THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2016, Vol. 71, No. 5, 601–609
doi:10.1093/gerona/glv064
Advance Access publication May 19, 2015

OXFORD

Original Article

Aerobic Exercise Recovers Disuse-induced Atrophy Through the Stimulus of the LRP130/PGC-1α Complex in Aged Rats

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Received January 27, 2015; Accpeted April 21, 2015

Decision Editor: Rafael de Cabo, PhD

Abstract

Physical training has been shown to be important to the control of muscle mass during aging, through the activation of several pathways including, IGF1-AKT and PGC-1α. Also, it was demonstrated that LRP130, a component of the PGC-1α complex, is important for the PGC-1α-dependent transcription of several mitochondrial genes in vivo. To explore the role of physical training during aging, we investigated the effects on muscle recovery after short-term immobilization followed by 3 or 7 days with aerobic or resistance training. Using morphological (myofibrillar adenosine triphosphatase activity, to assess the total muscle fiber cross-sectional area (CSA) and the frequency of specific fiber types), biochemical (myosin heavy chain), and molecular analyses (quantitative real-time PCR, functional pathways analyses, and Western blot), our results indicated that after an atrophic stimulus, only animals subjected to aerobic training showed entire recovery of cross-sectional area; aerobic training reduced the ubiquitin–proteasome system components involved in muscle atrophy after 3 days of recovery, and the upregulation in PGC-1α expression enhanced the process of muscle recovery by inhibiting the FoxO pathway, with the possible involvement of LRP130. These results suggest that aerobic training enhanced the muscle regeneration process after disuse-induced atrophy in aged rats possibly through of the LRP130/PGC-1α complex by inhibiting the ubiquitin–proteasome system.

Key Words: Aging—Muscle mass—Immobilization—Recovery—Exercise training

During aging, loss of muscle mass has a significant impact on muscle function (frailty, strength, weakness) and contributes to elevated morbidity and mortality (1,2). In addition, the negative health consequences, such as impaired functional capacity and strength, predispose the elderly adults to periods of prolonged bed rest, which exacerbate muscle loss (3) and worsen clinical status. Although the maintenance of muscle mass is important for the health of the elderly adults, who are prone to long periods of inactivity, very little is known about the mechanisms that control the regenerative capacity of the skeletal muscle in these individuals.

Although some atrophy models (hind-limb suspension and immobilization) have been used to better understand the response of muscle to aging and prolonged bed rest (4,5), few studies have investigated muscle recovery in these circumstances (4,6,7). Muscle recovery from disuse is slower during aging (6), so strategies to alleviate progressive loss of muscle mass need to be tested. In this regard, resistance training (RT) has been used in an attempt to enhance muscle regeneration after atrophy because this intervention is known to induce hypertrophy in skeletal muscle (8). However, RT protocols have not been found to restore old muscle to normal conditions (4),

suggesting that some processes that promote muscle atrophy may not be responsive to RT protocols.

The mechanisms that control the loss of muscle mass during disuse atrophy have been shown to consist mainly of protein degradation through the ubiquitin–proteasome system, a specific pathway for the selective degradation of muscle proteins under catabolic conditions (9,10). Corroborating these findings, numerous studies have demonstrated that two important components of this pathway, the E3 ubiquitin ligases, MAFbx or atrogin-1, and Murf-1, were highly elevated in several models of muscle disuse (4,8).

Notably, various studies have shown that the upregulation of atrogin-1 and MuRF1 depends on the activity of the FoxO family (11,12). FoxO1 activity is modulated by interaction with several transcription factors, including PGC-1α, a critical transcriptional coactivator involved in mitochondrial biogenesis in cardiac and skeletal muscle (13). Furthermore, it has been suggested that high levels of PGC-1α spare muscle mass in models of denervation, fasting, and heart failure (14). Additionally, PGC-1α expression in skeletal muscle can be induced by a variety of stimuli, including aerobic training (AT) (15,16). Although some studies have found a relationship between PGC-1α and FoxO during atrophy, little is known about the mechanism involved (17).

In addition, Cooper and colleagues (18), in a model of the French Canadian type of Leigh syndrome, showed that LRP130 (also known as LRPPRC, leucine-rich pentatricopeptide repeat motif-containing protein), which was initially isolated as a lectin-binding protein of unknown function from HepG2 cells (19), is a component of the PGC-1α complex and is necessary for the PGC-1α-dependent transcription of several mitochondrial genes in vivo; these authors also observed that this protein interacted with FoxO1 via PGC-1α. Therefore, we hypothesized that the physical training could improve the muscle recovery possibly through of the LRP130/PGC-1α complex by inhibiting the ubiquitin–proteasome system after disuse-induced atrophy in aged rats. Given the importance of muscle mass in aging, we explored mechanisms related to muscle recovery from disuse-induced atrophy in old rats. This initial study investigated the effects on muscle recovery after short-term immobilization (lower limb cast immobilization) followed by 3 or 7 days of retraining with AT or RT.

