Time course of ubiquitin-proteasome and macroautophagy-lysosome pathways in skeletal muscle in rats with heart failure

Naoto FUJITA¹, Hidemi FUJINO², Hiroki SAKAMOTO², Jyunya TAKEGAKI³, and Masataka DEIE⁴

¹Department of Musculoskeletal Functional Research and Regeneration, Graduate School of Biomedicine and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan; ²Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan; ³Department of Life Science, Graduate School of Arts and Science, The University of Tokyo, 3-8-1 Komada, Meguro-ku, Tokyo 153-8902, Japan; and ⁴Department of Orthopaedic Surgery, Aichi Medical University, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan

(Received 17 September 2015; and accepted 29 September 2015)

ABSTRACT

Patients with heart failure have limited exercise capacity due to not only the myocardial dysfunction but also skeletal muscle atrophy. However, the mechanisms and time course of protein degradation in skeletal muscle during heart failure remain unclear, and there is no established standard treatment. The purpose of the present study was to investigate the time course of major protein degradation pathways in skeletal muscle during heart failure. Four-week-old male Wistar rats were randomly assigned to heart failure induced by monocrotaline or control groups. At 14 and 21 days after monocrotaline injection, the lungs, heart, and gastrocnemius and soleus muscles were removed and analyzed. There was no significant difference in body weight between the groups at 14 days after monocrotaline injection. Although there were no morphological changes in the skeletal muscle of the monocrotaline group at this time point, ubiquitin-proteasome and macroautophagy-lysosome pathways were activated in the monocrotaline group. Additionally, the pathways were less strongly activated in the soleus muscle than in the gastrocnemius muscle. These results suggest that physical exercise that shifts to slow muscle characteristics should begin when there is no indication of skeletal muscle atrophy to prevent exercise intolerance with heart failure.

Patients with heart failure have limited exercise capacity due to not only myocardial dysfunction, but also skeletal muscle atrophy (6) and reduced strength (8). Skeletal muscle atrophy and consequent strength reduction result from various conditions such as muscular inactivity, sarcopenia, and cachexia, which decrease protein synthesis and increase protein degradation (17). Similarly, decreased protein synthesis (27) and increased protein degradation (4) after

heart failure contribute to skeletal muscle atrophy, and protein degradation is especially common in chronic cases.

Some protein degradation pathways involved in skeletal muscle atrophy, namely ubiquitin-proteasome and macroautophagy-lysosome pathways are delineated as major protein degradation system. Ubiquitin-proteasome pathway activity is increased in atrophied

Address correspondence to: Naoto Fujita, Ph.D., Department of Musculoskeletal Functional Research and Regeneration, Graduate School of Biomedicine and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
Tel: +81-82-257-5423, Fax: +81-82-257-5423
E-mail: fujitan@hiroshima-u.ac.jp

Abbreviations: atrogin-1, atrogin-1/MAFbx; MuRF-1, muscle RING finger 1; LC3, microtubule-associated protein 1 light chain 3; p62, p62/SQSTM1; MCT group, heart failure induced by monocrotaline group; Cont group, age-matched control group; ATPase, adenosine triphosphatase; SDH, succinate dehydrogenase; qPCR, quantitative polymerase chain reaction; TNF- α , tumor necrosis factor-alpha; ROS, reactive oxygen species

skeletal muscle (14). In this pathway, proteins ubiquitinated by muscle-specific ubiquitin ligases, atrogin-1/MAFbx (atrogin-1) and muscle RING finger 1 (MuRF-1), are digested by the 26S proteasome (15). However, the 26S proteasome cannot digest aggregated proteins (26). The macroautophagy-lysosome pathway is implicated as a compensatory pathway for proteasomal protein degradation (20) and can remove aggregated proteins. In this pathway, autophagosomes labeled with microtubule-associated protein 1 light chain 3 (LC3) are delivered to the lysosome for protein degradation (12). Labeled autophagosomes are tagged by p62/SQSTM1 (p62) with both LC3- and ubiquitin-binding domains (3); therefore, p62 is known as a mediator for targeting ubiquitinated proteins to the autophagy system. Activation of the ubiquitin-proteasome and macroautophagy-lysosome pathways has been reported in atrophied skeletal muscle induced by heart failure (28). However, the mechanisms of protein degradation in skeletal muscle during heart failure remain unclear, and there is no established standard treatment.

