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ARTICLE

# Resistance training minimizes catabolic effects induced by sleep deprivation in rats

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**Abstract:** Sleep deprivation (SD) can induce muscle atrophy. We aimed to investigate the changes underpinning SD-induced muscle atrophy and the impact of this condition on rats that were previously submitted to resistance training (RT). Adult male Wistar EPM-1 rats were randomly allocated into 1 of 5 groups: control, sham, SD (for 96 h), RT, and RT+SD. The major outcomes of this study were muscle fiber cross-sectional area (CSA), anabolic and catabolic hormone profiles, and the abundance of select proteins involved in muscle protein synthesis and degradation pathways. SD resulted in muscle atrophy; however, when SD was combined with RT, the reduction in muscle fiber CSA was attenuated. The levels of IGF-1 and testosterone were reduced in SD animals, and the RT+SD group had higher levels of these hormones than the SD group. Corticosterone was increased in the SD group compared with the control group, and this increase was minimized in the RT+SD group. The increases in corticosterone concentrations paralleled changes in the abundance of ubiquitinated proteins and the autophagic proteins LC3 and p62/SQSTM1, suggesting that corticosterone may trigger these changes. SD induced weight loss, but this loss was minimized in the RT+SD group. We conclude that SD induced muscle atrophy, probably because of the increased corticosterone and catabolic signal. High-intensity RT performed before SD was beneficial in containing muscle loss induced by SD. It also minimized the catabolic signal and increased synthetic activity, thereby minimizing the body's weight loss.

*Key words:* sleep loss, resistance exercise, autophagy, proteasome, muscle atrophy.

**Résumé :** La privation de sommeil (SD) peut causer l'atrophie des muscles. Cette étude se propose d'analyser les modifications sous-jacentes à l'atrophie musculaire causée par SD et examine l'impact de cette condition chez des rats soumis au préalable à un entraînement contre résistance (RT). On répartit aléatoirement des rats adultes Wistar EPM-1 dans cinq groupes : Contrôle (CTRL), Simulation (SHAM), SD (durant 96 h), RT et RT+SD. Les principaux résultats sont observés dans la surface de section transversale des fibres musculaires (CSA), le bilan des hormones catabolisantes et anabolisantes et l'abondance de protéines choisies pour leur implication dans les voies de l'anabolisme et du catabolisme des protéines. SD suscite l'atrophie musculaire; combinée à RT, on observe une moins grande diminution de la CSA des fibres musculaires. Chez les animaux du groupe SD, on observe une diminution du facteur de croissance 1 analogue à l'insuline (IGF-1) et de la testostérone; chez le groupe RT+SD, les concentrations de ces variables sont plus élevées que dans le groupe SD. On observe une augmentation de la corticostérone dans le groupe SD comparativement au groupe CTRL, mais cette augmentation est minimisée dans le groupe RT+SD. L'augmentation de la concentration de corticostérone dans certains groupes suit l'abondance des protéines autophagiques LC3, p62/SQSTM1 et des protéines ubiquitinées, suggérant ainsi un rôle déclencheur à la corticostérone. SD suscite la perte de masse corporelle, mais celle-ci est moindre dans le groupe préalablement entraîné. Selon nous, l'atrophie musculaire causée par SD est probablement due à l'augmentation de la corticostérone et du signal catabolique. Un RT d'intensité élevée effectué avant SD est bénéfique pour atténuer la perte de masse musculaire suscitée par SD; cet entraînement atténue aussi le signal catabolique et augmente l'activité de synthèse, minimisant ainsi la perte de masse corporelle. [Traduit par la Rédaction]

*Mots-clés :* perte de sommeil, exercice contre résistance, autophagie, protéasome, atrophie musculaire.

## Introduction

Skeletal muscle is one of the largest tissues, comprising ~40% of total body mass. The main function of skeletal muscle is movement, but it also plays an important role in storing amino acids and is an important site of postprandial glucose uptake. Thus, the maintenance of skeletal muscle mass is vital for optimal health. For example, the mass of skeletal muscle has been used as a predictor of functionality and quality of life in cachectic syndromes in cancer, chronic obstructive pulmonary disease, and chronic heart disease (Li et al. 2008). Moreover, muscle mass is positively

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correlated with resting metabolic rate (Wang et al. 2000) and inversely related to adiposity (Dâmaso et al. 2013). Muscle mass is determined by the balance between protein synthetic and degradative pathways (Sandri 2008). Several factors can influence these pathways, such as nutritional status (Mizushima et al. 2004), mechanical stimuli (Luo et al. 2013), and hormonal profile (Sandri 2011). More recently, sleep has also been highlighted as an important factor that regulates muscle trophism (Dattilo et al. 2011).