Methods

All procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Biosciences Institute Ethics Committee, São Paulo State University, Botucatu, SP, Brazil (protocol no. 343-CEEA). Old male (18 mo) Wistar rats (20) were purchased from the central animal facility at São Paulo State University. They were housed in polypropylene cages in a temperature-controlled room under a 12-h light–dark cycle and received food and water ad libitum. Animals were divided into 10 experimental groups (n = 6): immobilized for 1 week (I), with their corresponding controls (C); immobilized and allowed to recover for 3 or 7 days (R3 and R7, respectively), with their corresponding controls (C3 and C7, respectively); immobilized and then subjected to AT for 3 or 7 days (AT3 and AT7, respectively); and immobilized and then subjected to RT for 3 or 7 days (RT3 and RT7, respectively).

Initially, the animals underwent a week of adaptation to the training protocol and the water to minimize the effects of stress during the process of recovery after the atrophic stimulus. The immobilization process was based on a procedure adapted from Frimel and colleagues (21). Briefly, the animals in groups I, R3, AT3, RT3, R7, AT7, and RT7 were anesthetized with a mixture of ketamine (50 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal) to ensure complete sedation of animals during the procedure. After the animals regained consciousness, their hind limbs were fixed with a fast drying plaster bandage (see Supplementary Figure 1a). The extent of the immobilization was from the hip to the ankle so that the plantaris (PL) muscle would remain in a neutral position during the 7 days of immobilization, to promote muscle atrophy (see Supplementary Figure 1b) (22).

After the immobilization period, the animals in the R3 and R7 groups were kept in their cages for muscle recovery, and the animals in the AT3, AT7, RT3, and RT7 groups underwent a program of AT or RT for 3 or 7 days, to modulate muscle recovery. All animals in the C3, R3, C7, and R7 groups were kept in shallow water during the training session. At the end of the experiments (1 day after the following procedures: immobilization, recovery periods, and training session), fed animals were euthanized, the PL muscle was removed, frozen in liquid nitrogen, and stored at −80°C for further analyses.

Anatomical Data

The body weight (BW) and feed intake of the animals were monitored daily throughout the experiment. The PL muscle was collected, weighed, and subjected to morphological, biochemical, histochemical, and molecular analyses. Furthermore, the visceral, epididymal, and retroperitoneal fat pads were excised and weighed to assess the animal's body composition at the end of the experiment. Additionally, three parameters were used to evaluate the rate of muscle atrophy/hypertrophy: muscle weight (milligram), muscle-to-tibia length ratio, and muscle fiber cross-sectional area (CSA; square micrometers).

Analysis of Serum Cortisol

To assess the level of stress present in the animals after the period of muscle immobilization and recovery of 3 and 7 days, serum cortisol levels were measured. Blood was collected after decapitation and centrifuged (3,000g for 15 min), and the serum was collected and stored at −80°C. Cortisol concentrations were quantified by enzyme-linked immunosorbent assays, following the manufacturer's instructions (R & D Systems).

Training Protocol

Aerobic training

Aerobic or resistance exercise training was used in an attempt to enhance the process of muscle recovery after the atrophic stimulus. The AT (swimming) bouts were individually performed in an aquarium subdivided into four compartments and filled with 40 cm of water at 28–30°C. The animals in groups C and R were kept in shallow water throughout the training period, whereas animals in groups AT3 and AT7 were subjected to an aerobic swimming training program for 3 and 7 days, respectively (23). Initially, all the animals completed five sessions to adapt to the water in a progressive volume (0–15 minutes) and intensity (expressed as a load of 0%–2% of BW). To define an appropriate intensity of training, the anaerobic threshold was measured, which represents the limit of the metabolic transition between the zone of aerobic to anaerobic predominance (24). The protocol has a nonexhaustive test double effort (25) comprising two efforts of swimming for 5 minutes at the same intensity, with 2 minutes rest between them, with blood collection for lactate

analysis at the end of the first and second efforts. The intensities were varied by loads of 2%, 3%, and 4% of the animal's BW. Animals performed the test at the three intensities with an interval of 24 hours between the tests. These intensities were chosen based on study that determined the anaerobic threshold (5% of BW) in adult rats (26).

After determining the anaerobic threshold of animals, volume (time) and intensity (load) training took 20 minutes with 2.3% of BW (anaerobic threshold) for 3 (AT3) and 7 (AT7) days of training. The training sessions were performed at the same time of day, between 14:00 and 16:00 hours.

