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Rheumatoid arthritis-increased gene expressions in muscle atrophy are restored back to control as a response to acute resistance exercise

Estímulo agudo de musculação promove regulação de genes alterados pela atrofia muscular

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ABSTRACT: This study had as objective to analyze the acute effects of resistance exercise (RE) on the mRNA levels of the following genes (MyoD, myogenin, IGF-1, atrogin-1, MuRF-1, and myostatin) in rheumatoid arthritis (experimental arthritis). Therefore, 26 females rats were randomly allocated into four groups, control (CT, n=7), exercise (Ex, n=6), rheumatoid arthritis (RA, n=6) and RA with exercise (RAEx, n=7). Met-BSA was injected into the tibiotarsal joint in the RA and RAEx groups. After 15 days from injection, the animals were submitted to an acute bout of RE and six hours post protocol the animals were euthanized. We evaluated the joint thickness, inflammation score, cross-sectional area (CSA) of gastrocnemius muscle fibers and mRNA expression of the IGF-1, MyoD, myogenin, myostatin, MuRF-1, atrogin-1 and GAPDH. It was observed that the joint thickness and score strongly increased in arthritic rats ($p < 0.001$) while the CSA decreased ($p \leq 0.05$). Increased mRNA levels of IGF-1 (2.0 fold), myostatin (4.5 fold), atrogin-1 (2.5 fold), MyoD (3.7-fold) and myogenin (5 fold) were observed in muscle of arthritic rats. The mRNA expression of myostatin, atrogin-1, MyoD and myogenin decreased in the RAEx group. In this way, we can conclude that experimental arthritis-increased gene expressions in muscle atrophy (myostatin, atrogin-1, MyoD and myogenin) are restored back to control as a response to acute RE.

Key Words: Cachexia; Atrogin-1; Myostatin; MyoD; Myogenin.

RESUMO: O presente estudo teve como objetivo analisar o efeito agudo do Exercício com pesos sobre os níves de mRNA de genes envolvidos no anabolismo ou catabolismo muscular em um modelo experimental de Artrite Reumatóide. Para tanto, 26 ratas fêmeas foram randomicamente alocadas em quatro grupos, controle (CT, n=7), Exercício (Ex, n=6), Artrite Reumatóide (AR, n=6) e Artrite Reumatóide com exercício (AREx, n=7). Uma substância contendo Albumina bovina metilada foi injetada na articulação tíbio-tarsal nos grupos AR e AREx para indução da Artrite Reumatóide. Após 15 dias da injeção, os animais foram submetidos a um estímulo agudo de treinamento com pesos e 6 horas após o exercício os animais foram eutanasiados. Nós avaliamos a espessura da articulação, escore de inflamação, a área de secção transversa (AST) das fibras do músculo Gastrocnêmio e a mRNA de IGF-1, MyoD, Myogenina (genes envolvidos no anabolismo muscular), e MuRF-1, atrogina-1 (genes envolvidos no catabolismo muscular), além do gene controle , GAPDH. Foi observado que a espessura articular e o escore de inflamação aumentaram fortemente nas ratas induzidas a Artrite Reumatóide ($p < 0,001$), enquanto a AST reduziu ($p \leq 0,05$). Um aumento nos níveis de mRNA de IGF-1 (2,0 vezes), miostatina (4,5 vezes), atrogina-1 (2,5 vezes), MyoD (3,7 vezes) e miogenina (5 vezes) foi observado no músculo das ratas induzidas a Artrite Reumatóide. mRNA de miostatina, atrogina-1, MyoD e miogenina reduziu no grupo RAEx. Desta forma, podemos concluir, que o modelo experimental de Artrite Reumatóide induziu um aumento da expressão de genes durante a atrofia muscular (myostatin, atrogin-1, MyoD and myogenin) e que estas alterações foram reguladas pelo Exercício com peso.

Palavras-chave: Caquexia; Atrogina-1; Miostatina; Miogenina.

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Introdução

Cachexia is a serious consequence of rheumatoid arthritis[1,2]. It is characterized by skeletal muscle wasting and has been postulated to be an important contributor in loss of function and quality of life, beyond increase morbidity and mortality[2,3,4]. Experimental rat models of rheumatoid arthritis (experimental arthritis), such as adjuvant-induced arthritis, has been of critical importance for current understanding of the complex nature of muscle wasting, characterized by disruption of balance in muscle protein synthesis and degradation. Experimental arthritis studies have been determined catabolic markers. It involves two E3 ubiquitin ligases, which are muscle ring finger 1 (MuRF-1) and muscle atrophy F-box protein (atrogin-1 or MAFbx) and myostatin, a member of the transforming growth factor (TGF)-β family that negatively regulates skeletal muscle growth by inhibiting the activation of satellite cells (i.e. MyoD)[5,6,7,8]. Furthermore, IGF-1, MyoD and myogenin (myogenic regulatory factors and therefore anabolic factors) also have been reported to changes in muscle atrophy associated with experimental arthritis[5,6,9]. Therefore, studies with animal models of experimental arthritis have been of critic importance for defining regulatory pathways that could be manipulated therapeutically for improved treatment options.

