Chronic Exercise Training Down-Regulates TNF-α and Atrogin-1/MAFbx in Mouse Gastrocnemius Muscle Atrophy Induced by Hindlimb Unloading

Saad Al-Nassan¹, Naoto Fujita¹, Hiroyo Kondo², Shinichiro Murakami³ and Hidemi Fujino¹

¹Department of Rehabilitation Science, Kobe University, Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan, ²Department of Food Sciences and Nutrition, Nagoya Women’s University, 3-40 Shioji-cyo, Mizuho-ku, Nagoya 467-8610, Japan and ³Department of Physical Therapy, Himeji Dokkyo University, 7-2-1 Kamiono, Himeji 670-8524, Japan

Received June 25, 2012; accepted September 11, 2012; published online October 27, 2012

The purpose of this study was to investigate the effect of chronic moderate-intensity training in order to prevent muscle atrophy with a focus on TNF-α and atrogin-1/MAFbx as main proteolytic indicators. Hindlimb unloading model of mice received treadmill running exercise for 1 hr per day during hindlimb unloading period of 6 weeks. The gastrocnemius muscle mass, muscle fiber cross-sectional area, and succinate dehydrogenase (SDH) activity in the muscle fiber were higher in the exercised group, while TNF-α and atrogin-1/MAFbx mRNA expressions were significantly lower. Results in the present study showed that chronic exercise could prevent over expression of TNF-α and atrogin-1/MAFbx in the atrophied skeletal muscle, providing further support to the effects of chronic exercise training on muscle atrophy.

Key words: TNF-α, atrogin-1/MAFbx, gastrocnemius, muscle atrophy

I. Introduction

Tumor necrosis factor-α (TNF-α) is a polypeptide cytokine that has been associated with muscle wasting and weakness in inflammatory disease [35]. It has been reported that TNF-α stimulates muscle catabolism by activating the ubiquitin/proteasome pathway [23–25], which is the major pathway for selective protein degradation implicated in muscle atrophy, resulting from conditions such as unloading, denervation, and immobilization [34]. In the ubiquitin/proteasome pathway, the targeted protein is labeled by E3 ligases: in skeletal muscle, two E3 proteins: atrogin-1/MAFbx and MuRF1 are up-regulated and appear to be essential for accelerated muscle protein loss in a variety of experimental models of catabolism including hindlimb unloading [6]. In the study by Li et al., they suggest that TNF-α acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin-1/MAFbx in the skeletal muscle [20].

Hindlimb unloading has been used in rodents as a model of disuse to induce muscle atrophy [19, 38]. Previous studies have shown that exercise is an effective countermeasure to attenuate muscle loss induced by hindlimb unloading [1, 8, 10, 13]. In these studies, short-term resistance exercise had been used to attenuate the proteolytic process. However, there haven’t been any studies regarding the effect of chronic exercise training on muscle atrophy induced by hindlimb unloading, focusing on the regulation of muscle proteolytic mediators. Exercise training in moderate intensity can have an influence on skeletal muscles to secrete cytokines in healthy rats [22]. We hypothesized that chronic moderate intensity exercising would down-regulate TNF-α in atrophied skeletal muscle and also down-regulate the atrogin-1/MAFbx level, attenuating the overall ATP dependent Ubiquitin-proteasome muscle proteolysis pathway. In this study, a program of moderate-intensity exercises was used on a hindlimb unloading model of mice, to investigate the effects of chronic exercise on down-regulation of proteolytic mediators like TNF-α, and atrogin-1/MAFbx, which are main atrophy mediators.
II. Materials and Methods