Resistance training

RT was based on the protocol described by Cunha and colleagues (27). Initially, the animals underwent 1 week of training to acclimatize them to the water and training protocol. In this acclimation stage, the animals jumped into a vat containing water to a depth of 38 cm with a temperature between 28 and 32°C. The depth was appropriate to allow the animal to breathe on the surface of the water during successive jumps. The jump was recorded as successful only when the animals jumped to the edge of the container up to the water surface in successive movements. The adaptation process was gradual: the number of sets was increased (2–4), as was the number of repetitions (5–10), and the animals carried an added load of 50% BW in a vest. After the acclimation period and to ensure the same training intensity among animals, the loads were adjusted for a one repetition maximum test. The test was performed with a gradual increase in loads performed with a 5-minute interval between the attempts. Four trials were carried out within 1 day to determine the load that represents the one repetition maximum test (the heaviest load that the animal can achieve only once). Subsequently, the animals were submitted to 3 (RT3) or 7 (RT7) days of strength training after the atrophic stimulus. The training comprised 3 sets of 10 jumps (1 min of rest) using 60% of the one repetition maximum test, which corresponds to strength training (28). The training sessions were performed at the same time of day, between 14:00 and 16:00 hours.

Histochemical Analysis

The PL muscles were dissected, weighed, and a portion was quickly frozen in liquid nitrogen-cooled isopentane and stored at −80°C before sectioning. Histological sections (12 µm) were cut, and the total CSA and the frequency of specific fiber types were determined by myofibrillar adenosine triphosphatase histochemistry (29). CSA was measured on 400 fibers of the muscle in each animal, and the mean was calculated.

Myosin heavy chain analysis

Biochemical analysis of myosin heavy chain (MyHC) was performed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis in duplicate. Histological sections were collected from each sample and placed in an extraction solution (10% glycerol, 5% 2-mercaptoethanol, and 2.3% sodium dodecyl sulfate in a 0.9% Tris-HCl buffer [pH 6.8]). For electrophoretic separation, 15–20 µL of each extract was loaded onto a 7%–10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gradient gel over a period of 26 hours at 185 V. The gels were stained with Coomassie Blue (30) to identify the MyHC isoforms according to their molecular weights. The relative content of the different MyHC isoforms was determined using the densitometry readings of the bands that corresponded to each MyHC isoform using Image Master VDS Software (Pharmacia Biotech, EUA).

Quantitative real-time–PCR analysis

RT–qPCR (quantitative RT–PCR) experiments were performed following the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (31). Total RNA was isolated from PL muscle previously frozen in liquid nitrogen by homogenization using TRIzol according to the manufacturer's instructions in a tissue homogenizer (Bullet Blender, Next Advance Inc., Averill Park, NY).

The RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) (32) and quantified using a NanoVue Plus spectrophotometer (GE HealthCare). Following isolation, RNA samples were treated with TURBO DNase (Ambion, Life Technologies). Complementary DNA (cDNA) was synthesized from 2 µg of total RNA using the High Capacity RNA-to-cDNA kit (Life Technologies Corporation, Carlsbad, CA) according to the manufacturer's instructions. The PCR reactions consisted 50-fold dilution of the cDNA, using microfluidics platform TaqMan Low Density Arrays (TLDA) v1.0 (Life Technologies) according to the manufacturer's instructions. The cDNA was then transferred to TLDA containing 44 target genes and 4 endogenous genes (see Supplementary Table 1) and the relative quantification of gene expression was performed by the comparative Cq method (33) using Data Assist 2.0 (Life Technologies).

Pathway analysis

To further understand the biological relevance of the messenger RNA interactions, we performed a search for additional genes that are related to our differentially expressed gene set, in the context of functional association data, such as protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity, using the GeneMANIA Cytoscape plugin (34).

Western blot analysis

Protein lysates were prepared from the gastrocnemius muscle according to the following protocol. Muscle samples were homogenized in lysis buffer (1% Triton X-100, 10 mM of sodium pyrophosphate, 100 mM of NaF, 10 mg/mL of aprotinin, 1 mM of phenylmethyl sulfonylfluoride, 0.25 mM of Na_3VO_4, 150 mM of NaCl, and 50 mM of Tris-HCl, pH 7.5). The samples were centrifuged and 50 mL of the homogenate fraction were resuspended in 25 mL of Laemmli loading buffer (2% sodium dodecyl sulfate, 20% glycerol, 0.04 mg/mL of bromophenol blue, 0.12 M Tris-HCl pH 6.8, and 0.28 M β-mercaptoethanol). Tissues from all the groups were used and 50 µg of protein lysate was loaded per lane. The membranes were incubated overnight at 4°C with primary antibodies: from Cell Signaling Technology (Beverly, MA), anti-GAPDH (2118), anti-FoxO1 (9454), and anti-pFoxO1 (9461); from Abcam (Cambridge, MA), anti-PGC-1α (106814); and from Santa Cruz Biotechnology (Heidelberg, Germany), anti-MURF1 (27642), anti-Mafbx (27644), and anti-LRP130 (66845), as well as appropriate secondary antibodies. Finally, immunoreactive protein signals were detected using the SuperSignal West Pico Chemiluminescent Substrate Kit (Thermo Fisher Scientific, Rockford, IL), according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean ± standard deviation. The Kolmogorov–Smirnov normality test was used to verify data normal distribution. The differences between C and I group parameters were tested with unpaired Student's *t* tests. The muscle fiber type

frequency and MyHC data were analyzed using the Kruskal–Wallis test. Two-way analysis of variance using treatment and time as factors followed by a posteriori Bonferroni multiple comparison test was used for cortisol, CSA, gene, and protein expression analysis. Linear regression was used to assess the relationship between PGC-1α and LRP130 protein expression with the 3- and 7-day recovery process. Statistical significance was considered achieved when the value of p was <.05.