Sleep is an important determinant for the homeostasis of several physiological systems. Sleep deprivation (SD) is a recognized risk factor for metabolic syndromes, cardiovascular diseases (Chaput et al. 2013), cancer (Haus and Smolensky 2013), diabetes mellitus, and obesity (Van Cauter et al. 2008). SD increases plasma corticosterone levels and chronically reduces the levels of anabolic hormones such as testosterone (Dattilo et al. 2012), growth hormone, and insulin-like growth factor 1 (IGF-1) (Everson and Crowley 2004). These hormonal alterations could interfere with the activation of the phosphatidylinositol-3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) signaling pathway, the ubiquitin-proteasome system (UPS), and the autophagy-lysosomal system (ALS) (Bonaldo and Sandri 2013). The PI3K/Akt/mTOR pathway is the major synthetic pathway of myofibrillar proteins; it is activated by IGF-1, testosterone, and mechanical stimuli, among other signals. UPS is a protein complex involved in protein degradation, and its increased activity can produce muscle atrophy. ALS effects the degradation of cellular content (protein aggregates, organelles, and glycogen) in lysosomes through the process of autophagy, which is the sequestration of material to be degraded by a double-membrane vesicle, the autophagosome. An increase in ALS activity can induce muscle atrophy, but suppression of ALS activity below basal levels has the same effect owing to the accumulation of autophagosomes in the cytoplasm, which is lethal to the cell (Sandri 2011). As a consequence, SD may disturb the balance between protein synthesis and degradation, favoring catabolism (Mônico-Neto et al. 2013). In fact, SD for 96 h induces muscle atrophy in adult rats, but the underlying mechanisms are unknown (Dattilo et al. 2012).

Based on the hormonal environment that accompanies SD, it is possible that a potent countermeasure would be high-intensity resistance training (RT) (Mônico-Neto et al. 2013). RT is able to modulate muscle autophagy (Luo et al. 2013), generating a greater protein turnover and ensuring a more efficient utilization and production of energy. Thus, when performing RT regularly, the muscle has a greater capacity to store and mobilize energy substrate, and these effects could antagonize the catabolic environment generated by SD and minimize the deleterious atrophic effects on skeletal muscle (Mônico-Neto et al. 2013).

The present study had 2 objectives. The first objective was to ascertain whether RT prior to SD would be an effective intervention to counter the atrophic muscle loss observed in rats. The second objective was to characterize the changes in contents of protein components of UPS and ALS during SD in rats. We hypothesized that rats undergoing SD would lose muscle mass but that RT would attenuate the loss of muscle mass, and that the molecular changes associated with the preservation of lean mass would involve activation of protein synthesis and suppression of proteolytic pathways.

## Materials and methods

### Animals

Conventional male Wistar EPM-1 rats that were 75 days old, weighed 300–390 g, and were from the Center for Development of Models for Medicine and Biology (Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia) of Federal University of São Paulo (UNIFESP) were used. The animals were maintained on a 12-h light/dark cycle (light starting at 0700) with controlled temperature (22 ± 1 °C). Animals received water and food ad libitum. The NUVILAB CR-1 feed had the following composition: humidity 12.5%, crude protein 22%, ether extract 4%, mineral material 9%, crude fiber 7%, calcium 1.4%, phosphorus 0.8%, vitamins (A, D3, E, K3, B1, B2, B6, B12), niacin, pantothenic acid, folic acid, biotin, choline, minerals (sodium, iron, manganese, zinc, copper, iodine, selenium, cobalt fluoride), and the amino acids lysine (12 g/kg) and methionine (4 g/kg). All experimental procedures complied with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, NIH Publication No. 85-23, 2011 revision) and Canadian Council on Animal Care guidelines. The study was approved by the ethics committee of UNIFESP/São Paulo Hospital (#0764/10). A total of 50 animals were allocated into 5 groups: control (CTRL, $n$ = 10), SD ($n$ = 10), RT ($n$ = 10), SHAM (trained on the RT apparatus but without load; $n$ = 10), and RT plus SD (RT+SD, $n$ = 10).

Following the experimental protocols, the animals were euthanized by decapitation, blood samples were collected and immediately processed for hormone analysis, and the plantaris muscle of the right hind leg was dissected for molecular and histological analyses.

### Resistance training protocol

To investigate how resistance training can interfere with the negative effects of sleep deprivation, we submitted animals from the RT and RT+SD groups to training for 8 weeks. The training was performed with a previously described experimental setup (Yarasheski et al. 1990) with some modifications. In brief, the apparatus consisted of stairs 110 cm high and 18 cm wide, with 2 cm between steps, placed at an 80° angle. The animals had to climb the stairs carrying a cylinder that was fixed to the base of the tail by self-fusing tape and was also connected to wires to increase the cylinder's load as training progressed. The animals were allowed to familiarize themselves with the stairs for 3 consecutive days and then tested for their maximum load (ML). Both the familiarization period and the ML test followed the protocol described by Shiguemoto and colleagues (Shiguemoto et al. 2012). Every Monday throughout the training period, the ML was reassessed and the load was adjusted.