**Animal model and experimental design**

Twenty-two adult male ICR mice weighing in 32–34 g (Japan SLC, Shizuoka) were used in the present study. The animals were housed in individual cages in a temperature-controlled room at 22±2°C with a light-dark cycle of 12 hr and maintained on mice chow and water ad libitum. The animals were randomly divided into four groups: hindlimb unloading (HU, n=5), hindlimb unloading with exercise (HU+Ex, n=5), control with exercise (Con+Ex, n=7), and age-matched control (Con, n=5) groups. The animals in the HU and HU+Ex groups were applied hindlimb unloading for 6 weeks as described previously [26, 27]. The suspended height was adjusted to allow freely, and to prevent any contact between the hind limbs and any cage surface. Animals in the Con+Ex, and HU+Ex freely, and to prevent any contact between the hind limbs were sacrificed via an overdose of sodium pentobarbital. The right GAS muscle of each animal was homogenized in an extraction kit (QuickGene RNA tissue kit, Foster (Applied Biosystems), CA) and stored at −80°C until the histological and immunological analyses. For total RNA isolation, the medial portion of GAS muscle was immersed in RNAlater solution (Ambion, TX) and stored at −80°C with 2% CoCl$_2$ for 20 hr. Expression levels of TNF-α was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer’s instructions (eBioscience, San Diego, CA).

**ELISA for TNF-α**

The right GAS muscle of each animal was homogenized in ice-cooled homogenization buffer (10 mM NaCl and 10 mM Tris-HCl, pH 7.4), containing a protease inhibitor cocktail (1:200, Sigma, MO). The homogenates were centrifuged at 15000 g for 15 min at 4°C. The supernatant was saved, and protein concentration was determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as a standard. Quantitative assessment of TNF-α protein was carried using an ELISA kit to determine the expression levels of TNF-α (i.e., a high oxidative) and IIB (low oxidative) fibers. Results were compared with the homogenates of muscle fibers categorized as types I (fast twitch) and II (slow twitch) and were normalized to the expression levels of TNF-α.

**Real-time quantitative polymerase chain reaction (qPCR) analyses**

Total RNA was extracted from ~10 mg of GAS muscle using an extraction kit (QuickGene RNA tissue kit, SII, Fujifilm, Tokyo, Japan). Reverse transcription was carried out using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA), and cDNA samples were stored at −20°C. Expression levels of TNF-α (Mn00443258_m1), atrogin-1/MAFbx (Mn00499518_m1), and IL-6 (Mn00446790_m1) mRNA were quantified by TaqMan Gene Expression Assays (Applied Biosystems). Each TaqMan probe and primer set was validated by performing qPCR with a series of cDNA template dilutions to obtain standard curves of threshold cycle time against relative concentration using the normalization gene 18S. The qPCR was performed using PCR Fast Universal Master
Mix (Applied Biosystems) in a MicroAmp 96-well reaction plate. Each well contained 1 μL cDNA template, 10 μL PCR Fast Master Mix (Applied Biosystems), 8 μL RNase-free water, and 1 μL TaqMan Gene Expression Assays in a reaction volume of 20 μL. All samples and non-template control reactions were performed in a 7500 Fast Sequence Detection System (Applied Biosystems) at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min.

**Statistical analyses**

The data are expressed as mean±SE. Overall differences were determined using a one-way analysis of variance (ANOVA). When ANOVA was significant, group differences were determined using the Tukey’s post-hoc test. The statistically significant level was set at P<0.05.

### III. Results

**Body and muscle wet weights**

The mean body weights of the animals reflected a continuous weight gain through the six weeks period of the experiment. During the first week after start of unloading, the mean body weights increased in the Con, HU, and HU+Ex groups, i.e. 40.2±0.3, 36.4±0.7, and 35.8±0.3 g, respectively; while the Con+Ex group remained a mean of 32.4±0.6 g in the first week. After 6 weeks of unloading, the mean body weights in the Con, Con+Ex, HU, and HU+Ex groups were 44.6±0.9, 37.7±0.7*, 39.7±1.2*, and 38.6±1.0 g, respectively. The mean body weights were significantly less than Con group (Table 1). The mean absolute and relative GAS muscle weights in the HU group were 34% and 26% smaller than those in the Con group, respectively, whereas the values in the HU+Ex group were 24% and 12% smaller than those in the Con group, respectively. The absolute GAS muscle weight in the HU+Ex group was significantly larger than that in the HU group, and the relative GAS muscle weight in the HU+Ex group showed no significant difference from the Con group. The mean absolute and relative GAS muscle weights in the Con+Ex group were 19% and 2% smaller than those in the Con group, respectively.