Results

BW After Immobilization and Recovery

There was no significant difference in BW ([initial BW (g): C: 607.8 ± 79.7; I: 597.0 ± 86.7—3 days: C: 574.2 ± 55.1; R: 583.8 ± 42.6; AT: 578.2 ± 68.1; RT: 579.7 ± 56.1—7 days: C: 587.0 ± 58.9; R: 583.5 ± 58.9; AT: 569.8 ± 59.3; RT: 598.0 ± 32.5] [final BW (g): C: 550.3 ± 68.7; I: 531.0 ± 65.5—3 days: C: 534.8 ± 44.2; R: 506.0 ± 29.2; AT: 493.0 ± 45.5.1; RT: 484.3 ± 50.4—7 days: C: 546.0 ± 40.1; R: 503.5 ± 38.9; AT: 500.6 ± 49.7; RT: 516.8 ± 36.2]) and body fat (see Supplementary Figures 2 and 3) among the groups after 7 days of immobilization and the recovery period (3 and 7 days). These data demonstrate that all animals displayed similar healthy levels during experiment. Furthermore, no significant difference in weekly food intake was found among the groups (data not shown).

Muscle Parameters After Immobilization and Recovery

After 7 days of immobilization, there was significant 11% reduction in CSA (square micrometer) of total PL muscle in the I (1,955 ± 56.9) compared with C (2,205 ± 52.8) groups. This resulted in a significant decrease in CSA of all fiber types (I: 1,984 ± 182 vs 2,613 ± 154.1; IIA: 1,227 ± 92.6 vs 1,899 ± 153.1; IID: 1,561 ± 51.4 vs 1,862 ± 161.5, and IIB: 2,930 ± 129.6 vs 3,529 ± 282.1) in the I compared with C groups. After the experimental period, only the AT group recovery the CSA (after 7 days) while the others groups had a lower CSA compared with C group (Figure 1), indicating that the AT has enhanced muscle recovery. In addition, our results showed that there were no interaction between the procedures (recovery method) and the time.

Fiber type–specific CSA analysis revealed that type I and IIA fibers were completely recovered in both AT and RT groups after the recovery periods (Figure 2A and B). This adaptation suggest that the training (regardless of the type) enhanced the recovery process as only 3 days were sufficient to fully recover the I and IIA CSA. The glycolytic fibers needed more time to recover (just after 7 days): the IID fibers were recovered in both groups submitted to exercise (AT and RT), whereas the type IIB fibers had normal pattern of CSA only in AT group (Figure 2C and D). The R group recovered only the type I and IIA fibers after 7 days (Figure 2A–D). Moreover, there was no change in the frequency of the muscle fiber types or in the MyHC analysis (see Supplementary Figure 4) in all groups, demonstrating that neither the immobilization nor the recovery process changed the phenotypic profile of the PL muscle.

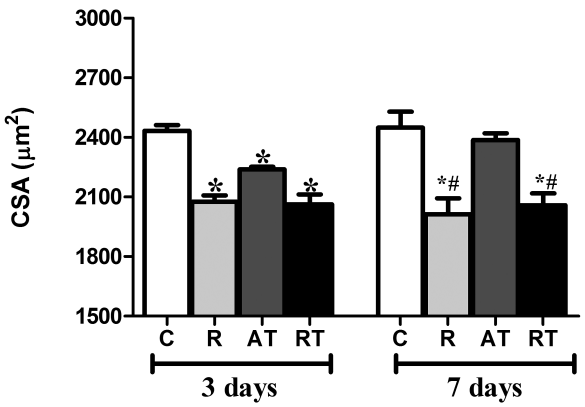

Figure 1. Cross-sectional area after the recovery period (3 and 7 days). Values are expressed as the mean ± standard deviation. $n = 6$ rats per group. *Significant difference compared with respective C group; #Significant difference compared with aerobic training (AT) group ($p < .05$).

Analysis of Serum Cortisol

There were no differences in serum cortisol levels (ng/mL) between the groups (C: 0.95 ± 0.1; I: 0.90 ± 0.6—3 days: C: 0.77 ± 0.5; R: 0.90 ± 0.2; AT: 0.75 ± 0.2; RT: 0.94 ± 0.2—7 days: C: 0.79 ± 0.3; R: 0.66 ± 0.1; AT: 0.61 ± 0.1; RT: 0.76 ± 0.3), showing that all animals remained in the same stress condition throughout the experimental period.