During the training sessions, the animals had to climb the stairs 4–8 times while carrying a load, with 60-s intervals between climbs. Initially, animals were loaded with 50% of their ML, and this load was gradually increased to 75%, 95%, and 100% of the ML on climbing attempts 2, 3, and 4, respectively. In subsequent climbing attempts, the load was increased by 30 g at each attempt until failure. The sessions were conducted 5 times per week (Monday–Friday). Animals took 8–12 steps per leg to climb from the base to the top of the stairs. Because of the high loads applied, prophylactic rest was introduced on weeks 6, 7, and 8; during these weeks, the animals trained on Monday, Tuesday, Thursday, and Friday and rested on Wednesday. The animals' body mass was measured on a weekly basis throughout the training period. The SHAM group performed the training protocol with no load other than their body weight. Forty-eight hours after the last training session, the RT and SHAM groups were euthanized, and the RT+SD and SD groups were subjected to SD as described below.

### Sleep deprivation

SD was imposed using a modification of the multiple-platform method (Suchecki and Tufik 2000), in which 5 (socially stable) animals are placed on 8 round platforms (6.5 cm diameter) 10 cm apart and located in a stainless steel reservoir (123 cm long, 44 cm wide, and 44 cm high). The reservoir was filled with water up to 1 cm below the surface of the platforms, and the animals were forced to remain on the platforms for the duration of the experiment. In this setup, whenever the animals entered the paradoxical sleep phase (a specific phase of sleep characterized by the presence of muscle atonia, similar to REM sleep in humans), they would make contact with the water owing to the muscle atonia

and wake up. The animals were subjected to SD for 96 continuous hours. Before the actual SD protocol, the animals were allowed to adapt to the deprivation reservoir for 1 h on each of 3 consecutive days. The animals' body weight was measured every 24 h throughout the SD period. Previous studies have shown that the modified multiple-platform method completely inhibits paradoxical sleep and promotes a 37% decrease in slow-wave sleep (a specific phase of non-REM sleep) (Machado et al. 2004).

### Histological analysis

Following dissection of the plantaris muscle, the tissue was dried for a few seconds on filter paper and then weighed using a precision scale. The tibia of the same leg was extracted, and its length was measured using a caliper (plateau to plateau) to normalize the data among animals. The distal fragment of the plantaris muscle was embedded in a mass made of powdered milk and an optimal cutting temperature compound (Tissue-Tek, Sakura, Japan) and oriented for obtaining cross-sectional cuts. Serial 8-μm sections were obtained using a cryostat (CM1850, Leica Microsystems, Cambridge, UK) at –22 °C; then, the sections were placed on silanized glass slides, submerged in acetone, and left to dry at room temperature for 10 min.

For morphological analysis, the samples were subjected to hematoxylin–eosin staining and analyzed using a BX50 bright-field microscope and DP71 camera (Olympus, Melville, N.Y., USA) under 40× magnification. Muscle fiber cross-sectional area (CSA) was analyzed in a blind manner, and 300 fibers per muscle were measured using AxioVision 4.6 software (Carl Zeiss MicroImaging GmbH). This variable was assessed in 5 animals randomly chosen from each group. Both muscle weight and CSA were normalized to the tibia length using the following equation:

$$\text{Trophism or weight} = \frac{\text{g or } \mu\text{m}^2}{\text{tibia length (cm)}} \times 1000$$

### Western blotting

The muscles (6 animals per group, randomly selected) were homogenized in a lysis buffer (100 mmol/L Tris, 150 mmol/L NaCl, 10 mmol/L EDTA (ethylenediaminetetraacetic acid), 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS (sodium dodecyl sulfate), 10% glycerol, pH 8.0) containing Complete Protease Inhibitor Cocktail Tablets (Roche, Penzberg, Germany) and PhosSTOP Phosphatase Inhibitor Cocktail Tablets (Roche) (10% of the tissue weight/volume). The homogenized tissue was incubated on ice for 10 min and centrifuged at 7000*g* for 5 min; then, the supernatant was collected and the pellet was discarded. The protein content of the supernatant was measured using BCA Protein Assay Reagent (Thermo Scientific Pierce Protein Biology). Aliquots (50 μg) from each sample were subjected to SDS-PAGE and transferred to a polyvinylidene fluoride membrane, which was incubated in a blocking solution (5% bovine serum albumin in Tris-buffered saline containing Tween 20 (TBS-T: 50 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, and 0.1% Tween 20)) for 60 min at room temperature. The membrane was then incubated with primary antibodies diluted in blocking solution for 2 h at room temperature and then rinsed 3 times (5 min each) with TBS-T. A secondary antibody conjugated to peroxidase and a substrate (SuperSignal West Pico, Thermo Scientific Pierce Substrates) were used to detect the bands of interest. A digital image of the membrane was acquired using a photo documentation device (Alliance Mini (4 megapixels), UVItec, Cambridge, UK), and the band intensity was measured using commercial software (UVIband, UVItec).