Physical exercise is an effective therapeutic intervention to prevent and ameliorate skeletal muscle atrophy induced by diverse conditions. Exercise attenuates enhanced protein degradation in atrophied skeletal muscle (1), and regular training prevents atrophy (13). However, exercises to combat skeletal muscle atrophy induced by heart failure can worsen myocardial dysfunction. Handoko *et al.* (16) reported that regular exercise after heart failure induced myocardial inflammation and increased the survival rate. In this study (16), exercise was found to be beneficial in the stable stage but detrimental in the progressive stage, suggesting that the effects of exercise on skeletal muscle atrophy could depend on the stage in heart failure. Therefore, determination of the time course of protein degradation affecting skeletal muscle atrophy in heart failure might allow identification of a stage with low risk for progression of myocardial dysfunction and high skeletal muscle adaptability to exercise. The purpose of the present study was to investigate the time course of major protein degradation pathway activities in skeletal muscle during heart failure.

MATERIALS AND METHODS

Experimental design. This study was approved by the Institutional Animal Care and Use Committee of Hiroshima University (A13-30) and was carried out according to the Hiroshima University Regulations for Animal Experimentation. All experiments were

conducted in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996).

Four-week-old male Wistar rats ($n = 28$) weighing 79–99 g were used in the study. All the animals were randomly assigned to heart failure induced by monocrotaline (MCT, $n = 14$) or control (Cont, $n = 14$) groups. Animals in the MCT group were given a single intraperitoneal injection of monocrotaline (30 mg/kg) to induce pulmonary arterial hypertension and subsequent right ventricular hypertrophy and failure; animals in the Cont group received an equivalent volume of saline. It is known that monocrotaline is metabolized in the liver and changed to the active form, which injures vascular endothelium of pulmonary vessels and causes pulmonary arterial hypertension (5–8). All animals were housed in a controlled environment with a fixed 12-h light:dark cycle at a constant temperature of $22 \pm 2^\circ\text{C}$. Food and water were provided *ad libitum*. The animals in the MCT group were sacrificed with an overdose of sodium pentobarbital at 14 days after monocrotaline injection ($n = 7$) or on the day that the body weight decreased $>9\%$ from its peak (21 ± 0.3 days after monocrotaline injection, $n = 7$). At 21 days after monocrotaline injection, animals in the MCT group expressed clinical signs of heart failure including weight loss, tachypnea, lethargy, cold extremities, and piloerection. These have been shown to correlate with decompensated right ventricular dysfunction in this model (2, 16). The animals in the Cont group were sacrificed at the same time points as the MCT group ($n = 7$ at each time point). The lungs, heart, and gastrocnemius and soleus muscles were removed immediately, weighed, frozen in liquid nitrogen, and stored at -80°C until further analysis.

Histological analysis. Serial transverse sections of thickness 10 μm were obtained using a cryostat from the middle part of the skeletal muscle sample and were mounted on glass slides. The sections were stained for myofibrillar adenosine triphosphatase (ATPase) and succinate dehydrogenase (SDH). For ATPase staining, the sections were preincubated in barbital acetate buffer (pH 4.2) for 5 min at room temperature. Following washing with 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 4 mM ATP (pH 9.4) for 45 min at room temperature. The sections were then washed in 1% CaCl_2 and 2% CoCl_2 every 3 min and, finally, in 0.01 M sodium barbital. Following washing with distilled water, the sections

Fig. 1 Time course of changes in body weight (A) and body weight at 14 and 21 days after monocrotaline injection (B). Cont: control group ($n = 14$), MCT: heart failure induced by monocrotaline group ($n = 14$). $n = 7$ in each group at each time point. Values represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

were visualized using 1% ammonium sulfide. For SDH staining, the sections were incubated in 50 mM phosphate buffer (pH 7.5) containing 0.05% nitro blue tetrazolium and 50 mM sodium succinate for 45 min at 37°C. The sections were analyzed, and images were acquired on a microscope (BZ-9000; Keyence, Osaka, Japan). Using the images from the serial sections stained for ATPase and SDH, the skeletal muscle fibers were categorized into types I, IIA, or IIB based on a previous study (21). The cross-sectional areas of skeletal muscle fibers were measured using ImageJ software (NIH, MD, USA). The cross-sectional areas of skeletal muscle fibers were quantified for five fields per muscle. At least 100 randomly selected values were analyzed for each skeletal muscle fiber type.