Muscle Atrophy and Hypertrophy Markers

The expression of muscle atrophy and hypertrophy markers was analyzed by TLDA, to obtain a more global perspective of the muscle recovery process in aging rats. Based on the genes grouped on the TLDA card, we focused on components of the following signaling pathways: cell proteolytic systems and cell death (ubiquitin–proteasome system, oxidative stress, autophagy–lysosome system, and apoptotic pathways); inflammatory cytokines (TNFα pathway), and muscle growth (IGF1-AKT signaling pathway and myostatin pathway). After 7 days of immobilization, there was a significant increase in gene and protein expression of ubiquitin–proteasome system components (% increase gene and protein; FoxO1: +18% and +20%; MAFbx: +20% and +23%, and Murf1: +22% and +25%, respectively) and decrease in LRP130 (% decrease gene and protein; −13% and −15%) and PGC-1α (% decrease gene and protein, −13% and −15%) expression in the I group compared with C group (Figure 3). The muscle growth-related factors remained unchanged after immobilization (see Supplementary Figures 5 and 6).

After 3 days of recovery period, the expression levels of MaFbx, Murf1 (just in protein expression), and FoxO1 remained elevated only in the R and RT compared with the C groups; after 7 days, R and RT groups presented a decreased expression in all gene (except the protein expression of MAFbx that was high in the R group) (Figures 4 and 5). Additionally, there was a significant decrease in PGC-1α and LRP130 expression in the R and RT groups.

Interestingly, the AT group showed reduced levels of the atrophy markers (just MAfbx protein expression remained elevated at 3 days) and an increase in the protein expression of PGC-1α and LRP130 after the recovery periods (Figures 4 and 5). Additionally, linear regression analysis between LRP130 and PGC-1α protein expression revealed a moderate correlation after 3 days (Pearson's $r = 0.67$, $p = .003$) and a strong correlation after 7 days (Pearson's $r = 0.98$, $p \leq .0001$).

Discussion

Although the regeneration capacity of atrophied muscle is known to decrease with age (35,36), the molecular mechanisms underlying

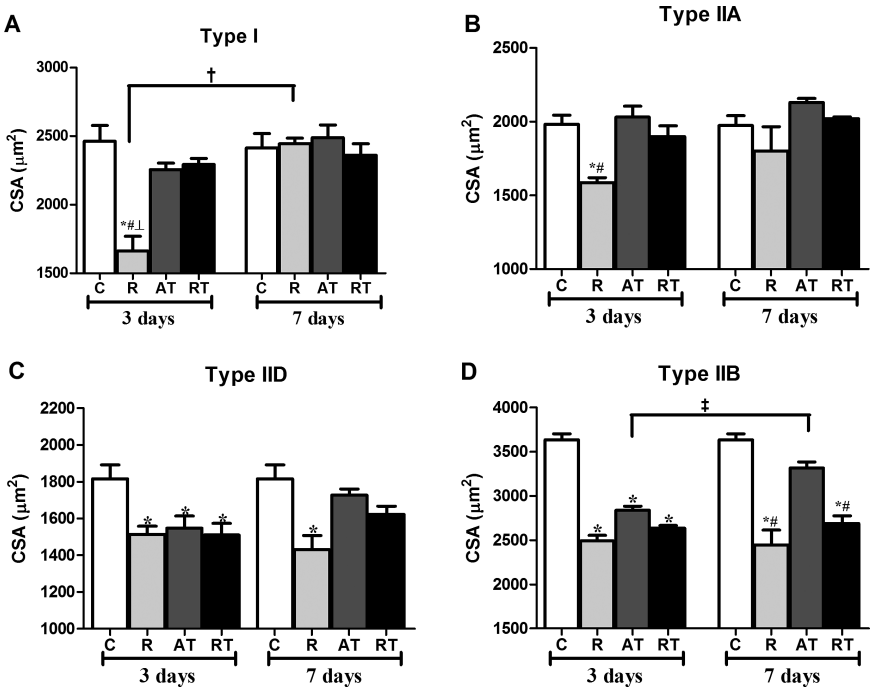

Figure 2. Cross-sectional area of muscle fiber type (**A**–**D**) after the experimental period. (A) Muscle fiber type I; (B) muscle fiber type IIA; (C) muscle fiber type IID; D muscle fiber type IB. The groups are represented as C: control group; R: recovery group; AT: aerobic training group; and RT: resistance training group. Values are expressed as the mean ± standard deviation. $n = 6$ rats per group. *Significant difference compared with respective C group. #Significant difference compared with AT group; ⊥Significant difference compared with RT group. †Significant difference in R group between 3 and 7 days. ‡Significant difference in AT group between 3 and 7 days ($p < .05$).