The complex nature of upregulation of catabolic and anabolic genes in muscle atrophy of experimental arthritis has been mainly attributed to chronic inflammation[5,6,10]. The rationale for linking the inflammation with muscle atrophy comes from the action of inflammatory mediators (such as cytokines, mainly TNF-α) on activation of proteolitc processes[11,10,12,13]. However, reduction in physical activity as a result of joint pain and stiffness has also been suggested as a mechanism involved in muscle atrophy[14,15,16]. Filippin *et al.* showed that rats reduced their spontaneous physical activity (locomotion: distance and velocity) after induction of arthritis. The same study showed that the reduction of spontaneous physical activity occurred before muscle atrophy. Moreover, mRNA level of IGF-1, atrogin-1, MuRF-1, myogenin, MyoD and myostatin have been reported to be increased in muscle atrophy of experimental rat models (without arthritis) of disuse/unload[16-21]. Collectively, these data strengthen the idea that reduced physical activity caused by experimental arthritis (muscle disuse) could provoke upregulation of those catabolic and anabolic genes in skeletal muscle.

Interestingly, a few days of "muscle reusing" (reloading) has been sufficient to restore myostatin, atrogin-1 and MuRF-1 mRNA levels back to control and to increase IGF-1 and MyoD in disuse-induced skeletal muscle atrophy[17,18,19,20,22]. Thus, it would seem reasonable to assume that muscle reusing through physical activity brings back to control those catabolic and anabolic genes associated to muscle atrophy caused by experimental arthritis. However, the muscle reusing through spontaneous locomotion is not possible in experimental arthritis because it reduces spontaneous physical activity, as mentioned previously. Therefore, to investigate this assumption, we have studied the acute effects of non-spontaneous physical activity (resistance exercise model) on the mRNA levels of MyoD, myogenin, IGF-1, atrogin-1, MuRF-1, and myostatin in muscle of an experimental arthritis model. This paper clarifies the acute molecular responses of skeletal muscle to "reusing/reloading" during experimental arthritis.

Materials and methods

Animal and Experimental Groups

This study was in accordance with the National Guide for Care and use of Laboratory Animals and had the approval of the University Ethics Committee (No. 275/2013). All procedures were performed in the Research Institute of Oncology (IPON) of the Federal University of Triângulo Mineiro (UFTM).

Female Wistar rats (126 ± 24 grams) with eight weeks of age were used in the present study. The rats were housed in plastic cages in standard conditions at 22°, 12h light-dark cycle, and had free access to water and standard food (ad libitum). Four experimental groups were used in this study: control (CT = 7); acute RE group without rheumatoid arthritis (Ex = 6); rheumatoid arthritis group without acute RE (RA = 6); acute RE and rheumatoid arthritis group (RAEx = 7). All rats were treated similarly in terms of daily manipulation. All groups performed one week (3 sessions) of exercise for familiarization. In the following weeks, RA and RAEx groups were immunized and then the groups were injected with Met-BSA into the tibiotarsal joint. Fifteen days after intra-articular injection, Ex and RAEx groups performed an acute resistance exercise (RE) model. All exercised rats were euthanized at the same period and day time, six hours after acute RE. The non-exercised rats were euthanized at same period and time point of the exercised rats. The gastrocnemius muscle samples of the left hind paw were removed, cleaned and their white portions were obtained and stored in TRIzol at -80°C for molecular analysis. We investigated the gastrocnemius white portion because it possesses more fast-twitch

fibers. Gastrocnemius muscles of the right hind paw were weighed and sectioned in the middle portion for morphometric analysis.

Experimental Arthritis protocol

The rats were anesthetized with an intraperitoneal injection solution containing ketamine (40 mg/kg) and Xylazine (5 mg/kg) for experimental arthritis procedures. Initially, the rats were immunized with two subcutaneous injections of 50μL Bovine Albumin methylated (Met-BSA) (40 mg/mL) diluted in glucose 5% emulsified with Complete Freund's Adjuvant (CFA) (supplemented with 1 mg/ml of inactivated Mycobacterium tuberculosis) into the base tail, with an interval of seven days between the injections. After seven days from the last injection at base tail, an intra-articular injection of 25 μL Met-BSA, CFA and glucose 5% was applied in the tibiotarsal joints[23].

Acute resistance exercise protocol

The RE model was chosen because the stress is lower than other models, such as swimming (water), the squat RE model (electric shock) and treadmill (electric shock and noise).