**Muscle fiber cross-sectional area**

The fibers in superficial layer of GAS muscle were divided into types IID (high oxidative) and IIB (low oxidative) (Fig. 1). In the mATPase stain, type IID fibers were smaller in size and darker in stain than type IIB fibers, which were bigger in size and lighter in stain. Type IID fibers cross-sectional area mean values were 1257±46, 1313±31, 992±49, and 1215±49 μm², for the Con, Con+Ex, HU+Ex.

### Table 1. Changes in body weight and gastrocnemius muscle weight after six weeks

| Group     | Body weight (g) | Muscle wet weight (mg) | Muscle weight/body weight (mg/g) |
|-----------|-----------------|------------------------|---------------------------------|
| Con       | 44.6±0.9        | 188±6                 | 4.2±0.02                        |
| Con+Ex    | 37.7±0.7*       | 153±4*                | 4.1±0.1                         |
| HU        | 39.7±1.2*       | 124±5*†               | 3.12±0.2*‡                      |
| HU+Ex     | 38.6±1.0*       | 143±4*‡               | 3.7±0.1                         |

Fig. 1. Light microscope images of superficial layer of the gastrocnemius muscle. Transverse sections were assayed for myofibrillar adenosine triphosphatase (mATPase) at pH 4.55 preincubation (upper), and for succinate dehydrogenase (SDH) activity (lower). 1: type IID (high oxidative); 2: type IIB (low oxidative). A, E: control group; B, F: Con+Ex group; C, G: HU group; D, H: HU+Ex group. Bar=50 μm.
Hu, and HU+Ex groups, respectively; showing a significant decrease of 21% in the HU group when compared to Con group, also showing a significant increase of 18% in HU+Ex group when compared to the HU group (Fig. 2). There were no significant differences between the Con and Con+Ex groups. Type IIB fibers cross-sectional area mean values were 2639±61, 2249±51, 1959±52, and 2245±56 μm², for the Con, Con+Ex, HU, and HU+Ex groups, respectively; showing a significant decrease of 26% in the HU group when compared to Con group, also showing a significant increase of 13% in the HU+Ex group when compared to the HU group (Fig. 2). The Con+Ex group showed a significant decrease of 15% when compared to the Con group.

Succinate dehydrogenase activity

The Con+Ex group showed a significant increase of SDH activity in both types of fibers compared to other groups (Fig. 3). The SDH activity of types IID, and IIB fibers showed a significant decrease in the HU group when compared to the other groups, also the HU+Ex group showed a significant increase of SDH activity of types IID and IIB fibers when compared to HU group (Fig. 3).

TNF-α expression

The expression level of TNF-α protein concentration in the HU group significantly increased by 4-fold from the Con group (Fig. 4A). In contrast, there were no significant differences between the Con, Con+Ex and HU+Ex groups. The expression level of TNF-α mRNA in the HU group showed a significant increase by 3-fold from the Con group (Fig. 4B). The value in the HU+Ex group was significantly less than that in the HU group, maintained at control level.

Atrogin-1/MAFbx expression

The expression level of atrogin-1/MAFbx mRNA in the HU group showed a significant increase by 2-fold from the Con group, while the HU+Ex group expression level was significantly lower than the HU group, being maintained at the Con group level (Fig. 4C). There were no significant differences between the Con and Con+Ex groups.