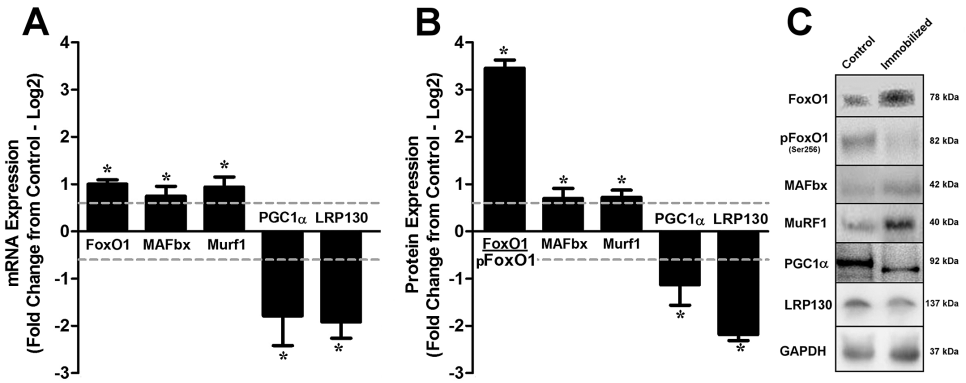

Figure 3. Relative messenger RNA (mRNA) and protein expression (log2 fold change from C group). (**A**) mRNA levels of immobilized group compared with control. (**B**) Protein content of immobilized group compared with control; (**C**) Representative blots of experimental groups after 1 week of immobilization. $n = 6$ rats per group. *Significant difference compared with C group (baseline; $p < .05$).

regeneration after muscle atrophy during aging are poorly understood. Therefore, this study was designed to evaluate the molecular mechanisms that mediate the muscle mass recovery after short-term cast immobilization in aged muscle. Moreover, we evaluated whether the AT or RT could affect differentially this process. The major findings of this study were that (a) only animals subjected to AT showed entire recovery of CSA after the recovery period, (b) the AT reduced the expression of ubiquitin–proteasome system components involved in muscle atrophy, and (c) upregulation in PGC-1α expression enhanced the process of muscle recovery by inhibiting the FoxO pathway, with the possible involvement of LRP130.

Consistent with previous muscle atrophy studies involving hindlimb suspension (37,38), our results showed a marked decrease in PL muscle fibers CSA (Figure 1) with reciprocal increase in expression of ubiquitin–proteasome system components (eg, FoxO1, MAFbx, and Murf1; Figure 3), which have been suggested to be important muscle atrophy markers (39). Furthermore, there was a decrease in LRP130 and PGC-1α expression (Figure 3), and no change in hypertrophy pathway components (see Supplementary Figures 5 and 6). These data confirm that 7-days immobilization model was effective to induce loss of muscle mass in our experimental groups.

Furthermore, to investigate whether muscle recovery in response to different stimuli (AT or RT) occurred in a fiber type–specific manner, we evaluated the CSA of the PL muscle fiber types. The muscle fiber types I and IIA CSA were completely recovered

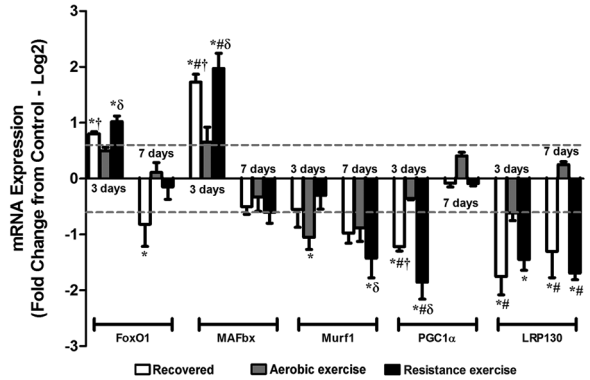

Figure 4. Relative messenger RNA (mRNA) expression (log2 fold change from C group) after the recovery period (3 and 7 days). The groups are represented as C: control group; R: recovery group; AT: aerobic training group; and RT: resistance training group. Values are expressed as the means ± standard deviation. $n = 6$ rats per group. *Significant difference compared with respective C group. #Significant difference compared with AT group; †Significant difference in R group between 3 and 7 days; δSignificant difference in RT group between 3 and 7 days ($p < .05$).

after 3 days in both AT and RT groups and just after 7 days in R group (Figure 2A and B). The type IID fibers CSA also were recovered in both AT and RT groups just after 7 days (Figure 2C). However, only the AT group recovered the type IIB fibers CSA after 7 days (Figure 2D). As a result, only the AT group had a total muscle recovery at the end of the experiment (as measured by PL muscle CSA) (Figure 1). Considering that RT has been advocated as the main stimulus for muscle regeneration (40,41), it is not clear why the AT was more effective for recovering aged muscle. A possible explanation for this is that only AT was able to attenuate the upregulation in expression of ubiquitin–proteasome system components (eg, FoxO1, MAFbx, and Murf1), which are involved in the control of muscle catabolism (Figures 4 and 5). Interestingly, in our study, this preventive effect of AT on atrophic proteins expression was early initiated, after 3 days of recovery, indicating that AT stimulus appears to be more efficient for creating a favorable cellular environmental and, thus, enhancing the recovery process in aged muscle. Beyond that, it has been shown that aerobic exercise (acute and chronic) also stimulates muscle protein synthesis in old individuals (42,43), suggesting that this kind of exercise really stimulate the skeletal muscle growth. Also, considering that, the