The antibodies were obtained from Cell Signaling Technology and were used at the following dilutions: Phospho-p70 S6 kinase (Thr389), 1:200; p70 S6 kinase (100), 1:200; Phospho-mTOR (Ser2448), 1:200; mTOR, 1:200; LC3, 1:1000; p62/SQSTM1, 1:1000; Phospho-FoxO3a (Ser253), 1:200; FoxO3a, 1:200; and anti-ubiquitin, 1:1000. The proteins were measured and normalized based on the amount of GAPDH (Cell Signaling Technology, 1:1000) or the phosphorylated protein/total protein ratio.

### Proteasome activity assay

The chymotrypsin-like activity of proteasome 20S of the plantaris muscle was assessed using a fluorometric assay based on the fluorogenic substrate *N*-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Suc-LLVY-AMC; Enzo Life Sciences item #P802-0005), as previously described (Cunha et al. 2012). Protein (50 μg) from each sample was incubated in 200 μL of 10 mmol/L 3-(*N*-morpholino)propanesulfonic acid, pH 7.5, containing 25 μmol/L Suc-LLVY-AMC as well as 25 μmol/L ATP and 5 mmol/L $Mg^{2+}$, which function as peptidase cofactors. When the substrate is degraded, amido-4-methylcoumarin is released and detected by a fluorometer (360 nm excitation and 460 nm emission). Peptidase activity was measured in the presence and absence of the specific proteasome inhibitor epoxomicin, and the difference between conditions was attributed to the proteasome.

### Blood assays

Blood samples were collected into EDTA-containing and additive-free tubes (Vacutainer, BD), and the samples were centrifuged to separate plasma and serum (respectively). The samples were stored at −80 °C until the time of analysis. The intra-assay coefficient of variation of the various tests is indicated in parentheses. The total testosterone concentration (7.7%) was measured with a chemiluminescent immunometric assay (UniCel DxI 800, Beckman Coulter). The corticosterone concentration (7.1%) was measured by radioimmunoassay using a commercial kit (MP Biomedicals), and IGF-1 was measured with an ELISA (R&D Systems) according to the manufacturer's instructions.

### Statistical analysis

Following the Shapiro–Wilk test for normality, the groups were compared using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test whenever a significant difference was found. In the case of measurements performed at various times, repeated-measures ANOVA was used, followed by Scheffé's post hoc test. Maximum loads were compared by *t* test, and correlation analyses were performed using Pearson's test. All data are expressed as the mean ± standard deviation, and significance was established as $p \leq 0.05$.

## Results

### Body mass, resistance training, and muscle trophism

The animals submitted to RT presented with an increase of 227% of maximum load in the eighth week compared with the first week (week 1, 352 ± 50 g; week 8, 1139 ± 127 g; $t = -31.97$, $p = 0.00$). During the training period, the body weight gain was higher in the groups that remained sedentary (CTRL and SD groups) compared with the trained groups (RT and RT+SD groups) ($F_{[2,45]} = 4.8141$, $p < 0.01$; Table 1). Forty-eight hours after the last training session, the SD and RT+SD groups were submitted to SD, which caused a reduction of body mass in both groups; however, the weight reduction exhibited by the RT+SD group was significantly less than that observed in the SD group ($F_{[2,27]} = 116.35$, $p < 0.01$; Table 1).

Resistance training increased muscle weight and muscle CSA, while SD resulted in a reduction in these variables, as expected. Animals submitted to resistance training prior to sleep deprivation (RT+SD) showed muscle weight and muscle CSA comparable to those in CTRL and SHAM ($F_{[4,20]} = 12.115$, $p > 0.2$; effect size $f = 1.93$; power = 0.99); however, the RT+SD group showed lower values than the RT group ($p = 0.02$) and higher values than the SD group ($p < 0.01$). The magnitude of muscle loss in the RT+SD group compared with the RT group was ~10% ($F_{[4,20]} = 12.115$, $p < 0.01$), nearly identical to the reductions in the SD group compared with the CTRL and SHAM groups (Figs. 1A and 1B).

**Table 1.** Changes in body mass during 8 weeks of resistance training and 96 h of sleep deprivation.

| | Body mass (g) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Resistance training period | | | | | | | | | | Sleep deprivation period | | |
| | Basal | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Δ% | Initial | Final | Δ% |
| CTRL | 353±28 | 364±27 | 376±29 | 384±27 | 398±28* | 405±29* | 415±32* | 425±35* | 429±38* | 23±4 | 429±38 | 440±40† | 2.48±1.18 |
| SHAM | 333±28 | 352±33 | 366±38 | 374±40 | 381±40* | 386±38* | 392±40* | 396±39* | 400±39* | 20±4 | — | — | — |
| RT | 349±39 | 374±48 | 384±55 | 389±55 | 394±55* | 399±55* | 409±57* | 411±51* | 410±46* | 17±4[a,b] | — | — | — |
| SD | 353±26 | 372±34 | 380±38 | 386±38 | 398±40* | 408±39* | 418±39* | 424±33* | 425±30* | 21±2 | 425±30[a] | 385±30[a,†] | −9.31±2.45[a] |
| RT+SD | 344±28 | 372±36 | 375±37 | 384±41 | 393±40* | 402±43* | 405±45* | 407±46* | 406±46* | 15±4[a,b,c] | 394±40[a] | 368±42[a,†] | −6.76±1.63[a,b] |

**Note:** CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. During the training period, the SD and CTRL groups remained in their home box and received no intervention. Δ value: weight variation (percentage from basal value). Data are expressed as the mean ± 2 standard deviations.

