doi: 10.4149/gpb_2018001

# Exercise preconditioning attenuates atrophic mediators and preserves muscle mass in acute sepsis

Saad Al-Nassan[1] and Hidemi Fujino[2]

[1] *Department of Physical and Occupational Therapy, Faculty of Allied Health Sciences, Hashemite University, 150459, Zarqa 13115, Jordan*
[2] *Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan*

**Abstract.** Sepsis is an inflammatory condition that causes a severe and rapid loss of body proteins, especially skeletal muscles. The ubiquitin-proteasome system plays a major role in skeletal muscle proteolysis. Understanding the effects of exercise preconditioning on septic-induced ubiquitin-proteasome activation plays a pivotal role in planning rehabilitation strategies for patients who are susceptible for developing cachexia. In this study, we applied mild preconditioning exercises in the form of treadmill running for adult mice for a period of two weeks, before they were injected with lipopolysaccharide to induce sepsis. Our results show that the body weight and cross-sectional area (CSA) of muscle fibers were preserved in the pre-exercised mice. The main finding in our study was that pre-exercised mice maintained a low level of tumor necrosis factor-alpha in the gastrocnemius muscle, which resulted in a down-regulated profile of main atrophic mediators: p38, FOXO3A, and multi-ubiquitin proteins. By these findings, we conclude that a mild program of preconditioning exercises can prevent atrophy and preserve muscle mass in acute sepsis. This provides further evidence to the importance of rehabilitation planning in acute illness.

**Key words:** Exercise — Sepsis — Muscle wasting — Atrophic-mediators

## Introduction

Muscle wasting is a major feature of the cachexia associated with diseases such as sepsis, cancer, AIDS, diabetes, uremia, congestive heart failure, and chronic obstructive pulmonary disease (Hasselgren 1995; Tisdale 1997). Muscle dysfunction occurs in 40–70% of septic patients (Bolton 2000). Studies in septic patients and experimental animals have provided evidence that the myofibrillar proteins are particularly sensitive to the effects of sepsis (Hasselgren and Fischer 1998), and the increased myofibrillar protein breakdown in sepsis, is thought to be mediated essentially through the activation of the ubiquitin-proteasome proteolytic system (Dehoux et al. 2003). When the ubiquitin is activated by an ubiquitin-activating enzyme (E1 family) (Hershko et al. 1983), and conjugated through the ubiquitin-conjugating enzymes (E2 family) (Jentsch 1992), the ubiquitin is stabilized by the two known ubiquitin ligases (E3 family) namely Atrogin-1 and Murf1 (Hershko and Ciechanover 1998), and attach ubiquitin to protein substrates according to the "N-end rule" (Lecker et al. 1999). Endotoxin or lipopolysaccharide (LPS) is a major component of the cell wall of Gram-negative bacteria recognized by Toll-like receptors (TLR4) of innate immune cells (Holececk 2012). LPS administration induces a number of catabolic factors in sepsis, including tumor necrosis factor-alpha (TNF-α), which promotes muscle wasting in diseased states (Ahmad et al. 1994; Jackman and Kandarian 2004). Studies have shown that TNF-α directly stimulates muscle loss through activation of the p38 mitogen-activated protein kinase (MAPK) pathway, which mediates the ubiquitin ligases (Li et al. 2005). P38 is a stress-activated protein kinase that responds to a variety of stimuli, including oxidative stress and TNF-α (Obata et al. 2000), and has been identified as a likely mediator of catabolic signaling in skeletal muscle (Tracey 2002). Not only the p38 MAPK pathway is activated

Correspondence to: Hidemi Fujino, Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan
E-mail: fujino@phoenix.kobe-u.ac.jp

by TNF-α in muscle proteolysis, it was also shown that the Akt/FOXO pathway plays a potential role in muscle protein degradation when mediated by elevated TNF-α as a result of LPS-induced endotoxaemia (Crossland et al. 2008).

Sepsis is a leading cause of death in intensive care units (ICU) (Annane et al. 2003, 2005). The rapid and severe loss of muscle proteins as a result of the inflammatory condition plays a major role in the increased mortality and morbidity in septic patients. Pharmacological interventions targeting the cachectic agents, mainly TNF-α and other pro-inflammatory cytokines aiming to mediate muscle protein loss have equivocal results (Gould et al. 2013). Regular exercise appears to induce anti-inflammatory effects (Brandt and Pedersen 2010); these anti-inflammatory effects of exercise may offer protection against TNF-α induced insulin resistance (Petersen and Pedersen 2005). There is limited but promising evidence that early exercise can attenuate myopathy and muscle wasting in patients with sepsis (Paratz and Kayambu 2011; Coelho et al. 2013), however, studies on the effect of electrical muscle stimulation (EMS) on muscles of septic patients have shown controversial results (Gervasili et al. 2009; Poulsen et al. 2011). We have conducted this study to further explore the exercise preconditioning effect on muscle wasting key mediators prior to septic stimulus. Our hypothesis was that a moderate program of exercise could guard the muscles of mice against septic-induced wasting, through attenuation of main catabolic mediators.