Quantitative polymerase chain reaction (qPCR) analysis. Total RNA was isolated from ~20 mg of each skeletal muscle using a lysis buffer containing 0.5 mL TRIzol® reagent (15596-026; Invitrogen, Tokyo, Japan). Reverse transcription was carried out using the High-Capacity cDNA Reverse Transcription Kit (4374966; Applied Biosystems, CA, USA), and the resultant cDNA samples were stored at -20°C. The mRNA expression levels of atrogin-1 (Rn00591730_m1), MuRF-1 (Rn00590197_m1), LC3 (Rn00563181_m1), and p62 (Rn00677357_g1) were quantified using TaqMan® Gene Expression Assays (Applied Biosystems). The TaqMan® probe and primer set were 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 (Rn03928990_g1). qPCR was performed using the

PCR Fast Advanced Master Mix (Applied Biosystems) in a 96-well reaction plate. Each well contained 1 μ L cDNA template, 5 μ L PCR Fast Advanced Master Mix, 3.5 μ L RNase-free water, and 0.5 μ L TaqMan® Gene Expression Assays in a reaction volume of 10 μ L. All sample and nontemplate control reactions were performed in a CFX96™ Real-Time PCR Detection System (BioRad) under the following conditions: 50°C for 2 min, 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s.

Statistical analysis. Data are expressed as mean $\pm$ standard error. The significance of differences between the groups was evaluated using one-way analysis of variance followed by Tukey's HSD post hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

Body weight

Body weight in the Cont group increased every day during the experimental period (Fig. 1A). In contrast, body weight in the MCT group peaked at 15 days after monocrotaline injection, flattened, and then gradually decreased after 17 days. At 21 days after monocrotaline injection, body weight in the MCT group decreased by an average of 11% from its peak. Although there were no significant differences in body weight between the Cont and MCT groups at 14 days after monocrotaline injection, the value was significantly lower in the MCT group than in the Cont group at 21 days (Fig. 1B). At 21 days after monocrotaline injection, body weight in the MCT group decreased by 31% from the value in the Cont group.

Fig. 2 Wet weight (A, C) and ratio of wet weight to body weight (B, D) of lung (A, B) and heart (C, D). $n = 7$ in each group at each time point. Values represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

Wet weight of lungs and heart

Wet weights of lungs and heart were measured as indicator of pulmonary congestion and cardiac hypertrophy, respectively. The lung wet weight and the ratio of lung wet weight to body weight were significantly higher in the MCT group than in the Cont group at 14 and 21 days after monocrotaline injection (Fig. 2A, 2B). Although the heart wet weight was almost the same between the Cont and MCT groups at 14 days after monocrotaline injection, the value was higher in the MCT group than in the Cont group at 21 days (Fig. 2C), but statistically insignificant. The ratio of heart wet weight to body weight was significantly higher in the MCT group than in the Cont group only at 21 days after monocrotaline injection (Fig. 2D).

Wet weight of skeletal muscles

There were no significant differences in the wet weights or ratios of wet weight to body weight of the gastrocnemius (Fig. 3A, 3B) and soleus (Fig. 3C, 3D) muscles between the Cont and MCT groups at 14 days after monocrotaline injection. At 21 days after

monocrotaline injection, the wet weights of the gastrocnemius and soleus muscles in the MCT group decreased by 33% and 25%, respectively, from the values in the Cont group. The ratio of wet weight to body weight of the gastrocnemius muscle was significantly lower in the MCT group than in the Cont group at 21 days after monocrotaline injection. In contrast, there was no significant difference at 21 days in the value for the soleus muscle between the Cont and MCT groups.