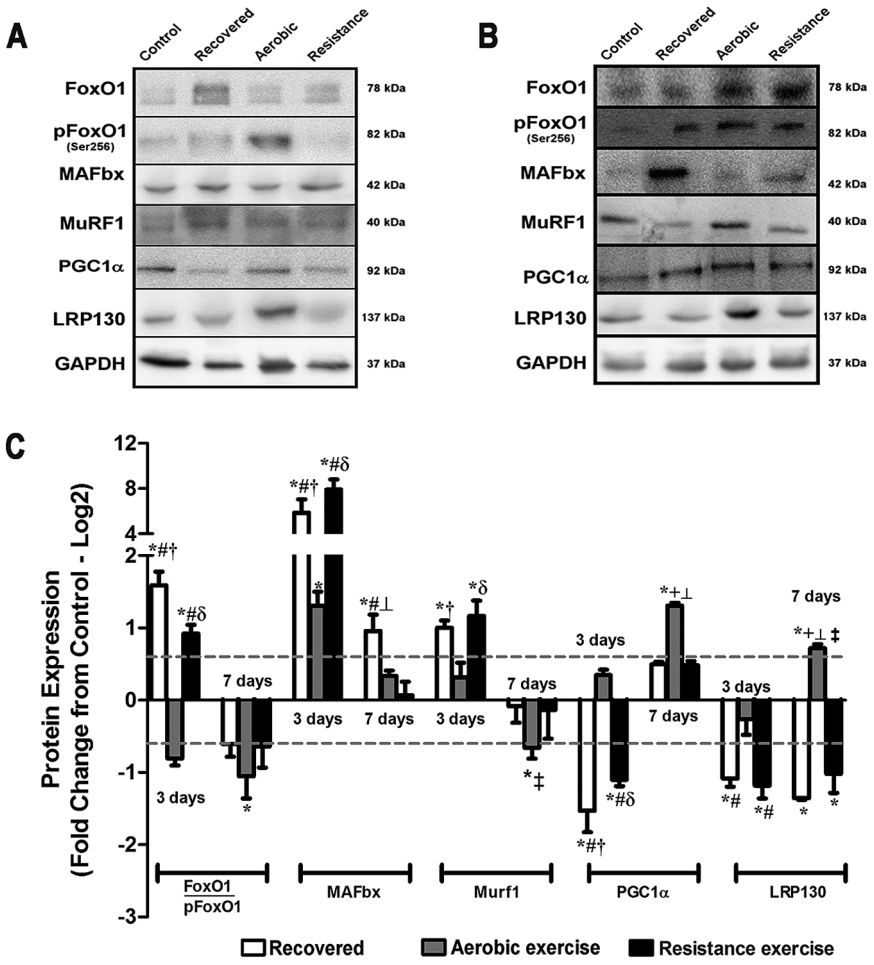

Figure 5. Relative protein expression (log2 fold change from C group) after the recovery period (3 and 7 days). (A) 3 days and (B) 7 days—representative blots of experimental groups. (C)—Protein content of recovery groups after 3 and 7 days; the groups are represented as C: control group; R: recovery group; AT: aerobic training group; and RT: resistance training group. Values are expressed as the means ± standard deviation. $n = 6$ rats per group. *Significant difference compared with respective C group; +Significant difference compared with R group; #Significant difference compared with AT group; ⊥Significant difference compared with RT group; †Significant difference in R group between 3 and 7 days; ‡Significant difference in AT group between 3 and 7 days; δSignificant difference in RT group between 3 and 7 days ($p < .05$).