## Materials and Methods

### *Animal model and experimental design*

Twenty-four adult male ICR mice weighing 30~32 g (Japan SLC, Shizuoka) were used in our study. The animals were housed in a temperature-controlled room at 22 ± 2°C with a light-dark cycle of 12 h and maintained on mice chow and water *ad libitum*. Initially, the animals were randomly assigned to sedentary and exercised groups ($n = 12$ *per* group). Body weights of animals in all groups were checked twice; once after the first week of exercise protocol, and once again prior to LPS or PBS injections. The exercised animals had an increase in body weight of 1.6 ± 0.5 g at the end of the exercise protocol. After 24 h from completing the last exercise session, animals in the exercised group were randomly subdivided into two groups: Ex+LPS group ($n = 6$), which received single injection of *E. coli* lipopolysaccharide (LPS; 20 mg/kg, *i.p.*, serotype O111:B4, Sigma-Aldrich, Saint Louis, Missouri, USA), and Ex+PBS group ($n = 6$), which received equal volume *i.p.* injections of phosphate-buffered saline (PBS). The sedentary group animals were also subdivided into two groups: control sedentary (Con, $n = 6$), and LPS sedentary (LPS, $n = 6$). Animals in the LPS group received single *i.p.* LPS injections, equal in concentration to those injected into the Ex+LPS group animals. This study was approved by the Institution of Animal Care and Use Committee and carried out according to Animal Experimentation Regulation of Kobe University. All experiments were conducted in accordance with the principles of laboratory animal care (NIH publication No. 85-23, revised 1985).

### *Exercise preconditioning protocol*

Upon initial assignment of animals into sedentary or exercised groups, animals in the trained group received 3 familiarization sessions over a period of 3 days, on a rodent treadmill (Osaka Micro System, Osaka, Japan), with a speed intensity ranging between 10~15 m/min, for 30~45 min a day, to get accustomed to the treadmill and to the exercise protocol. The actual protocol consisted of running for 60 min a day, with an intensity of 18 m/min, six days a week, for two weeks. The blood lactate level in the trained group animals was measured from blood collected from tail vein by using a blood lactate test meter (Lactate Pro; Arkray, Kyoto, Japan) pre and post exercise. Levels of blood lactate did not change significantly, remaining below 2 mmol/l.

### *Muscle sample preparation*

All animals were euthanized with sodium pentobarbital 24 h after final LPS or PBS injections. The gastrocnemius (GAS) muscle was rapidly excised and weighed, then immediately flash-frozen in pre-cooled acetone to be stored at −80°C until the histological and immunological analyses.

### *mATPase staining for fiber type differentiation and fiber cross-sectional area*

The mid-belly portion of frozen GAS muscle was attached to cryostat chuck, and transverse sections (11 μm in thickness) were cut on a cryostat microtome (CM1510S, Leica Instruments, Heidelberg, Germany) at −20°C. The sections were then stained for myofibrillar adenosine triphosphatase (mATPase) at pH 4.45 pre-incubation to differentiate the muscle fibers as types I, IIA, IID, or IIB as described by Punkt (Punkt et al. 2004). The sections were pre-incubated in barbital acetate buffer (pH 4.45) for 5 min at room temperature. Following wash by 0.1 M barbital buffer containing 0.18 M $CaCl_2$ (pH 9.4) for 30 s, the sections were incubated in 0.1 M barbital buffer containing 0.18 M $CaCl_2$ and 4m M ATP (pH 9.4) for 45 min at room temperature. The sections were then washed in 1% $CaCl_2$ and 2% $CoCl_2$ for 3 min, and washed by 0.01 M sodium barbital. Following distilled water wash, the sections were visualized by 1% ammonium sulfide. The myosin ATPase reaction was used to

identify the muscle fiber type and to measure cross-sectional area of each type. Images were visualized with a light microscope and photographed by an attached CCD camera (BX51, Olympus, Tokyo, Japan). Cross-sectional areas from at least 100 muscle fibers *per* type were measured using the ImageJ software program (NIH, Bethesda, MD).

*ELISA for TNF-α*

A portion of approximately 25 mg of GAS muscle 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 homogenate was 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 in GAS muscle was carried out using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instruction (eBioscience, San Diego, CA).

*Western blotting for atrophic mediators*

Total protein concentration was determined by the use of a protein determination kit (Bio-Rad Laboratories, Hercules, CA). Western blotting was conducted by loading 20 μg of sample protein into 10–12.5% SDS-PAGE, then transferred into PVDF membrane. Following 1 h blocking in 5% skimmed milk, membranes were incubated overnight with anti-p38, anti-phospho-p38, anti-FOXO3A, anti-phospho-FOXO3A, or anti-multi-ubiquitin at 4°C. All antibodies except mutli-ubiquitin (Enzo Life sciences, NY, USA) were obtained from Cell Signaling Technology (MA, USA). PVDF membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Inc. PA, USA). Equal loading of proteins was confirmed by stripping membranes and re-probing with anti-GAPDH antibody (Biochain Institute, Hayward, CA). The images were analyzed with the LAS-1000 (Fujifilm, Tokyo, Japan) using a chemiluminscent image analyzer. The digitized signals were quantified using the Multi-Gauge Analysis Software program (Fujifilm) against the relative concentration of GAPDH as an internal control.

*Statistical analyses*

The data are expressed as means ± 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 $p < 0.05$

**Results**

*Muscle wet weight and fiber cross-sectional area*

Body weight, muscle wet weight, and ratio of muscle wet weight to body weight are shown in Table 1. Post 24 h from LPS injections, the mean body weight value of LPS group showed a significant decrease of 12.3% from Con, and 17% from Ex+PBS group, while the mean body weight in Ex+LPS group dropped only by 2%, and 7% from Con, and Ex+PBS; respectively. Mean muscle wet weight value of LPS group showed a significant decrease of 23% from mean value of Con group, while both exercised groups values were significantly higher than the LPS group. Ratios of muscle wet weights to body weights were significantly higher in both exercised groups compared to LPS.