Skeletal muscle fiber cross-sectional area

ATPase and SDH staining revealed that the gastrocnemius muscles were composed of type I, IIA, and IIB fibers (Fig. 4A–H), and the soleus muscles were composed of type I and IIA fibers (Fig. 4L–S). More than 90% of the fibers in the gastrocnemius muscle were type IIA and IIB, whereas the soleus muscle was mostly type I fiber.

In both the gastrocnemius (Fig. 4I, 4J, 4K) and soleus (Fig. 4T, 4U) muscles, no fiber types showed significant differences in cross-sectional area between the Cont and MCT groups at 14 days after

Fig. 3 Wet weight (A, C) and ratio of wet weight to body weight (B, D) of the gastrocnemius (A, B) and soleus (C, D) muscles. $n = 7$ in each group at each time point. Values represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

monocrotaline injection. However, in both the gastrocnemius and soleus muscles, the values were significantly lower in the MCT group than in the Cont group at 21 days. At this time point, the mean cross-sectional areas of the type I, IIA, and IIB fibers in the gastrocnemius muscle decreased by 17%, 21%, and 20%, respectively, from the values in the Cont group. In the soleus muscle, the mean cross-sectional areas of the type I and IIA fibers decreased by 28% and 33%, respectively, from the value in the Cont group.

Expression levels of mRNA for the ubiquitin-proteasome pathway

At 14 days after monocrotaline injection, although statistically insignificant, the mRNA expression levels of atrogin-1 (Fig. 5A) and MuRF-1 (Fig. 5B) in the gastrocnemius muscle in the MCT group were 2.1- and 2.4-fold higher than in the Cont group, respectively. In contrast, these expression levels in the soleus muscle were almost identical between the Cont and MCT groups at this time point (Fig. 5C, 5D). At 21 days after monocrotaline injection, the mRNA

expression levels of atrogin-1 and MuRF-1 in the gastrocnemius and soleus muscles were significantly higher in the MCT group than in the Cont group.

Expression levels of mRNA for the macroautophagy-lysosome pathway

At 14 days after monocrotaline injection, although statistically insignificant, the mRNA expression levels of LC3 (Fig. 6A) and p62 (Fig. 6B) in the gastrocnemius muscle in the MCT group were 1.7- and 3.1-fold higher than in the Cont group, respectively. In contrast, these expression levels in the soleus muscle were almost identical between the Cont and MCT groups at this time point (Fig. 6C, 6D). At 21 days after monocrotaline injection, the mRNA expression levels of LC3 and p62 in the gastrocnemius and soleus muscles were significantly higher in the MCT group than in the Cont group.

DISCUSSION

Although monocrotaline injection increased the ratio of lung wet weight to body weight after 14 days,

Fig. 4 Transverse sections of the gastrocnemius (A–H) and soleus (L–S) muscles stained with myofibrillar adenosine triphosphatase (A–D, L–O) and succinate dehydrogenase (E–H, P–S) in the Cont (A, C, E, G, L, N, P, R) and MCT (B, D, F, H, M, O, Q, S) groups at 14 (A, B, E, F, L, M, P, Q) and 21 (C, D, G, H, N, O, R, S) days after monocrotaline injection. 1: type I fiber; 2: type IIA fiber; 3: type IIB fiber. Scale bar = 100 μm . Cross-sectional area of type I (I, T), IIA (J, U), and IIB (K) fibers in the gastrocnemius (I–K) and soleus (T, U) muscles. Two sections per animal and five randomly chosen fields per section were evaluated. For each muscle fiber type, over 100 muscle fibers were measured. Values represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

Fig. 5 Expression levels of atrogin-1 (A, C) and MuRF-1 mRNA (B, D) in the gastrocnemius (A, B) and soleus (C, D) muscles. $n = 7$ in each group at each time point. Values were calculated as fold changes relative to the soleus muscle in the Cont group at 14 days and represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

there was no concurrent effect on the skeletal muscle wet weight or the fiber cross-sectional area. In contrast, monocrotaline injection increased the ratio of wet weight to body weight for both lungs and heart and decreased the skeletal muscle wet weight and fiber cross-sectional area after 21 days. These results suggest that skeletal muscle atrophy occurs only in the stage of right ventricular failure, not in the stage of pulmonary arterial hypertension. Steffen *et al.* (24) reported that treatment with anti-tumor necrosis factor- α (TNF- α) from the onset of body weight decrease attenuated skeletal muscle atrophy in monocrotaline-induced heart failure but could not prevent it entirely, suggesting that treatment must begin before body weight loss. Together with our results, this indicates that skeletal muscle atrophy is already in progress at the stage of pulmonary arterial hypertension.