[a]Different from CTRL.
[b]Different from SD.
[c]Different from SHAM.
*Different from basal.
†Different from initial.

**Fig. 1.** (A) Ratio of muscle weight (g) to tibia length (cm) × 1000. (B) Ratio of cross-sectional area of the plantaris muscle (μm²) to tibia length (cm) × 1000. Data are expressed as the mean ± standard deviation. CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. [a]Different from CTRL, [b]different from SHAM, [c]different from SD, and [d]different from RT+SD.

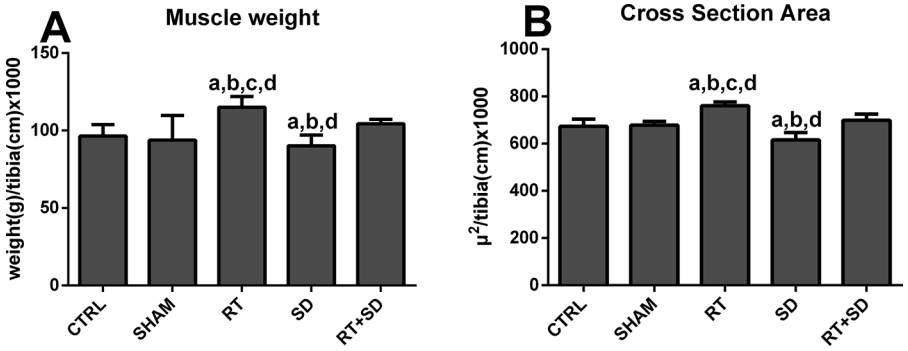
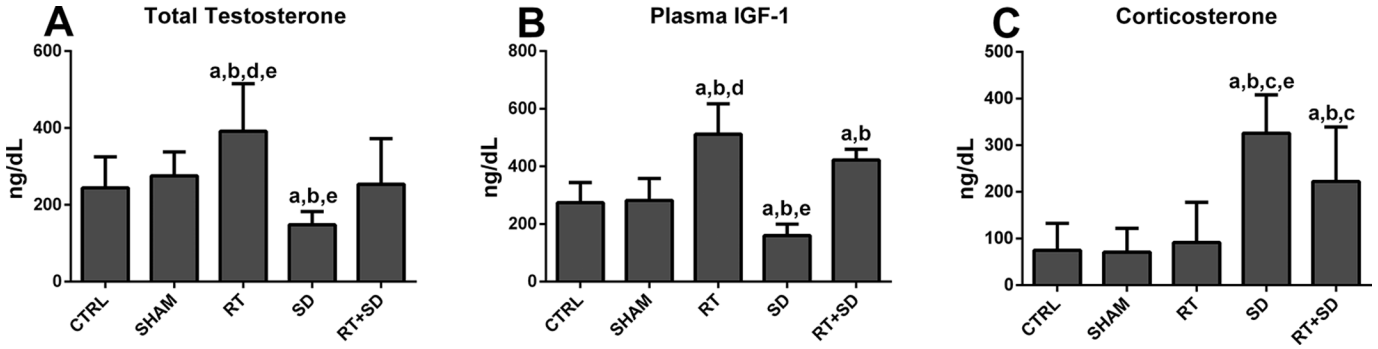



**Fig. 2.** (A) Serum testosterone levels, (B) plasma IGF-1 levels, and (C) plasma corticosterone levels. Data are expressed as the mean ± standard deviation. CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. [a]Different from CTRL, [b]different from SHAM, [c]different from RT, [d]different from SD, and [e]different from RT+SD.




## Hormonal responses

Total testosterone level was increased in the RT group and decreased in the SD group compared with CTRL. The RT+SD group showed a testosterone level similar to the control level and reduced values compared with the RT group ($F_{[4,45]} = 9.3744$, $p < 0.01$; Fig. 2A). Similarly, the level of IGF-1 was higher in the RT group and lower in the SD group compared with CTRL ($p < 0.001$), but the RT+SD group showed the same level of IGF-1 when compared with the RT group ($F_{[4,45]} = 16.286$, $p < 0.01$; Fig. 2B). These data demonstrate that RT was protective of the maintenance of IGF-1 levels against SD and minimized the reduction of testosterone. The plasma corticosterone level was increased in the SD and RT+SD groups compared with other groups. However, the augmentation in the RT+SD group was lower than that in the SD group ($F_{[4,45]} = 18.882$, $p < 0.01$; Fig. 2C), indicating that RT minimized the effect of sleep loss. No differences among the CTRL, SHAM, and RT groups were observed in relation to corticosterone.