For fiber CSA based on fiber type (Fig. 1A), the mean values in type I fibers did not show significant differences among the four groups, despite the decreased mean in LPS group. However, mean CSAs for types IIA, IID, and IIB, showed significant decreases in LPS group when compared to other groups, except for type IID, which showed significant decreases from Con, and Ex+PBS, but not from Ex+LPS group. Changes in total fibers CSA (Fig. 1B), reflected a significant decrease of 22% when compared to Con, while the exercised groups values were significantly higher than the LPS by 23% and 28%, for Ex+LPS and Ex+PBS groups, respectively. The total fiber CSA of Ex+LPS group did not show a notable change from Con group, while in Ex+PBS group it increased only by 6% of Con group.

*Muscle TNF-α protein concentration*

The expression level of TNF-α protein concentration in the LPS group significantly increased by 2.2-fold from Con, 1.7-fold from Ex+LPS, and 1.8-fold from Ex+PBS group (Fig. 2). In contrast, there were no significant differences between the Con, Ex+LPS, and Ex+PBS groups.

**Table 1.** Changes in body weight and gastrocnemius weight after LPS or PBS injection

| Group | Body weight (g) | Muscle wet weight (mg) | Muscle weight/ Body weight (mg/g) |
|---|---|---|---|
| Con | 36.5 ± 0.9 | 150.5 ± 4.0 | 4.1 ± 0.1 |
| LPS | 32 ± 1.1* | 115.2 ± 6.7* | 3.6 ± 0.2 |
| Ex+LPS | 35.8 ± 0.6 | 154.3 ± 5.3# | 4.3 ± 0.2# |
| Ex+PBS | 38.6 ± 1.3# | 165.1 ± 3.6# | 4.3 ± 0.1# |

Values are means ± SEM ($n$ = 6 mice/group). * $p < 0.05$ *vs.* Con group; # $p < 0.05$ *vs.* LPS group. Con, control; LPS, lipopolysaccharide; Ex, pre-exercised; PBS, phosphate-buffered saline.

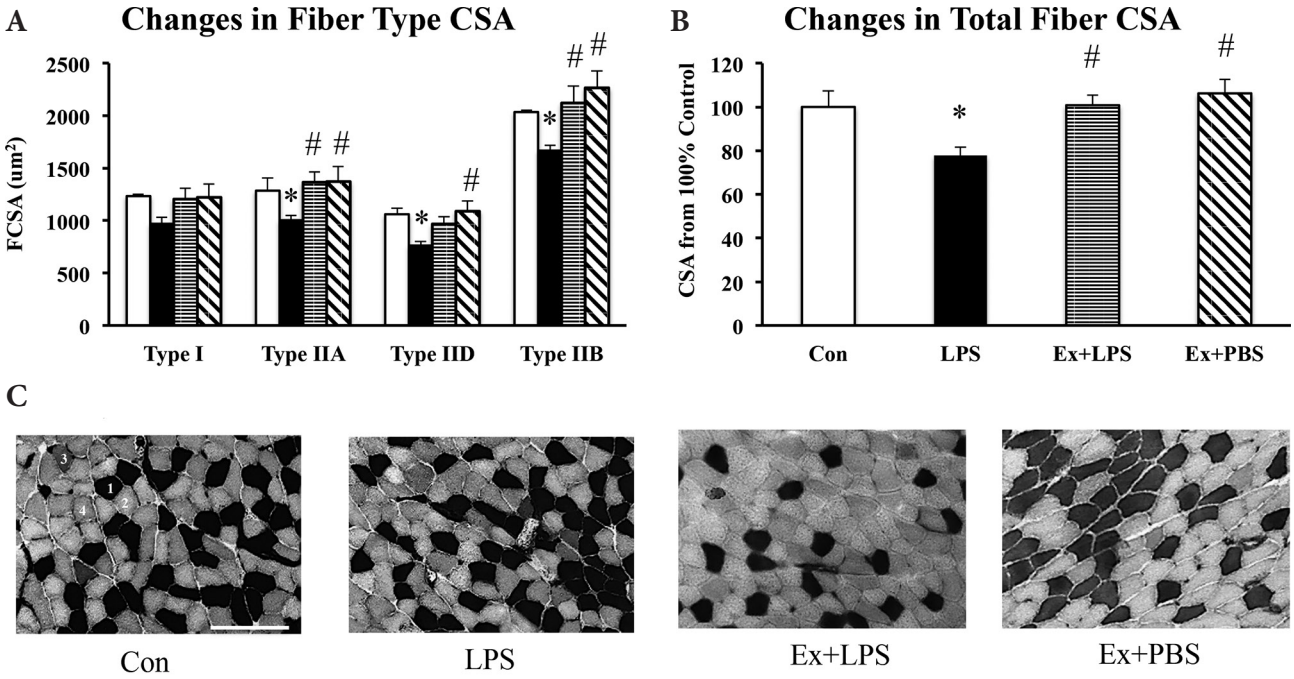


**Figure 1.** Changes in fiber type cross-sectional area (**A**), and in total fibers cross-sectional area (**B**). Values are means ± SEM. *, and # are significantly different from Con, and LPS groups, respectively, at $p < 0.05$. **C.** Light microscopic images of mATPase stain of gastrocnemius muscle. 1, type I; 2, type IIA; 3, type IID; 4, type IIB; Con, control; CSA, cross-sectional area; LPS, lipopolysaccharide. Scale bar: 100 μm

*Atrophic mediator´s protein expressions*

The phosphorylated p38 in LPS group showed a significant 3.3-fold increase from Con group, while Ex+LPS and Ex+PBS maintained significantly lower expression levels of phosphorylated p38 (Fig. 3A). However, expression levels of total p38 protein did not show significant changes among all groups (Fig. 3B). The ratio of phosphorylated p38/total p38 was significantly increased in LPS group compared to all other groups (Fig. 3C). Both exercised groups maintained an expression ratio similar to Con group level.

