was applied between the probe and skin. A 2.5-cm-diameter ultrasonic probe was used and was not moved around the area (Fig. 2).

Weight and sample preparation

Rats were sacrificed with an overdose of diethyl ether after 8 wk of treatment, and final weights were recorded. The gastrocnemius was dissected and weighed. A part of the gastrocnemius was fixed in 4% paraformaldehyde solution for morphologic analysis, and another part was quickly frozen in liquid nitrogen and stored at −80°C until analysis.

Biochemical analysis

To verify the effect of LIPUS on the metabolic capacity of skeletal muscle, activity levels of succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) (the marker enzymes in the Krebs cycle) in the gastrocnemius were analyzed with standard colorimetric tests using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the protocols provided by the manufacturers.

Morphometric analysis

Gastrocnemius muscles were dissected rapidly and fixed with 4% paraformaldehyde for 24 h. Muscle tissues were embedded in paraffin and mounted on ultrathin semiautomatic microtomes (Dako, Tokyo, Japan). After fixation, serial 8- to 10-μm transverse sections were