IL-6 expression

The expression level of IL-6 mRNA showed a significant decrease in the Con+Ex group from the Con group only, while there were no significant differences between the other groups (Fig. 4D).
IV. Discussion

The present study demonstrated the preventive effects of chronic exercise training on muscle atrophy, induced by hindlimb unloading for 6 weeks in mouse gastrocnemius muscle. In the present study, the gastrocnemius muscle showed a significant atrophy after 6 weeks of unloading; this is consistent with previous studies [21, 27, 29]. The exercise protocol used in the present study attenuated a decrease in muscle weight and cross-sectional area in types IID and IIB muscle fibers during unloading. Running exercise in the present study did not cause muscle hypertrophy in the Con+Ex group as shown in Table 1. But it was capable of attenuating muscle atrophy in the HU+Ex group through decreasing protein degeneration process, which continued in the non-exercised HU group, resulting in a significant decrease in HU group’s muscle weight, and muscle weight/body weight ratio. This suggests that moderate intensity treadmill training is capable of preventing muscle atrophy. Although most of the studies on exercise countermeasures for hindlimb unloading induced atrophy used resistance exercise protocols [5, 11, 14], our results in the present study are consistent with a study done by Gwang et al. [12] in the ability of endurance exercises in attenuating muscle atrophy induced by hindlimb unloading. Endurance exercise training is known to affect oxidative muscle fiber types, and transformation of glycolytic fibers into oxidative types [7]. This can explain why type IIB fibers cross-sectional area in Con+Ex group was smaller than Con group, also in HU+Ex group, type IIB fibers cross-sectional area was significantly bigger than HU group, but also significantly smaller than Con, and Con+Ex groups, meaning that exercise protocol did not affect type IIB fibers cross-sectional area in a hypertrophic manner. The chronic exercise training resulted in the inhibition of the over expressions of TNF-α and atrogin-1/MAFbx mRNA in the atrophied muscle, suggesting evidence for the role of chronic exercise training in regulating proteolytic pathway mediators (mainly TNF-α) which is considered as one of the triggering cytokines of many proteolytic pathways.

It has been reported that reactive oxygen species (ROS) and elevated proinflammatory cytokines, in particular TNF-α, mediate muscle atrophy; ROS can also initiate the mitochondrial-driven apoptosis pathway [4]. In the present study, the SDH activity of type IID (high oxidative) and type IIB (low oxidative) fibers in the HU group were significantly lower than the other groups. Mukhina et al. [28] stated that unloading inhibits SDH activity in muscles. The exercised groups (i.e. the Con+Ex and HU+Ex groups) showed significant increases in the SDH activity in both types of fibers, this is consistent with a previous study in which running exercises increased SDH activity in both
types of fibers of the mouse tibialis anterior [17]. In our study, chronic exercise program prevented the atrophic changes on the oxidative level, due to the ability of endurance exercises to increase oxidative capacity and decrease the effects of ROS, as stated by Wenz et al. [39].