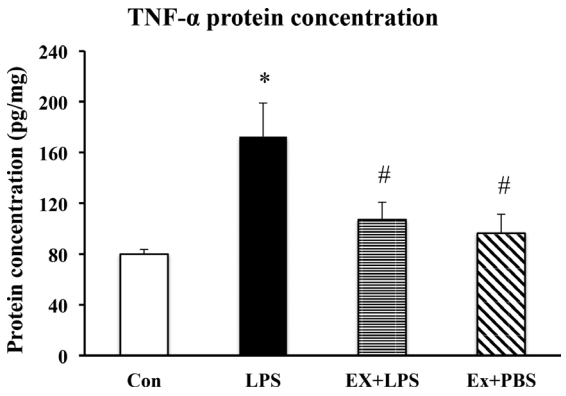


**Figure 2.** Tumor necrosis factor-alpha (TNF-α) protein concentration in gastrocnemius muscle evaluated by ELISA. Values are means ± SEM. *, and # are significantly different from Con, and LPS groups, respectively, at $p < 0.05$. For more abbreviations, see Fig. 1.

While the phosphorylated FOXO3A expression levels were not changed significantly in all groups (Fig. 4A), the total FOXO3A expression was significantly increased in LPS group compared to Con, Ex+LPS, Ex+PBS; by 2-fold, 1.5-fold, and 1.8-fold, respectively (Fig. 4B). Even though a decreased phospho-FOXO3A/total FOXO3A ratio was shown in LPS group (Fig. 4C), the ratio changes among all groups were not significant.

The expression level of multi-ubiquitin protein was significantly increased in LPS group by 2-fold from Con group, 1.6-fold from Ex+LPS group, and 1.8-fold from Ex+PBS group (Fig. 5A).

## Discussion

In our study, a program of exercise preconditioning managed to preserve muscles of trained mice from wasting which usually occurs as a consequence of sepsis. Our data presents a blunting effect of exercise preconditioning on atrophic mediators in cachectic conditions, such as severe sepsis,

adding further evidence to the importance of exercises in critical illness.

As LPS is known to rapidly induce muscle protein loss within 24 h (Premer et al. 2002), the 23% reduction of GAS muscle weight in LPS group is relevant with the study by Mofarrahi et al. (2012), which showed significant drops of muscle weight with a same dose concentration and at the same time of sacrifice. The exercise preconditioning program preserved GAS muscle weight in the Ex+LPS group, and did not result in significant hypertrophy in the Ex+PBS group. The exercise program also maintained the total fiber cross-sectional area near control values in the Ex+LPS group, while the untrained LPS group showed significant decreases in all fiber types, except for type I fibers, which are less prone to sepsis-induced muscle wasting (Wang and Pessin 2013). In accordance with the fiber CSA results, aerobically trained septic rats showed significant increases in the CSA of type II in plantaris muscle when compared to untrained septic rats (Coelho et al. 2013).

Cytokines are produced locally in muscles during endotoxemia (Borge et al. 2009), and TNF-α is a key mediator in activating proteolytic pathways in response to LPS. In the present study, TNF-α protein concentration of GAS muscle in the LPS group showed a significant increase in comparison with other groups. This is consistent with the work of (Frost et al. 2002); as they showed that LPS increases TNF-α in GAS muscle, and that local synthesis of pro-inflammatory cytokines in skeletal muscle promotes muscle wasting. On the other hand, a lesser amount of TNF-α was obtained in the exercised LPS group, as contracted muscles release epinephrine, which in turn decreases TNF-α (Starkie et al. 2003). Neither anti-inflammatory cytokines (*e.g.*, IL-10), nor serum TNF-α were detected in this study, as we have focused on the pathway involving TNF-α activation of the ubiquitin proteasome system *via* p38 MAPK (Li et al. 2005), and its response to preconditioning exercises upon septic stimulus. However, this does not exclude the role of anti-inflammatory cytokines in mediating catabolic pathways. Many studies have shown that exercises have anti-inflammatory effects on body tissues (Petersen and Pedersen 2005). Cross-sectional studies have demonstrated an association between physical inactivity and low-grade inflammation in healthy subjects and elderly people (Geffken et al. 2001; Abramson and Vaccarino 2002). The guarding effects of exercise preconditioning shown by the results of TNF-α in the present study are consistent with the study of Starkie et al; in which TNF-α response to endotoxin was blunted in subjects who performed 3 h of ergometer cycling before receiving the endotoxin (Starkie