## Anabolic signaling

mTOR$^{(Ser2448)}$ activity was increased in the RT+SD group compared with other groups ($F_{[4,25]} = 4.8868$, $p < 0.01$; Fig. 3A). Phosphorylated p70S6K$^{(Thr389)}$ did not change in the SHAM, RT, and SD groups, but the RT+SD group showed high values compared with these groups ($F_{[4,25]} = 2.9013$, $p < 0.04$; Fig. 3B).

**Fig. 3.** (A) Quantification of mTOR and (B) p70S6K. Data are expressed as the mean ± standard deviation. CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. [a]Different from CTRL, [b]different from SHAM, [c]different from RT, [d]different from SD, and [e]different from RT+SD.

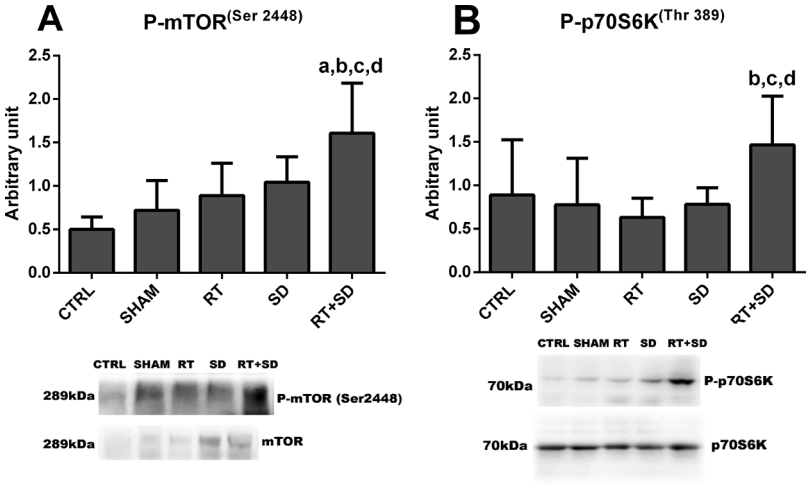
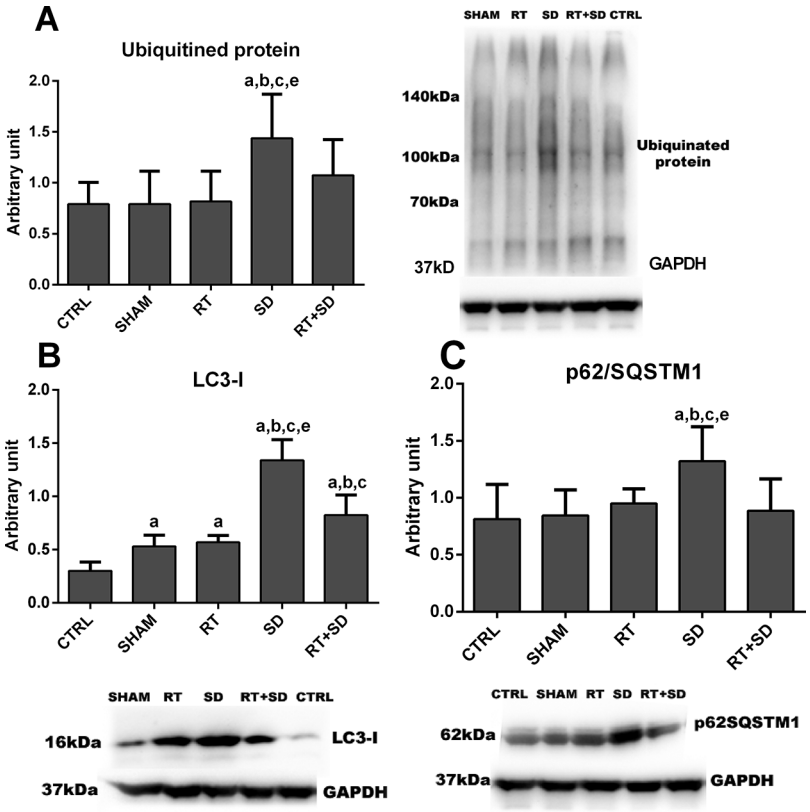



**Fig. 4.** (A) Representation of the ubiquitinated protein level, (B) protein expression of LC3, and (C) protein expression of p62/SQSTM1. Data are expressed as the mean ± standard deviation. CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. [a]Different from CTRL, [b]different from SHAM, [c]different from RT, [d]different from SD, and [e]different from RT+SD.