TNF-α is a polypeptide cytokine that has been associated with muscle wasting and weakness in inflammatory disease [35], it acts via p38 to increase atrogin-1/MAFbx gene expression in the skeletal muscle. atrogin-1/MAFbx gene product is up-regulated in response to TNF-α [20]. Furthermore, TNF-α is considered essential for accelerated muscle protein loss in a variety of experimental models of catabolism, including hindlimb suspension [20]. It has been reported that the decrease in the muscle fiber’s cross-sectional area with atrophy was attenuated in atrogin-1/MAFbx-deficient mice [6]; the functional importance of atrogin-1/MAFbx is evident. In both ELISA and qPCR results for TNF-α in our study, the HU group’s expression levels were significantly elevated compared to control groups; while exercises maintained a low level of TNF-α concentration, and a significantly low expression of TNF-α mRNA compared to the HU group. Exercise training is able to decrease the level of TNF-α through muscle contraction and release of epinephrine, which in turn decreases elevated TNF-α [36]. Additionally, atrogin-1/MAFbx mRNA level was significantly elevated in the HU group, while exercises maintained a significant decrease of atrogin-1/MAFbx mRNA expression in the HU+Ex group. According to Li et al. [20], down-regulation of TNF-α through exercise training resulted in down-regulation of atrogin-1/MAFbx production, reflecting the preventive effect of chronic exercise training on attenuation of muscle atrophy. IL-6 is an immunomodulatory cytokine, and increases 100-fold in skeletal muscle during acute exercise, remaining elevated in the circulation after prolonged exercise session (~1-h post-exercise, [30]). Additionally, it has been reported that IL-6 has a role in inhibiting TNF-α production [31]. IL-6 mRNA expression levels in the present study showed a significant difference in the Con+Ex group only, being decreased from the Con group; but not showing any differences among the other groups. This low level of IL-6 mRNA expression in mouse muscle tissue is consistent with a study on human muscle tissue done by Fischer et al. [9] as they stated that IL-6 derived from contracting skeletal muscle is required less in the trained state. A study by Lira et al. [22] showed the ability of chronic exercise in decreasing IL-6 in healthy rat skeletal muscle, and same result was obtained in our study in skeletal muscle of healthy mouse represented by Con+Ex group. Many studies on muscle derived IL-6 focused on immediate responses of resistive or eccentric bouts of exercises [18, 37]. It was speculated that the difference in exercise protocol between present (endurance exercise) and previous studies (resistance exercise) caused different results for IL-6. Jonsdottir et al. [18] concluded that IL-6 is locally produced in the skeletal muscle after muscle contractions, with no significant differences between the various muscle fiber types. While it was stated by Plomgaard et al. [32] that the cytokine expression is fiber-type specific, the protein expression of IL-6 is higher in the fast fiber than in the slow fiber.

Previous studies have been reported that resistance exercise protocols contribute to induce hypertrophy of skeletal muscle and decrease the loss of muscle mass induced by hindlimb unloading [2, 3, 16]. In the study by Lira et al., a program of chronic exercises has managed to decrease cytokine production including TNF-α in healthy rat skeletal muscles [22]. Our results in the present study showed that chronic exercise could prevent overexpression of TNF-α and atrogin-1/MAFbx in the atrophied skeletal muscle. This provides further evidence for the effect of exercise on muscle atrophy. It was found that not only resistance exercise protocols, but also chronic endurance exercises could attenuate skeletal muscle atrophy induced by hindlimb unloading through down-regulation of TNF-α levels and indirect down-regulation of atrogin-1/MAFbx gene expression.

V. Acknowledgment

This study was supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

VI. References

1. Adams, G. R., Haddad, F., Bodell, P. W., Tran, P. D. and Baldwin, K. M. (2007) Combined isometric, concentric, and eccentric resistance exercise prevents unloading-induced muscle atrophy in rats. J. Appl. Physiol. 103; 1644–1654.
2. Allen, D. L., Linderman, J. K., Roy, R. R., Bigbee, A. J., Grindeland, R. E., Mukku, V. and Edgerton, V. R. (1997) Apoptosis: a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting. Am. J. Physiol. 273; C579–C587.
3. Allen, D. L., Linderman, J. K., Roy, R. R., Grindeland, R. E., Mukku, V. and Edgerton, V. R. (1997) Growth hormone/IGF-I and/or resistive exercise maintains myonuclear number in hindlimb unweighted muscles. J. Appl. Physiol. 83; 1857–1861.
4. Andrianjafinony, T., Dupré-Aucourtier, S., Letexier, D., Couchoux, H. and Desplanches, D. (2010) Oxidative stress, apoptosis, and proteolysis in skeletal muscle repair after unloading. Am. J. Physiol. Cell Physiol. 299; C307–C315.
5. Bajotto, G. and Shimomura, Y. (2006) Determinants of disuse-induced skeletal muscle atrophy: exercise and nutrition countermeasures to prevent protein loss. J. Nutr. Sci. Vitaminol. (Tokyo) 52; 233–247.
6. Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K., Nunez, L., Clarke, B. A., Poueymirou, W. T., Panaro, F. J., Na, E. and Dharmarajan, K. (2001) Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294; 1704–1708.
7. Diaz-Herrera, P., Torres, A., Morcuende, J., Garcia-Castellano, J., Calbet, A. and Sarrat, R. (2001) Effect of endurance running on cardiac and skeletal muscle in rats. Histol. Histopathol. 16; 29–35.
8. Dupont-Versteegden, E. E., Fluckey, J. D., Knox, M., Gaddy, D. and Peterson, C. A. (2005) Effect of flywheel-based resistance exercise on processes contributing to muscle atrophy during unloading in adult rats. J. Appl. Physiol. 101; 202–212.
9. Fischer, C. P., Plomgaard, P., Hansen, A. K., Pilegaard, H., Saltin,
Gwag, T., Lee, K., Ju, H., Shin, H. J., Lee, W. and Choi, I. (2009) A rat resistance exercise regimen attenuates losses of muscular skeletal mass during hindlimb suspension. *Acta Physiol. Scand.* 176; 293–300.