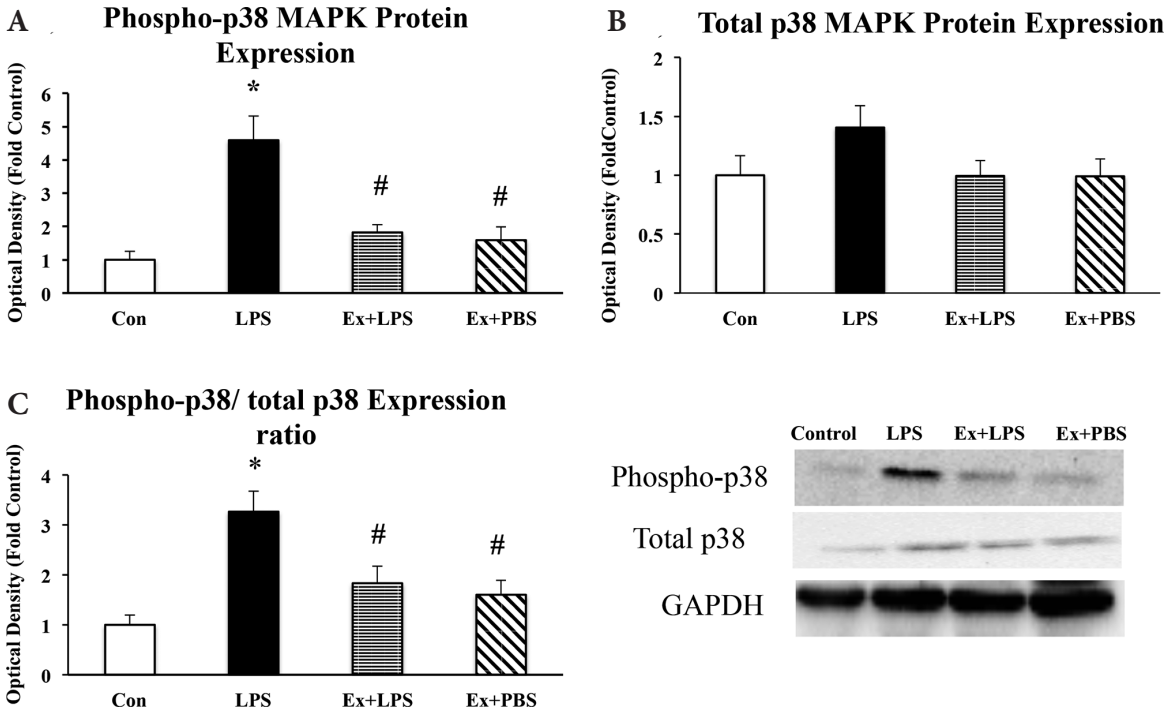


**Figure 3.** Expression levels of phospho-p38 (**A**), total p38 (**B**) and phospho-p38/total p38 ratio (**C**) in the gastrocnemius muscle and representative Western blots. Densitometric quantifications were normalized according to GAPDH level. Values (means ± SEM) were calculated as the fold changes relative to the Con group. *, and # are significantly different from Con, and LPS groups, respectively, at $p < 0.05$. For abbreviations, see Fig. 1.

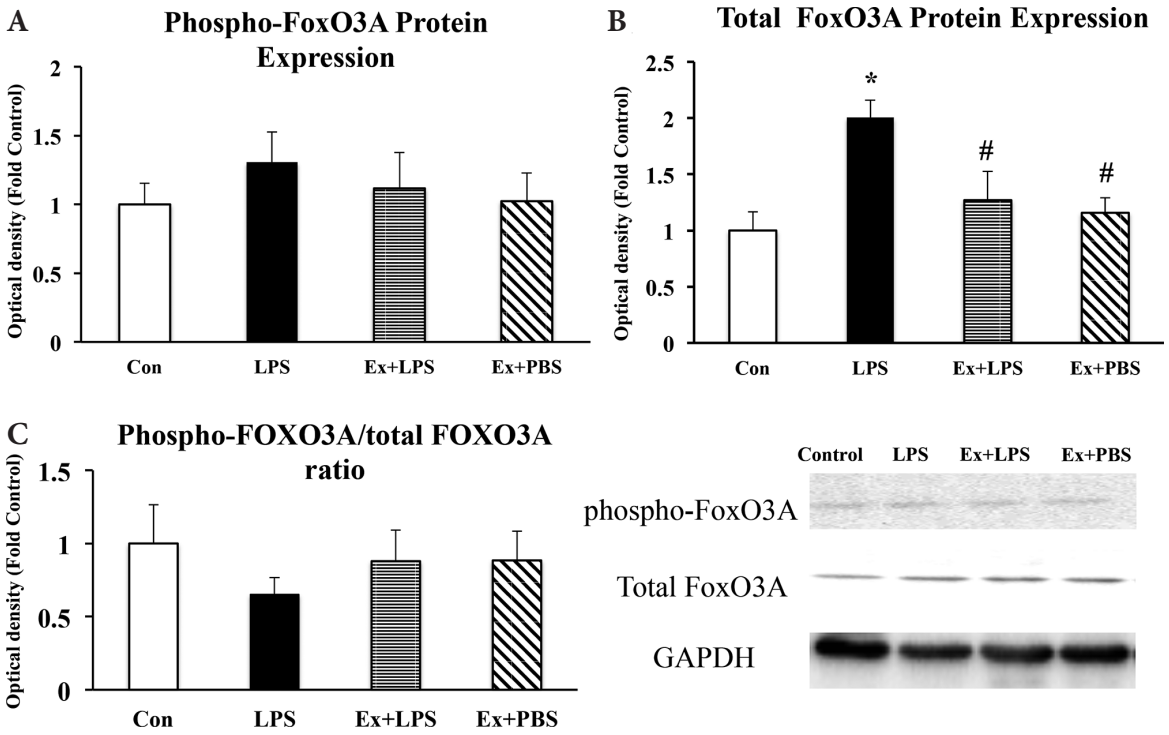


**Figure 4.** Expression levels of phospho-FoxO3A (**A**), total FoxO3A (**B**) and phospho-FoxO-3A/total FoxO-3A ratio (**C**) in the gastrocnemius muscle and representative Western blots. Densitometric quantifications were normalized according to GAPDH level. Values (means ± SEM) were calculated as the fold changes relative to the Con group. *, and # are significantly different from Con, and LPS groups, respectively, at $p < 0.05$. For abbreviations, see Fig. 1.

et al. 2003). Also, it was found that exercise normalizes the overexpression of TNF-α in TNF-R knockout mice (Keller et al. 2004). Although we expected the TNF-α level in muscles of Ex+PBS group to be lower than Con group level, the result showed a concentration higher than Con group. This result is not uncommon as continuous physical exercise, including treadmill running, can be a stressful event, and can elicit an influx of TNF-α (Pedersen et al. 2000).