Although skeletal muscle atrophy was not detected by morphological methods at 14 days after monocrotaline injection, the mRNA expression levels for the ubiquitin-proteasome (Cont vs. MCT, atrogin-1:

$P = 0.08$, MuRF-1: $P = 0.09$) and macroautophagy-lysosome (Cont vs. MCT, LC3: $P = 0.11$, p62: $P = 0.15$) pathways in the gastrocnemius muscle were marginally increased. Monocrotaline injection can cause physical inactivity (23), decrease food intake (10), and cause deconditioning with hypoxia (10), which activates the ubiquitin-proteasome pathway (14), the macroautophagy-lysosome pathway (20), or both (9) in the skeletal muscle, respectively. However, the Cont and MCT groups showed no differences in physical activity as measured by infrared sensor, food intake (Cont, 22 ± 0.4 g vs. MCT, 20 ± 1.5 g), or oxygen saturation of arterial blood (Cont, $97 \pm 0.3\%$ vs. MCT, 97 ± 0.1 g) for at least 14 days after monocrotaline injection in preliminary experiments. Overexpression of TNF- α was previously observed 2 weeks after monocrotaline injection (22), and increased circulating levels of TNF- α trigger apoptosis in skeletal muscle (5, 6). Additionally, overexpression of cytokines such as TNF- α has been reported in vascular inflammation of the lungs induced by monocrotaline injection (25). Therefore, we speculate

Fig. 6 Expression levels of LC3 (A, C) and p62 (B, D) mRNA in the gastrocnemius (A, B) and soleus (C, D) muscles. $n = 7$ in each group at each time point. Values were calculated as fold changes relative to the soleus muscle in the Cont group at 14 days and represent mean $\pm$ SE. * and † are significantly different ($P < 0.05$) from the Cont group at the same time point and within the same group at 14 days, respectively.

that vascular inflammation in the lungs could release upstream factors such as TNF- α and activate the ubiquitin-proteasome and macroautophagy-lysosome pathways at 14 days after monocrotaline injection, with subsequent skeletal muscle atrophy at 21 days.

The skeletal muscle atrophied less in the soleus muscle than in the gastrocnemius muscle after 21 days, but there was no significant difference in the ratio of soleus muscle wet weight to body weight between the Cont and MCT groups. These results suggested that atrophy in soleus muscle is inadequate to explain body weight loss with heart failure. Additionally, there was no significant difference in mRNA expression level for the ubiquitin-proteasome and macroautophagy-lysosome pathways in the soleus muscle at 14 days after monocrotaline injection between the Cont and MCT groups. In both the gastrocnemius and soleus muscles, the type I fiber cross-sectional area decreased less than those of type IIA and IIB fibers. These results suggest that type I fiber considered as oxidative slow-twitch fiber has high tolerance to protein degradation with heart

failure. Congestive heart failure is associated with apoptosis in skeletal muscles (5, 6) induced by elevated pro-inflammatory cytokines and reactive oxygen species (ROS) production (7). However, the magnitude of apoptosis is lower in slow muscle (19) and the region that composed mainly of type I fibers in fast muscle (29) than in fast muscle and the region that composed mainly of type II fibers in slow muscle due to the high anti-oxidative capacity of type I fibers (30). Therefore, because pro-inflammatory cytokines and ROS also activate the ubiquitin-proteasome and macroautophagy-lysosome pathways (28), the characteristics of slow-twitch fiber would inhibit protein degradation with heart failure.