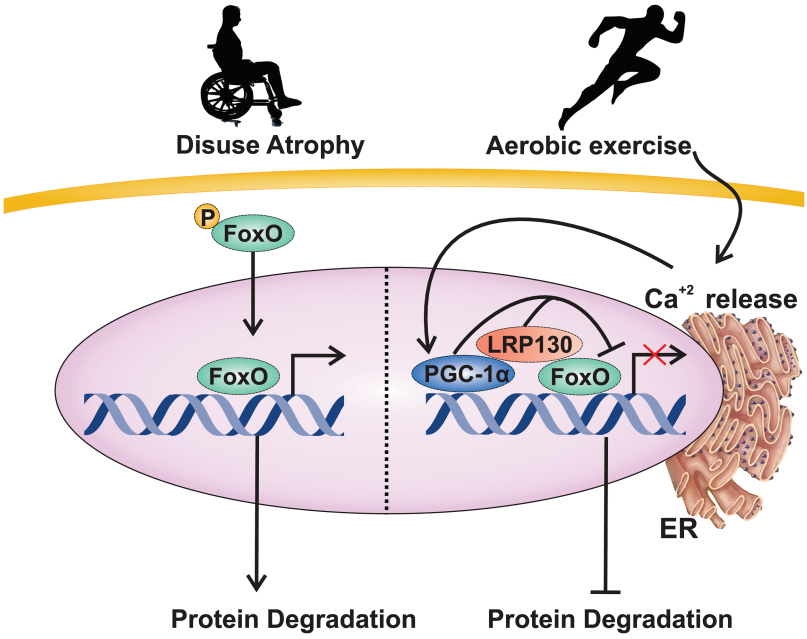

Figure 6. Model of inhibition of FoxO and atrophy by the complex PGC1α/RPL130 induced by aerobic exercise in aging. Aerobic exercise increase the Ca+ release of ER and drives PGC1α activation and translocation to nucleus by Ca+ signaling, where interact to form a complex with the protein RPL130 leading to inhibition of transcriptional activity of FoxO, which suppress the activation of atrophic genes and protein degradation.

aged skeletal muscle is oxidatively limited and that the AT improve the muscle metabolic capacity in aged condition (44), it seems that AT can create a favorable environment to stimulate the aging muscle recovery after atrophy.

The improvement of recovery in AT compared with RT, seems to be associated with the early attenuation in atrophic pathways by the expression of PGC-1α and LRP130 (Figures 4 and 5). In fact, it has been shown that increased PGC-1α expression can protect muscles from atrophy by inhibiting FoxO (14,45), and that repetitive muscle contractions, as occur in AT, could activate the AMPK pathway and, consequently, induce PGC-1α expression (46). Our results support this hypothesis and demonstrate that the activation of PGC-1α pathway may also be important for enhanced muscle regeneration process. From our results, it is possible to speculate that the improvement in muscle recovery is associated with an anabolic effect of the PGC-1α, induced by the AT, on the muscle fibers. However, the exact mechanisms need to be clarified.

Interestingly, the expression levels of PGC-1α and LRP130 during recovery after disuse-induced atrophy were positively correlated (3 days: $r = 0.67$, $p = .003$; 7 days: $r = 0.98$, $p < .0001$). Although our results cannot demonstrate a causal relationship between these factors, they support an previous study (18), demonstrating a strong correlation between LRP130 and PGC-1α. In fact, LRP130 has been shown to cooperate with PGC-1α in the liver to maintain glucose homeostasis in French Canadian Leigh syndrome (18). Furthermore, this study show that LRP130 may be required to increase or decrease the docking of PGC-1α and its concomitant transcriptional action on specific transcription factors genes and promoters. The authors showed that PGC-1α had lost its ability to co-activate FoxO1 in cells that lacked LRP130, whereas exogenous expression of LRP130 restored this co-activation function. Although the interaction between PGC-1α and LRP130 has been demonstrated only in the liver (18), and our study provides no direct information if the PGC–LPR130 complex is central to the aging muscle atrophy response to AT, we strongly believe that LRP130 could also bind to PGC-1α and, thus, blocking the function of FoxO1 during skeletal muscle recovery by AT after immobilization-induced atrophy. Interestingly, this relationship was only evident after 3 days of recovery (when the LRP130 and PGC-1α proteins returned to normal levels of expression) and was more pronounced after 7 days only in AT group. This suggest that AT stimulus is more efficient than RT for enhancing the muscle regeneration process in aged muscle, presumably by attenuating the down-regulation in PGC-1α and LRP130 expression (Figure 6). However, to confirm this preventive effect, more studies are needed.

Although the protein expression analysis does not allow us to verify the correct activity of MaFbx, Murf1, PGC-1α, and LPR130 during skeletal muscle recovery by AT after immobilization-induced atrophy, due to the difference between nuclear and cytoplasmic expression of proteins (47,48), however, our morphological and molecular data with others studies provide an important indicator of the real participation of these proteins in muscle regeneration process after immobilization-induced atrophy.

In conclusion, the main finding of this study is that AT enhanced the muscle regeneration process after disuse-induced atrophy in aged muscle possibly through of the LRP130/PGC-1α complex by inhibiting the ubiquitin–proteasome system. These results provide important information that improves our understanding of the mechanisms underlying muscle recovery during aging and could therefore help to identify treatment strategies for age-related loss of muscle mass.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

Funding

This study was supported by the São Paulo Research Foundation, Brazil (FAPESP, Proc. 2011/14484-4).

Conflict of Interest

Each author has participated intellectually or practically in the work to take public responsibility for the content of the article. The authors have read and approved the final manuscript. All authors of this study declare that they have no conflict of interest.

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