The increased phosphorylated form of p38 and phospho-p38/total p38 protein expression ratio was detected in the LPS group. Thus, the increased level of TNF-α in the investigated muscle enhanced a catabolic response of p38 protein,

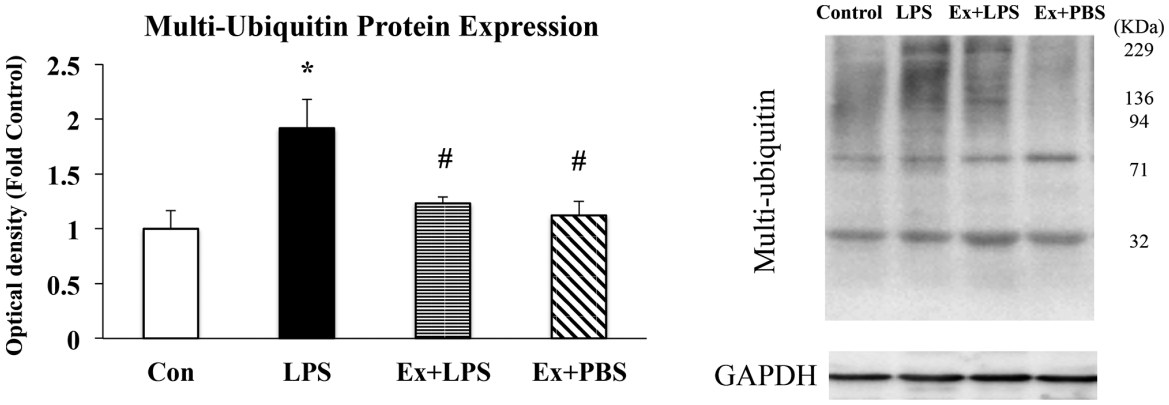


**Figure 5.** Expression level of multi-ubiquitin protein in the gastrocnemius muscle and representative Western blot. Densitometric quantifications were normalized according to GAPDH level. Values (means ± SEM) were calculated as the fold changes relative to the Con group. *, and # are significantly different from Con, and LPS groups, respectively, at $p < 0.05$. For abbreviations, see Fig. 1.

whereas this enhancement was attenuated in muscles of the Ex+LPS group, reflecting a blunting effect of exercise preconditioning on such a key mediating catabolic factor. It has been shown in several studies that pathologic elevation of p38 activity favors protein degradation and muscle atrophy (Li et al. 2005). This response is elicited not only in case of sepsis, but also in other pro-catabolic conditions including acute quadriplegic myopathy, cast immobilization, type 2 diabetes, and ageing (Childs et al. 2003; Koistinen et al. 2003; Williamson et al. 2003; Di Giovanni et al. 2004). Vichaiwong et al. (2009) showed that phospho-p38 level in the muscles of pre-exercised rats was reduced in comparison to sedentary rat´s level upon triggering by hydrogen peroxide. Another study also reported that peritoneal macrophages isolated from exercised mice and exposed to LPS showed reduced levels of TNF-α and p38 activity (Chen et al. 2010). In the present study, the p38 levels in pre-exercised group showed no significant difference from the Con group. These results can be explained by the nature of preconditioning program in terms of intensity, frequency, and duration, which did not elicit changes from healthy controls.

During LPS-induced endotoxemia, Akt-1 is predominantly inactivated, possibly due to elevated cytokine level such as TNF-α, enabling FOXO factors to up-regulate E3 ligases (MAFbx and MuRF-1) and promote muscle protein degradation through the ubiquitin-proteasome pathway (Crossland et al. 2008). In the LPS group, total FOXO-3A protein level was significantly higher compared to other groups in the present study. This is consistent, in part, with the results of Smith et al. (2010).

The phospho-FOXO-3A protein level showed no significant differences among the four groups in the present study. FOXO-3A is notably involved in both atrophy and autophagy (Zhao et al. 2007; Mammucari et al. 2008), and it has been shown that a decrease in phsopho-FOXO-3A/total FOXO-3A ratio is in correlation with decreased Akt-1 activity in muscles of septic rats (Crossland et al. 2008). Although Akt-1 was not investigated in the present study, we believe that elevated TNF-α has mediated Akt-1/FOXO signaling in the sedentary LPS group, and this promoted protein ubquitination and degradation, leading to muscle atrophy. The program of exercise preconditioning has managed to maintain muscle homeostasis, and attenuate muscle atrophy in septic mice. The down-regulated levels of TNF-α, and p38 in the Ex+LPS group have played a direct role in down-regulation of multi-ubiquitin expression, which resembles the conjugating part of protein degradation *via* the ubiquitin-proteasome pathway (Glickman and Ciechanover 2002). In a study by Kavazis et al. (2014), exercise training protected against doxorubicin-induced myopathy in rat skeletal muscle, through preventing the rise of FOXO-3. Thus, this mechanism is consistent with our findings of the ability of moderate exercise training on down-regulating FOXO mediation of the downstream targets of the ubiquitin-proteasome pathway.

The results from this study demonstrate an attenuating effect of exercise preconditioning on muscles of septic mice by keeping a down-regulated level of key atrophic mediators, and preserving muscle homeostasis and mass. Sepsis and systemic inflammation may lead to multiple organ failure, which is associated with loss of muscle mass and/or onset of ICU-acquired weakness syndrome (Schefold et al. 2010). This has urged scientists to find pharmacological and non-pharmacological approaches to countermeasure this problem. Our findings emphasize the role of early and proper rehabilitation planning for patients susceptible to undergo a cachectic condition such as sepsis. We also contributed evidence that being physically active promotes the immune system and guards one of the body's largest systems, the muscular system, against illness-induced deterioration.