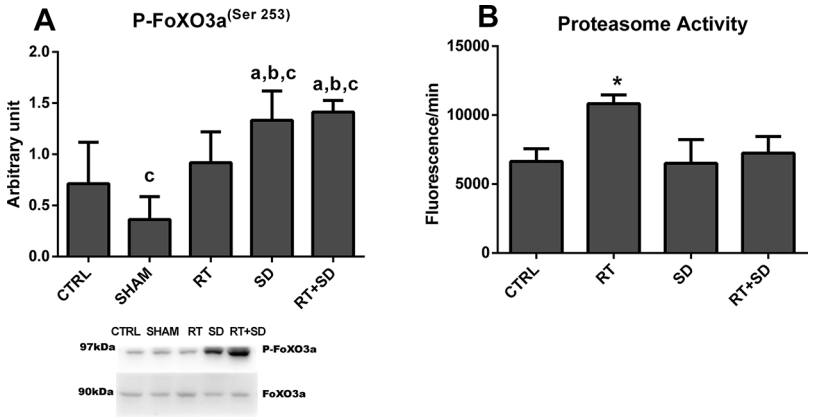



## Catabolic signaling

Animals of the SD group had increased levels of ubiquitinated proteins compared with other groups ($F_{[4,25]}$ = 3.5294, $p$ = 0.02; Fig. 4A). LC3 increased in all groups compared with CTRL; however, a more prominent increase was observed in the SD group ($F_{[4,25]}$ = 49.298, $p$ < 0.01; Fig. 4B). Protein sequestosome-1 (p62/SQSTM1) was increased in the SD group compared with the other groups ($F_{[4,25]}$ = 3.8769, $p$ < 0.01; Fig. 4C). These findings indicate that SD impairs autophagy, but resistance training prior to SD attenuates this effect.

FoxO3a is a transcription factor that regulates the transcription of LC3 and ubiquitin ligase genes. Moreover, the activity of FoxO3a is modulated by corticosterone (Schakman et al. 2013); thus, we evaluated the phosphorylation status of FoxO3a. As shown in Fig. 5, phosphorylated FoxO3a (inactive form) was increased in the SD and RT+SD groups compared with the CTRL, SHAM, and RT groups

**Fig. 5.** (A) Quantification of FoxO3a and (B) proteasome activity. Data are expressed as the mean ± standard deviation. CTRL, control; SHAM, resistance trained with no load; RT, resistance trained; SD, sleep deprived. [a]Different from CTRL, [b]different from SHAM, [c]different from RT, and *different from all groups.




($F_{[4,17]}$ = 10.668, $p$ < 0.01; Fig. 5A). Although the amount of ubiquitinated protein was increased in the SD group, higher proteasome activity was observed only in the RT group compared with the other groups ($F_{[3,16]}$ = 14.513, $p$ < 0.01; Fig. 5B).

### Correlation analysis

Positive correlations of corticosterone with LC3 ($r$ = 0.60, $p$ = 0.002), p62/SQSTM1 ($r$ = 0.53, $p$ = 0.008), and ubiquitinated protein ($r$ = 0.43, $p$ = 0.034) were found.

## Discussion

We have shown that SD induced reductions in muscle weight and CSA. The muscle atrophy observed in this study confirms our previous data obtained in another glycolytic muscle (tibialis anterior) (Dattilo et al. 2012). Resistance training before SD proved to act as a buffer against SD-induced muscle atrophy, because the muscle CSA of the RT+SD group was not different from that of the CTRL and SHAM groups (Figs. 1A and 1B).

The increase of glucocorticoids stimulates muscle autophagy, and the degradation products are recycled as metabolic substrates (Bonaldo and Sandri 2013; Vainshtein et al. 2014). In this study, the amounts of the ubiquitinated proteins LC3 and p62/SQSTM1 were increased in the SD group. In blot analysis, the lipidated form of LC3 (LC3-II) was not detected, suggesting that there was reduced conversion of LC3-I to LC3-II, which is required for the formation of autophagosomes. To confirm this theory, we analyzed the levels of p62/SQSTM1, a selective autophagy substrate capable of binding ubiquitin to LC3-II, and found an increased level in the SD group. Reduced levels of p62/SQSTM1 are associated with increased autophagy, while accumulation of this protein is a good marker of suppression of autophagy (Klionsky et al. 2008; Mizushima and Yoshimori 2007; Nakai et al. 2007; Wang et al. 2006). Together, these results suggest that muscle atrophy induced by SD was caused by the suppression of autophagy.

Interestingly, the RT+SD group showed no increase in p62/SQSTM1 and ubiquitinated protein, in addition to a minimized increase in LC3 and corticosterone levels, indicating that RT was protective against catabolic signal. As expected, the molecular behavior was sufficient to contain the muscle mass loss, and the decreased body weight reduction in the RT+SD group suggests a beneficial effect of RT for the whole organism. SD did not influence proteasome activity, because only the RT group showed increased activity of the proteasome, which is likely indicative of increased protein turnover in that group (Baehr et al. 2014).

One of the pathways that can mediate the induction of expression of LC3 and ubiquitin ligase is the activation of transcription factor FoxO3a; this pathway is under the influence of corticosterone (Schakman et al. 2013). Although corticosterone varied among groups similarly to the expression level of LC3 and the amount of ubiquitinated protein (as demonstrated by the correlation analysis), the activity of Foxo3a did not covary proportionately. Thus, it is possible that corticosterone contributes to the increase of autophagic markers but not through the activation of gene transcription mediated by Foxo3a. We speculate that reduction of the circulating concentration of testosterone by SD may have resulted in a synergistic increase in the activity of corticosterone because these hormones compete for the same receptor; besides, corticosterone induces apoptosis in Leydig cells, which produce testosterone (Mayer and Rosen 1977). The result would be a net increase in catabolic activity.

