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Low-intensity resistance training attenuates dexamethasone-induced atrophy in the flexor hallucis longus muscle

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ABSTRACT

This study investigated the potential protective effect of low-intensity resistance training (RT) against dexamethasone (DEX) treatment induced muscle atrophy. Rats underwent either an 8 week period of ladder climbing RT or remained sedentary. During the last 10 days of the exercise protocol, animals were submitted to a DEX treatment or a control saline injection. Muscle weights were assessed and levels of AKT, mTOR, FOXO3a, Atrogin-1 and MuRF-1 proteins were analyzed in flexor hallucis longus (FHL), tibialis anterior (TA), and soleus muscles. DEX induced blood glucose increase (+46%), body weight reduction (−19%) and atrophy in FHL (−28%) and TA (−21%) muscles, which was associated with a decrease in AKT and an increase in MuRF-1 proteins levels. Low-intensity RT prevented the blood glucose increase, attenuated the FHL atrophy effects of DEX, and was associated with increased mTOR and reductions in Atrogin-1 and MuRF-1 in FHL. In contrast, TA muscle atrophy and signaling proteins were not affected by RT. These are the first data to demonstrate that low-intensity ladder-climbing RT specifically mitigates the FHL atrophy, which is the main muscle recruited during the training activity, while not preventing atrophy in other limb muscle not as heavily recruited. The recruitment-dependent prevention of atrophy by low intensity RT likely occurs by a combination of attenuated muscle protein degradation signals and enhanced muscle protein synthesis signals including mTOR, Atrogin-1 and MuRF-1.

1. Introduction

Dexamethasone (DEX) is used as an anti-inflammatory and anti-allergic drug, however, high doses or chronic use may lead to several side effects including hyperglycemia, hypertension, dyslipidemia and cachexia [1–4]. With respect to skeletal muscle, some studies have shown that chronic treatment with DEX promotes muscle atrophy [2–6] which, in some circumstances, is also associated with body weight loss [6–8]. As reviewed by Egerman and Glass [9], normal maintenance of muscle mass occurs *via* a tight control of signaling processes involved in both synthesis (including IGF-1, insulin growth factor-1; PI3k, phosphoinositide 3 kinase; AKT/PKB, protein kinase B; mTOR, protein kinase mammalian target of rapamycin and P70S6K, ribosomal protein S6 kinase) and degradation (including FOXO3a, forkhead box 3A; MAFbx, muscle atrophy Fbox (also named Atrogin-1), and MuRF-1, muscle ring finger-1) of muscle proteins. Atrogin-1 and MuRF-1 were the first proteins considered crucial for regulation of muscle atrophy [10]. Although skeletal muscle atrophy induced by DEX has been shown to be associated with both increased catabolism [6,7] and reduced

Abbreviations: AKT/PKB, protein kinase B; AMPK, adenosine monophosphate kinase; BSA, bovine serum albumin; CaMKK, calcium/calmodulin-dependent protein kinase; CEUA, Committee for Ethical Use of Animals; DEX, dexamethasone; EDTA, ethylenediaminetetraacetic acid; FHL, flexor hallucis longus; FOXO3a, forkhead box 3A; GLUT-4, glucose transporter type 4; IGF-1, insulin growth factor-1; *i.p.*, intraperitoneal injection; MHC, miosin high chain; mTOR, protein kinase mammalian target of rapamycin; MuRF-1, muscle ring finger-1; MVCC, maximum voluntary carrying capacity; NaCl, sodium chloride; PI3k, phosphoinositide 3 kinase; PMSF, phenylmethylsulfonyl fluoride; P70S6K – 70 kDa, ribossonal protein S6 kinase; RIPA, radioimmunoprecipitation assay; RT, resistance training; S, sedentary; SC, sedentary control; SD, sedentary treated with DEX; SE, standard error; SOL, soleus; T, training; TBST, tris-buffered saline with Tween; TC, resistance training; TD, resistance training treated with DEX; Tris–HCl, Tris hydrochloride.

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synthesis of muscle proteins [5,9], the possible alterations in molecular signaling mechanisms causing this are not fully understood.

Low intensity physical exercise has been used as a non-pharmacological intervention to control several side effects of DEX including diabetes, hypertension and sarcopenia [11,12,13]. Pinheiro et al. [1] have shown that endurance exercise is effective in preventing alterations in lipid metabolism induced by DEX. Similarly, Barel et al. [2] demonstrated that 8 weeks of treadmill endurance training attenuated DEX-induced increases in blood glucose, but was not able to prevent muscle atrophy induced by DEX. In contrast, other studies demonstrate that aerobic continuous exercise has been shown to attenuate [14], or prevent [15], the muscle mass reduction induced by glucocorticoids. It is important to investigate different types of exercise in order to better understand whether exercise can prevent muscle mass reductions induced by DEX, and to identify mechanisms that might be involved. Resistance exercise training has been shown to be efficient in the attenuation of muscle mass reduction in some pathologies [4,14,15]. However, the role of resistance training in preventing muscle atrophy induced by DEX is not completely understood. The purpose of the current study is to examine the hypothesis that low intensity resistance training will protect against DEX-induced muscle atrophy and the underlying molecular mechanisms responsible for this effect, by attenuation of signals associated with muscle protein degradation and elevation of signals associated with muscle protein synthesis. These responses will be specific to the flexor hallucis longus (FHL) muscle that is heavily recruited during the training protocol used in this study, and will not occur in other limb muscles that are not as heavily recruited during this type of training [16]. To our knowledge, this is the first study to investigate the effects of this type of low intensity resistance training on FHL muscle atrophy induced by DEX.