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

## References

Abramson JL, Vaccarino V (2002): Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. Arch. Intern. Med. **162,** 1286–1292 https://doi.org/10.1001/archinte.162.11.1286

Ahmad S, Karlstad MD, Choudhry MA, Sayeed MM (1994): Sepsis-induced myofibrillar protein catabolism in rat skeletal muscle. Life Sci. **55,** 1383–1391 https://doi.org/10.1016/0024-3205(94)00752-7

Annane D, Aegerter P, Jars-Guincestre MC, Guidet B (2003): Current epidemiology of septic shock: the CUB-Rea Network. Am. J. Respir. Crit. Care Med. **168,** 165–172 https://doi.org/10.1164/rccm.2201087

Annane D, Bellissant E, Cavaillon JM (2005): Septic shock. Lancet **365,** 63–78 https://doi.org/10.1016/S0140-6736(04)17667-8

Bolton CF (2000): Evidence of neuromuscular dysfunction in the early stages of the systemic inflammatory response syndrome. Intensive Care Med. **26,** 1179–1180 https://doi.org/10.1007/s001340000622

Borge BA, Kalland KH, Olsen S, Bletsa A, Berggreen E, Wiig H (2009): Cytokines are produced locally by myocytes in rat skeletal muscle during endotoxemia. Am. J. Physiol. Heart Circ. Physiol. **296,** H735–744 https://doi.org/10.1152/ajpheart.01309.2008

Brandt C, Pedersen BK (2010): The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. J. Biomed. Biotechnol. **2010,** 520258 https://doi.org/10.1155/2010/520258

Chen MF, Chen HI, Jen CJ (2010): Exercise training up-regulates macrophage MKP-1 and affects immune responses in mice. Med. Sci. Sports Exerc. **42,** 2173–2179

https://doi.org/10.1249/MSS.0b013e3181e2158d
Childs TE, Spangenburg EE, Vyas DR, Booth FW (2003): Temporal alterations in protein signaling cascades during recovery from muscle atrophy. Am. J. Physiol. Cell. Physiol. **285,** C391–398
https://doi.org/10.1152/ajpcell.00478.2002
Coelho CW, Jannig PR, Souza AB, Fronza HJr, Westphal GA, Petronilho F (2013): Exercise training prevents skeletal muscle damage in an experimental sepsis model. Clinics (Sao Paulo) **68,** 107–114
https://doi.org/10.6061/clinics/2013(01)OA17
Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, Greenhaff PL (2008): A potential role for Akt/FOXO signaling in both protein loss and the impairment of muscle carbohydrate oxidation during sepsis in rodent skeletal muscle. J. Physiol. **586,** 5589–5600
https://doi.org/10.1113/jphysiol.2008.160150
Dehoux MJ, Van Beneden RP, Fernandez-Celemin L, Lause PL, Thissen JP (2003): Induction of MafBx and Murf ubiquitin ligase mRNAs in rat skeletal muscle after LPS injection. FEBS Lett. **544,** 214–217
https://doi.org/10.1016/S0014-5793(03)00505-2
Di Giovanni S, Molon A, Broccolini A, Melcon G, Mirabella M, Hoffman EP, Servidei S (2004): Constitutive activation of MAPK cascade in acute quadriplegic myopathy. Ann. Neurol. **55,** 195–206
https://doi.org/10.1002/ana.10811
Frost RA, Nystrom GJ, Lang CH (2002): Lipopolysaccharide regulates pro-inflammatory cytokine expression in mouse myoblasts and skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. **283,** R698–709
https://doi.org/10.1152/ajpregu.00039.2002
Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP (2001): Association between physical activity and markers of inflammation in a healthy elderly population. Am. J. Epidemiol. **153,** 242–250
https://doi.org/10.1093/aje/153.3.242
Gerovasili V, Stefanidis K, Vitzilaios K, Karatzanos E, Politis P, Koroneos A (2009): Electrical muscle stimulation preserves the muscle mass of critically ill patients: a randomized study. Crit. Care. **13,** R161
https://doi.org/10.1186/cc8123
Glickman MH, Ciechanover A (2002): The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol. Rev. **82,** 373–428
https://doi.org/10.1152/physrev.00027.2001
Gould DW, Lahart I, Carmichael AR, Koutedakis Y, Metsios GS (2013): Cancer cachexia prevention via physical exercise: molecular mechanisms. J. Cachexia Sarcopenia Muscle **4,** 111–124
https://doi.org/10.1007/s13539-012-0096-0
Hasselgren PO (1995): Muscle protein metabolism during sepsis. Biochem. Soc. Trans. **23,** 1019–1025
https://doi.org/10.1042/bst0231019
Hasselgren PO, Fischer JE (1998): Sepsis: stimulation of energy-dependent protein breakdown resulting in protein loss in skeletal muscle. World J. Surg. **22,** 203–208
https://doi.org/10.1007/s002689900370
Hershko A, Ciechanover A (1998): The ubiquitin system. Annu. Rev. Biochem. **67,** 425–479
https://doi.org/10.1146/annurev.biochem.67.1.425
Hershko A, Heller H, Elias S, Ciechanover A (1983): Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. J. Biol. Chem. **258,** 8206–8214
Holecek M (2012): Muscle wasting in animal models of severe illness. Int. J. Exp. Pathol. **93,** 157–171
https://doi.org/10.1111/j.1365-2613.2012.00812.x
Jackman RW, Kandarian SC (2004): The molecular basis of skeletal muscle atrophy. Am. J. Physiol. Cell Physiol. **287,** C834–843
https://doi.org/10.1152/ajpcell.00579.2003
Jentsch S (1992): The ubiquitin-conjugation system. Annu. Rev. Genet. **26,** 179–207
https://doi.org/10.1146/annurev.ge.26.120192.001143
Kavazis AN, Smuder AJ, Powers SK (2014): Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle. J. Appl. Physiol. **117,** 223–230
https://doi.org/10.1152/japplphysiol.00210.2014
Keller C, Keller P, Giralt M, Hidalgo J, Pedersen BK (2004): Exercise normalises overexpression of TNF-alpha in knockout mice. Biochem. Biophys. Res. Commun. **321,** 179–182
https://doi.org/10.1016/j.bbrc.2004.06.129
Koistinen HA, Chibalin AV, Zierath JR (2003): Aberrant p38 mitogen-activated protein kinase signalling in skeletal muscle from Type 2 diabetic patients. Diabetologia **46,** 1324–1328
https://doi.org/10.1007/s00125-003-1196-3
Lecker SH, Solomon V, Price SR, Kwon YT, Mitch WE, Goldberg AL (1999): Ubiquitin conjugation by the N-end rule pathway and mRNAs for its components increase in muscles of diabetic rats. J. Clin. Invest. **104,** 1411–1420
https://doi.org/10.1172/JCI7300
Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, Reid MB (2005): TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. FASEB J. **19,** 362–370
https://doi.org/10.1096/fj.04-2364com
Mammucari C, Schiaffino S, Sandri M (2008): Downstream of Akt: FoxO3 and mTOR in the regulation of autophagy in skeletal muscle. Autophagy **4,** 524–526
https://doi.org/10.4161/auto.5905
Mofarrahi M, Sigala I, Guo Y, Godin R, Davis EC, Petrof B (2012): Autophagy and skeletal muscles in sepsis. PLoS One **7,** e47265
https://doi.org/10.1371/journal.pone.0047265
Obata T, Brown GE, Yaffe MB (2000): MAP kinase pathways activated by stress: the p38 MAPK pathway. Crit. Care Med. **28,** N67–77
https://doi.org/10.1097/00003246-200004001-00008
Paratz JD, Kayambu G (2011): Early exercise and attenuation of myopathy in the patient with sepsis in ICU. Phys. Ther. Rev. **16,** 59–65
https://doi.org/10.1179/1743288X11Y.0000000002
Pedersen BK, Bruunsgaard H, Ostrowski K, Krabbe K, Hansen H, Krzywkowski (2000): Cytokines in aging and exercise. Int. J. Sports Med. **21,** Suppl **1,** S4–9
https://doi.org/10.1055/s-2000-1444