An objective of this study was to verify the efficacy of RT in minimizing deleterious effects of SD. The increases of testosterone and IGF-1 and the muscle hypertrophy observed in the RT group demonstrated that training was effective. Moreover, training performed before SD inhibited the IGF-1 alterations and minimized the decrease in testosterone induced by SD. The RT+SD group also presented levels of p62/SQSTM1 and ubiquitinated proteins comparable to those in the CTRL group and lower levels of LC3 when compared with the SD group, suggesting that resistance training was capable of controlling the shift toward catabolic pathways induced by SD. It is noteworthy that the RT+SD group showed increases in phosphorylated mTOR and p70S6K compared with the other groups, suggesting that trained rats maintained an increased capacity for protein synthesis during the subsequent SD compared with the RT and SD groups. Long-term adaptation to RT allows the organism to react differently when exposed to catabolic conditions. Previous studies have shown that in a catabolic condition (such as muscle denervation), there is increased synthetic activity, observed as phosphorylation of mTORC1. It is known that mTORC1 is able to "sense" amino acids present in the lysosomal lumen and is recruited through the Rag GTPase complex, increasing protein synthesis; however, blocking this mechanism increases muscle loss (Quy et al. 2013; Sandri 2013; Zoncu et al. 2011). It is possible that this could be occurring in our model, explaining the rise in the phosphorylation of mTOR and p70S6K and suggesting maintenance of muscle autophagy, which would provide more amino acids as substrate, in the RT+SD group. The accumulation of autophagy markers observed in the SD group, associated with a possible reduction of the amount of amino acids generated through this pathway, could explain the unchanged mTOR and p70S6K phosphorylation. Conversely, chronic RT decreases the phosphorylation of mTOR (Luo et al. 2013), and the acute effect of RT in muscle endures for 24–48 h (Karagounis et al. 2010). In the present study, muscles were analyzed 48 h after the

last RT session, which could explain the absence of changes in mTOR and p70S6K phosphorylation in the RT group.

RT was able to minimize the magnitude of muscle loss in animals submitted to SD. We believe that the high phosphorylation rates of mTOR and p70S6K and the low levels of p62/SQSTM1, ubiquitinated protein, and corticosterone in the RT+SD group, associated with less body mass loss, suggest that RT had a beneficial systemic effect. This may be due to more effective substrate utilization and better assimilation of stress induced by SD. Furthermore, RT prevented the testosterone level from falling below the baseline (as in the SD group), which could reduce a heightened catabolic activity.

In response to stress induced by SD, high activity of the hypothalamic–pituitary–adrenal (HPA) axis is observed. Previous studies have shown that the loss of body weight varies together with the activity of the HPA axis, being higher at the beginning of SD and lower once the animals acclimate to the stress (Galvão et al. 2009; Hipolide et al. 2006; Martins et al. 2008, 2010). Tiba et al. (2008) inhibited the synthesis of corticosterone in animals submitted to SD and did not observe a reduction in body weight. In the present study, the body weight loss and corticosterone levels were minimized in the RT+SD group. Thus, HPA axis activity may be a primary driver of muscle atrophy and weight loss during SD in rats, and RT can minimize the stress induced by SD. In a study by Vollert and colleagues, endurance exercise performed for 4 weeks before 24 h of SD in rats prevented an increase of corticosterone (Vollert et al. 2011). This study suggests better assimilation of stress induced by SD in trained rats.

The SD protocol we used is thought to result in stressful stimuli beyond SD alone, such as changes in the environment, humidity, noise, water contact, and others (Horne and McGrath 1984; Siegel 2001; Tiba et al. 2008; Vertes and Eastman 2000). These additional stressors may potentiate the effects of SD, and thus the effects of SD may be overestimated in this model compared with other SD models. In the present study, SD was induced by a modified multiple-platform method that does not include the stress of social isolation or social instability, as present in other models, and the 3-day adaptation period before SD results in the animals having a smaller increase in corticosterone (Suchecki and Tufik 2000). Despite the methodological rigor, it is impossible to eliminate all stressors in addition to SD. Sleep loss per se represents a psychological (Palma et al. 2000) and physiological stress (Mônico-Neto et al. 2015); thus, this is a limitation of the study with our particular SD animal model.

In summary, our data show that SD induces muscle atrophy by increasing the catabolic signal, which can be driven by an increase in corticosterone. RT (8 weeks) before SD (96 h) in rodents increased muscle protein synthetic activity (i.e., mTOR$^{(Ser2448)}$ and p70S6K$^{(Thr389)}$), reduced the catabolic signal, contained plantaris muscle mass loss, and reduced body weight losses. Thus, RT could be used to reduce the losses of body and muscle mass due to sleep deprivation.

## Conflict of interest statement

The authors have no conflicts of interest to disclose.

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