2. Materials and methods

2.1. Animals

All methods used were approved by the Committee for Ethical Use of Animals (CEUA) of the UNESP-São Paulo State University, Araçatuba (protocol # 2012-02253). Forty-four male rats (250–300 g) were housed in group cages and maintained under controlled environmental conditions (12 h dark–light cycle, 22 °C of temperature) with *ad libitum* access to standard diet (Biobase, Brazil) and water. Body weight was measured weekly during the exercise protocol and daily during DEX treatment.

2.2. Maximum voluntary carrying capacity (MVCC)

Initially, the animals were adapted to the climbing apparatus which included a vertical ladder (110 cm, 80° incline) and a tail weight attachment as previously described by Hornberger and Farrar [16], Prestes et al. [17] and Sanches et al. [18]. Briefly, after a 10 day familiarization period, each rat performed a test in order to evaluate its maximum voluntary carrying capacity (MVCC). In this test, each rat started to climb the ladder carrying 75% of its own body weight and was allowed to rest at the top of the ladder for 120 s. After each completed climb 30 more grams were added to the total carried mass. This procedure was successively repeated until the rat failed to climb the entire length of the ladder on three consecutive attempts; MVCC was defined as the highest load successfully carried in this protocol.

2.3. Blood glucose determination

After overnight fasting (12 h), animals were gently restrained, tail blood samples were obtained from tail nicks, and blood glucose levels were determined using a digital glucometer system (One Touch Ultra – Johnsons & Johnsons). This procedure was performed for each animal on three occasions during the protocol: (1) at the beginning of the sedentary/exercise protocol; (2) before DEX/saline administration, and (3) just prior to euthanasia. For occasion 2, the blood sample was obtained 24 h after the last training session.

2.4. Experimental treatment protocol

After preliminary assessments were completed, rats were allocated into four experimental groups balanced to ensure equal initial body weight, fasting blood glucose and MVCC across groups: (1) sedentary control (SC); (2) sedentary treated with DEX (SD); (3) resistance trained control (TC) and (4) resistance trained treated with DEX (TD). DEX-treated rats received daily injections (at 9 a.m.) of DEX (Decadron®, 0.5 mg/kg of body weight, *i.p.*, dissolved in saline) during the last 10 days of the entire experimental protocol (8 weeks + 10 days). Control animals received daily injections of saline during the last 10 days of the experimental protocol (same volume as DEX treated rats). In the trained group, resistance training continued throughout the DEX or saline administration period.

Trained groups performed 8 weeks of ladder resistance training at 60% of MVCC, 5 days a week [19]. Each training session consisted of 14–20 ladder climbs. The maximal voluntary carrying test was repeated after 4 weeks (MVCC-2) and after 8 weeks (MVCC-3) of training. An additional MVCC (MVCC-4) was carried out after the 10 day DEX (or saline) treatment period. Sedentary rats also performed MVCC tests at the same time intervals as indicated for the trained animals, but otherwise remained in their cages and did not undergo the training protocol.

2.5. Tissue harvesting

After euthanasia by excess of anesthesia (Ketamine 160 mg/kg and Xylazine 20 mg/kg), entire FHL, tibialis anterior (TA), and soleus muscles were removed, weighed, immediately frozen in dry ice and then stored at −80 °C until used for Western blotting analysis. Muscle weights were normalized to tibia bone length since DEX treatment is known to affect body weight. The tibia bone length was measured from the proximal extremity of the lateral condyle up to the posterior process, in the distal extremity. The three muscles selected for study have different fiber type composition. While FHL is composed predominantly of myosin heavy chain 2a/x (MHC IIa/x) with a small percentage of myosin heavy chain 2b (MHC IIb) [16,20], TA muscle is composed mainly of MHCIIb [16,20]. Soleus muscle is composed mainly by slow-fiber type I. It has been previously demonstrated that, of these three limb muscles, FHL muscle is the heavily recruited muscle during the type of ladder-climbing resistance exercise training protocol used in this study [16,20].

2.6. Western blotting procedures

Samples of TA, FHL and SOL were homogenized in RIPA solution (10X, Millipore) containing 0.5 M Tris–HCl, pH 7.4, 1.5 M NaCl, 2.5% deoxycolic acid, 10% NP-40, 10 mM EDTA using a polytron homogenizer. Just prior to homogenization, a protease inhibitor cocktail (Pic, Sigma) and 1% PMSF were added to the samples. Samples were centrifuged at 10,000 × *g* for 5 min and the supernatant was collected and stored in −20 °C freezer for future analysis. Bradford assays were used to determine the protein concentration of the samples (Bio-Rad Kit, Protein Assay Standard II, Hercules, CA) as previously published [21]. Absorbance values were determined using a spectrophotometric plate reader (BMG Labtech, Spectro Star Nano). Western blotting was performed according to previously reported procedures [22]. In summary, samples containing 50–80 μg of protein (depending on the specific target in