Petersen AM, Pedersen BK (2005): The anti-inflammatory effect of exercise. J. Appl. Physiol. **98,** 1154–1162

Poulsen JB, Moller K, Jensen CV, Weisdorf S, Kehlet H, Perner A (2011): Effect of transcutaneous electrical muscle stimulation on muscle volume in patients with septic shock. Crit. Care Med. **39,** 456–461 https://doi.org/10.1097/CCM.0b013e318205c7bc

Premer DM, Goertz R, Georgieff MK, Mammel MC, Schwarzenberg SJ (2002): Muscle proteolysis and weight loss in a neonatal rat model of sepsis syndrome. Inflammation **26,** 97–101 https://doi.org/10.1023/A:1014840412552

Punkt K, Naupert A, Asmussen G (2004): Differentiation of rat skeletal muscle fibers during development and ageing. Acta Histochem. **106,** 145–154 https://doi.org/10.1016/j.acthis.2003.11.005

Schefold JC, Bierbrauer J, Weber-Carstens S (2010): Intensive care unit-acquired weakness (ICUAW) and muscle wasting in critically ill patients with severe sepsis and septic shock. J. Cachexia Sarcopenia Muscle **1,** 147–157 https://doi.org/10.1007/s13539-010-0010-6

Smith IJ, Alamdari N, O'Neal P, Gonnella P, Aversa Z, Hasselgren PO (2010): Sepsis increases the expression and activity of the transcription factor Forkhead Box O 1 (FOXO1) in skeletal muscle by a glucocorticoid-dependent mechanism. Int. J. Biochem. Cell. Biol. **42,** 701–711 https://doi.org/10.1016/j.biocel.2010.01.006

Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK (2003): Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J. **17,** 884–886 https://doi.org/10.1096/fj.02-0670fje

Tisdale MJ (1997): Biology of cachexia. J. Natl. Cancer Inst. **89,** 1763–1773 https://doi.org/10.1093/jnci/89.23.1763

Tracey KJ (2002): Lethal weight loss: the focus shifts to signal transduction. Sci. STKE **130,** pe21 https://doi.org/10.1126/stke.2002.130.pe21

Vichaiwong K, Henriksen EJ, Toskulkao C, Prasannarong M, Bupha-Intr T, Saengsirisuwan V (2009): Attenuation of oxidant-induced muscle insulin resistance and p38 MAPK by exercise training. Free Radic. Biol. Med. **47,** 593–599 https://doi.org/10.1016/j.freeradbiomed.2009.05.036

dWang Y, Pessin JE (2013) Mechanisms for fiber-type specificity of skeletal muscle atrophy. Curr. Opin. Clin. Nutr. Metab. Care **16,** 243–250 https://doi.org/10.1097/MCO.0b013e328360272d

Williamson D, Gallagher P, Harber M, Hollon C, Trappe S (2003): Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. J. Physiol. **547,** 977–987 https://doi.org/10.1113/jphysiol.2002.036673

Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S (2007): FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell. Metab. **6,** 472–483 https://doi.org/10.1016/j.cmet.2007.11.004

Received: October 5, 2017
Final version accepted: January 15, 2018
First published online: June 29, 2018