In conclusion, although skeletal muscle atrophy did not occur before heart failure, the protein degradation pathways were activated at an early stage. Additionally, protein degradation pathways were activated less in slow muscle than in fast muscle. This study demonstrates that physical exercises that shift to slow muscle such as endurance training (11, 18) should begin before any indication of skeletal mus-

cle atrophy to prevent exercise intolerance with heart failure. However, this study has some limitations. Although we speculate that vascular inflammation in the lungs was induced by monocrotaline injection and the proinflammatory molecules activate protein degradation pathways, we did not analyze the upstream molecules of the ubiquitin-proteasome and macroautophagy-lysosome pathways such as TNF- α and ROS. Additionally, although exercise at the stage of pulmonary arterial hypertension in the monocrotaline model might worsen myocardial dysfunction, we did not investigate the effects herein. Therefore, further research is required to determine the stage at which exercise has low risk and high efficacy for heart failure.

Acknowledgements

This work was carried out at the Analysis Center of Life Science, Natural Science for Basic Research and Development, Hiroshima University. We would like to thank staff members for technical support. Additionally, this study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, and Technology (25750202).

REFERENCES

- Al-Nassan S, Fujita N, Kondo H, Murakami S and Fujino H (2012) Chronic exercise training down-regulates TNF- α and atrogin-1/MAFbx in mouse gastrocnemius muscle atrophy induced by hindlimb unloading. *Acta Histochem Cytochem* **45**, 343–349.
- Benoist D, Stones R, Drinkhill M, Bernus O and White E (2011) Arrhythmogenic substrate in hearts of rats with monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. *Am J Physiol Heart Circ Physiol* **300**, H2230–H2237.
- Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H and Johansen T (2005) p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol* **171**, 603–614.
- Carvalho RF, Castan EP, Coelho CA, Lopes FS, Almeida FL, Michelin A, de Souza RW, Araújo JP Jr, Cicogna AC and Dal Pai-Silva M (2010) Heart failure increases atrogin-1 and MuRF1 gene expression in skeletal muscle with fiber type-specific atrophy. *J Mol Histol* **41**, 81–87.
- Dalla Libera L, Ravara B, Angelini A, Rossini K, Sandri M, Thiene G, Battista Ambrosio G and Vescovo G (2001) Beneficial effects on skeletal muscle of the angiotensin II type 1 receptor blocker irbesartan in experimental heart failure. *Circulation* **103**, 2195–2200.
- Dalla Libera L, Sabbadini R, Renken C, Ravara B, Sandri M, Betto R, Angelini A and Vescovo G (2001) Apoptosis in the skeletal muscle of rats with heart failure is associated with increased serum levels of TNF- α and sphingosine. *J Mol Cell Cardiol* **33**, 1871–1878.
- Dalla Libera L, Ravara B, Gobbo V, Danieli Betto D, Germinario E, Angelini A and Vescovo G (2005) Skeletal muscle myofibrillar protein oxidation in heart failure and the protective effect of Carvedilol. *J Mol Cell Cardiol* **38**, 803–807.
- Dalla Libera L, Ravara B, Gobbo V, Betto DD, Germinario E, Angelini A, Evangelista S and Vescovo G (2010) Skeletal muscle proteins oxidation in chronic right heart failure in rats: can different beta-blockers prevent it to the same degree? *Int J Cardiol* **143**, 192–199.
- de Theije CC, Langen RC, Lamers WH, Gosker HR, Schols AM and Köhler SE (2015) Differential sensitivity of oxidative and glycolytic muscles to hypoxia-induced muscle atrophy. *J Appl Physiol* (1985) **118**, 200–211.
- Enache I, Charles AL, Bouitbir J, Favret F, Zoll J, Metzger D, Oswald-Mammoser M, Geny B and Charloux A (2013) Skeletal muscle mitochondrial dysfunction precedes right ventricular impairment in experimental pulmonary hypertension. *Mol Cell Biochem* **373**, 161–170.
- Feng H, Kang C, Dickman JR, Koenig R, Awoyinka I, Zhang Y and Ji LL (2013) Training-induced mitochondrial adaptation: role of peroxisome proliferator-activated receptor γ coactivator-1 α , nuclear factor- κ B and β -blockade. *Exp Physiol* **98**, 784–795.
- Fujita N, Hayashi-Nishino M, Fukumoto H, Omori H, Yamamoto A, Noda T and Yoshimori T (2008) An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol Biol Cell* **19**, 4651–4659.
- Fujita N, Murakami S, Arakawa T, Miki A and Fujino H (2011) The combined effect of electrical stimulation and resistance isometric contraction on muscle atrophy in rat tibialis anterior muscle. *Bosn J Basic Med Sci* **11**, 74–79.
- Fujita N, Murakami S and Fujino H (2011) The combined effect of electrical stimulation and high-load isometric contraction on protein degradation pathways in muscle atrophy induced by hindlimb unloading. *J Biomed Biotechnol* **2011**, 401493.
- Glickman MH and Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* **82**, 373–428.
- Handoko ML, de Man FS, Happé CM, Schaliij I, Musters RJ, Westerhof N, Postmus PE, Paulus WJ, van der Laarse WJ and Vonk-Noordegraaf A (2009) Opposite effects of training in rats with stable and progressive pulmonary hypertension. *Circulation* **120**, 42–49.
- Jackman RW and Kandarian SC (2004) The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol* **287**, C834–C843.
- Kang C, Chung E, Diffie G and Ji LL (2013) Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1 α . *Exp Gerontol* **48**, 1343–1350.
- Libera LD, Zennaro R, Sandri M, Ambrosio GB and Vescovo G (1999) Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. *Am J Physiol* **277**, C982–C986.
- Matsumoto G, Wada K, Okuno M, Kurosawa M and Nukina N (2011) Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol Cell* **44**, 279–289.
- Punkt K, Naupert A and Asmussen G (2004) Differentiation of rat skeletal muscle fibres during development and ageing. *Acta Histochem* **106**, 145–154.
- Sadamura-Takenaka Y, Ito T, Noma S, Oyama Y, Yamada S,