Graham, S. C., Roy, R. R., West, S. P., Thomason, D. and Knox, M., Tesch, P., Peterson, C. A. and Gaddy-Kurten, D. (2002) Exercise effects on the size and metabolic properties of soleus fibers in hindlimb-suspended rats. *Aviat. Space Environ. Med.* 60; 226–234.

Gwag, T., Lee, K., Ju, H., Shin, H. J., Lee, W. and Choi, I. (2009) Stress and signaling responses of rat skeletal muscle to brief endurance exercise during hindlimb unloading: a catch-up process for atrophied muscle. *Cell. Physiol. Biochem.* 24; 537–546.

Harada, F., Adams, G. R., Bodell, P. W. and Baldwin, K. M. (1989) Isometric resistance exercise fails to counteract skeletal muscle atrophy processes through the initial stages of unloading. *J. Appl. Physiol.* 100; 433–441.

Herbert, M. E., Roy, R. R. and Edgerton, V. R. (1988) Influence for atrophied muscle. *Endurance exercise during hindlimb unloading: a catch-up process for atrophied muscle. Cell. Physiol. Biochem.* 24; 537–546.

Haddad, F., Adams, G. R., Bodell, P. W. and Baldwin, K. M. (1989) Exercise effects on the size and metabolic properties of soleus fibers in hindlimb-suspended rats. *Aviat. Space Environ. Med.* 60; 226–234.

Hurst, J. E. and FITTS, R. H. (2003) Hindlimb unloading-induced atrophy in skeletal muscle and loss of function: protective effect of isometric exercise. *J. Appl. Physiol.* 95; 1405–1417.

Ishihara, A., Hirofui, C., Nakatani, T., Itoh, K., Itoh, M. and Katsuta, S. (2002) Effects of running exercise with increasing loads on tibialis anterior muscle fibers in mice. *Exp. Physiol.* 87; 113–116.

Jonsdottir, I. H., Schjerling, P., Ostrowski, K., Asp, S., Richter, E. A. and Pedersen, B. K. (2000) Muscle contractions induce interleukin-6 mRNA production in rat skeletal muscles. *J. Physiol.* 528; 157–163.

Kaser, C. E., McNulty, A. L., Otto, A. J. and Thomas, D. P. (1993) Alterations in skeletal muscle related to impaired physical mobility: an empirical model. *Res. Nurs. Health.* 16; 265–273.

Li, Y. P., Chen, Y., John, J., Moylan, J., Jin, B., Mann, D. L. and Reid, M. B. (2005) TNF-alpha acts via Pho MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J.* 19; 362–370.

Liantonio, A., Pierno, S., Digennaro, C., Giannuzzi, V., Grameniga, G., Desaphy, J. F. and Camerino, D. C. (2009) The hindlimb unloading model of microgravity differently affects skeletal muscle properties of young and adult mice. *Basic Applied Myology* 19; 107–112.

Lira, F. S., Koyama, C. H., Yamashita, A. S., Rosa, J. C., Zanchi, N. E., Batista, M. L. Jr. and Seelaender, M. C. (2009) Chronic exercise decreases cytokine production in healthy rat skeletal muscle. *Cell Biochem. Funct.* 27; 458–461.

Llovera, M., Martinez, C. G., Agell, N., Lopez-Soriano, F. J. and Argilés, J. M. (1997) TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. *Biochem. Biophys. Res. Commun.* 230; 238–241.

Martinez, C. G., Agell, N., Llovera, M., Lopez-Soriano, F. J. and Argilés, J. M. (1993) Tumor necrosis factor-α increases the ubiquitinization of rat skeletal muscle proteins. *FEBS Lett.* 323; 211–214.

Martinez, C. G., Llovera, M., Agell, N., Lopez-Soriano, F. J. and Argilés, J. M. (1995) Ubiquitin gene expression in skeletal muscle is increased during sepsis: involvement of TNF-α but not IL-1. *Biochem. Biophys. Res. Commun.* 217; 839–844.

Morey, E. R., Sabelman, E. E., Turner, R. T. and Baylink, D. J. (1979) A new rat model simulating some aspects of space flight. *Physiologist* 22; S23–S24.

Morey-Holton, E. R. and Globus, R. K. (2002) Hindlimb unloading rodent model: technical aspects. *J. Appl. Physiol.* 92; 1367–1377.

Mukhina, A. M., Nemirovskaiia, T. I., Larina, I. M., Pastushkova, L. Kh., Vasil’eva, G., Istomina, V. E., Velsova, O. M., Turtikova, O. V. and Shenkman, B. S. (2008) Effects of creatine phosphokinase competitive inhibitor on system and tissue energy metabolism in rats in the norm and during unloading. *Avitakosmol. Ekolog. Med.* 42; 35–39.

Parco, M. S., Emidio, E. P. and Stephen, E. A. (2008) Age-dependent increase in oxidative stress in gastrocnemius muscle with unloading. *J. Appl. Physiol.* 105; 1695–1705.

Pedersen, B. K., Akreström, T. C., Nielsen, A. R. and Fischer, C. P. (2007) Role of myokines in exercise and metabolism. *J. Appl. Physiol.* 103; 1093–1098.

Petersen, A. M. and Pedersen, B. K. (2006) The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J. Physiol. Pharmacol.* 57; 43–51.

Plomgaard, P., Kenkowa, M. and Pedersen, B. K. (2005) Fiber type specific expression of TNF-alpha, IL-6 and IL-18 in human skeletal muscles. *Exerc. Immunol. Rev.* 11; 53–63.

Punkt, K., Naupert, A. and Asmussen, G. (2004) Differentiation of rat skeletal muscle fibres during development and ageing. *Acta Histochem.* 106; 145–154.

Reid, M. B. (2005) Response of the ubiquitin-proteasome pathway to changes in muscle activity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288; R1423–R1431.

Reid, M. B. and Li, Y. P. (2001) Tumor necrosis factor-α and muscle wasting: a cellular perspective. *Respir. Rev.* 2; 269–272.

Starkie, R., Ostrowski, S., Jauffred, S., Febbraio, M. and Pedersen, B. (2003) Exercise and IL-6 inhibition inhibits endotoxin-induced TNF-α production in humans. *FASEB J.* 17; 884–886.

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A. and Pedersen, B. K. (2002) IL-6 and TNF-alpha expression in and release from, contracting human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 283; E1272–E1278.

Thomason, D. B. and Booth, F. W. (1990) Atrophy of the soleus muscle by hindlimb unweighting. *J. Appl. Physiol.* 68; 1–12.

Wenz, T., Diaz, F., Hernandez, D. and Moraes, C. T. (2009) Endurance exercise is protective for mice with mitochondrial myopathy. *J. Appl. Physiol.* 106; 1712–1719.

This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.