- Kawahara K, Inoue H and Maruyama I (2014) HMGB1 promotes the development of pulmonary arterial hypertension in rats. *PLoS One* **9**, e102482.
23. Simonini A, Long CS, Dudley GA, Yue P, McElhinny J and Massie BM (1996) Heart failure in rats causes changes in skeletal muscle morphology and gene expression that are not explained by reduced activity. *Circ Res* **79**, 128–136.
 24. Steffen BT, Lees SJ and Booth FW (2008) Anti-TNF treatment reduces rat skeletal muscle wasting in monocrotaline-induced cardiac cachexia. *J Appl Physiol* (1985) **105**, 1950–1958.
 25. Tsai BM, Turrentine MW, Sheridan BC, Wang M, Fiore AC, Brown JW and Meldrum DR (2006) Differential effects of phosphodiesterase-5 inhibitors on hypoxic pulmonary vasoconstriction and pulmonary artery cytokine expression. *Ann Thorac Surg* **81**, 272–278, 2006.
 26. Venkatraman P, Wetzel R, Tanaka M, Nukina N and Goldberg AL (2004) Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol Cell* **14**, 95–104.
 27. Vescovo G, Ravara B, Gobbo V, Angelini A and Dalla Libera L (2005) Skeletal muscle fibres synthesis in heart failure: role of PGC-1alpha, calcineurin and GH. *Int J Cardiol* **104**, 298–306.
 28. von Haehling S, Steinbeck L, Doehner W, Springer J and Anker SD (2013) Muscle wasting in heart failure: an overview. *Int J Biochem Cell Biol* **45**, 2257–2265.
 29. Wüst RC, Myers DS, Stones R, Benoist D, Robinson PA, Boyle JP, Peers C, White E and Rossiter HB (2012) Regional skeletal muscle remodeling and mitochondrial dysfunction in right ventricular heart failure. *Am J Physiol Heart Circ Physiol* **302**, H402–H411.
 30. Xu R, Andres-Mateos E, Mejias R, MacDonald EM, Leinwand LA, Merriman DK, Fink RH and Cohn RD (2013) Hibernating squirrel muscle activates the endurance exercise pathway despite prolonged immobilization. *Exp Neurol* **247**, 392–401.