In the week before arthritis induction, all groups (including control groups) performed 3 sessions, 10 climbs in each session, without extra load, for familiarization with the ladder protocol. To simulate an acute resistance exercise protocol, the progressive loading protocol proposed by Matheny et al. was adapted for the research needs[24]. The model consisted in animals climbing up a ladder (1.1 x 0,18m, with 2 cm spacing between grid steps, 80º inclination grades) with a fixed load attached to the tail. The ladder's length and space between grid steps forced the rats to perform 8-12 movements (in each paw) in each climb. The apparatus attached to the tail consisted of cylindrical tubes containing spherical lead weights inside. It was attached to the proximal part of the animal's tail by a self-adhesive tape (1.5cm, Tartan 3M). The acute RE initially consisted of no load and was progressively increased by an additional 25% of their own body weights every three climbs. For instance, the rats started doing three climbs with no load; next, three climbs with a load at 25% of their body weight (BW) added to the apparatus; then, three climbs with 50% of BW; after that three climbs with 75% of BW and finally three climbs with 100% of BW, totaling 15-climbs with recovery of 120s between them. When the animal was unable to complete a climb with the stipulated load (failure) the load was decreased to complete the total of 15-climbs. Failure was defined as the inability to lift the weight even after two successive stimuli (pinch) at the tail. The average of pinch in arthritis groups (RA and RAEx) was mimicked in control groups (CT and Ex). The RE session was performed in the morning.

Euthanasia

Animals were anesthetized with intraperitoneal injection solution containing Ketamine (80 mg/kg) and Xylazine (10 mg/kg), six hours post RE session. Euthanasia was performed by cardiac puncture and hypovolemic shock. The non-exercised rats were anesthetized and euthanized at same period and time point of the exercised rats.

Muscle morphometric analysis

Morphological analyses were performed from gastrocnemius histological sections (6μm thickness). The gastrocnemius histological sections were obtained in a microtome (Leica Biosystems, Nussloch, Germany) and stained with hematoxylin and eosin (HE) method. The stained sections were used for photographic documentation of six random histological fields (20 x lens) (Nikon Evolucion MP 5.0). An image analysis software (Image J 1.46r) was used to determine cross-sectional area (CSA) of 200 fibers per muscle.

RNA extraction and qPCR

Muscles samples were extracted (50-70mg) from gastrocnemius white portion. Total RNA extraction was performed using TRIzol (SIGMA-ALDRICH, Sto Louis MO, USA), following the manufacturers protocol. After extraction, a dry pellet was resuspended in RNase-free water, treated with DNase I (Life Technologies, Carlsbad, CA, EUA) to remove any possible DNA presence in sample. Total RNA was quantified using a high precision fluorometer QUBIT 2.0 (Life Technologies, Carlsbad, CA, EUA) and a RNA BR Assay kit (Life Technologies, Carlsbad, CA, EUA). All Samples had RNA concentrations between 20-100 ng/μl. When RNA higher values were found, samples were diluted in RNase-free water.

Quantification of mRNA was obtained by 7900HT Fast Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) using Quantifast SYBR Green RT-PCR one-step kits (QIAGEN, Hilden, Alemanha). Thus, 1µl RNA samples treated with DNAse were added to a mixture containing, 10µl 2 x Quantifast SYBR GREEN RT-PCR Master Mix, 0.2µl Quant Fast, 0.6µl primer sense and anti-sense and completed with RNAse Free water to reach 20µl. Annealing temperature and curve were used as measures of quality. The qPCR reaction conditions and cycles performed in the 7900HT Fast Real-Time PCR System apparatus (Life Technologies, Carlsbad, CA, USA) were as follows: 50ºC per 10 min, 95ºC per 5 min for initial denaturation and amplification of 40 cycles (95ºC per 10 seconds for denaturation, 60ºC per 30 seconds for annealing and extension). Fluorescence values were obtained between the annealing/extension stages and *threshold cycle numbers* (CT) were determined using the SDS version 1.2.3 software (Applied Biosystems, USA). mRNA MyoD, myogenin, IGF-1, MuRF-1, atrogina and miostatin were normalized for GAPDH values (reference gene) and calculated by Livak method ($\Delta\Delta C_T$). Primers for all genes were obtained from previous studies and constructed from sequences published in GenBank (www.pubmed.com) to ensure the specificity of target sequences, avoid generation of secondary structures of primers and also to avoid dimerization in each primer and between primers sense and anti-sense[5,25].

Clinical severity and measurement of edema

Arthritis index score, joint edema, body weight and food intake were examined weekly. Evaluation of arthritis severity was performed by measuring the arthritis index and joint edema of each animal. Arthritis severity was quantified clinically according to a graded scale of 0–4 as follows: 0, no evidence of erythema and swelling; 1, erythema and mild swelling confined to the tarsals or ankle joint; 2, erythema and mild swelling extending from the ankle to the tarsal; 3, erythema and moderate swelling extending from the ankle to metatarsal joints; and 4, erythema and severe swelling encompassing the ankle, foot, and digits, or ankylosis of the limb[16]. Joint edema was assessed by size of latero-lateral extent of the height of the tarsal rats hind paws with analogic calipers (Starfer, São Paulo, SP, Brazil)[16]. For severity rheumatoid arthritis index score and joint edema was performed with mean values (average arithmetic) of two hind paws.

Statistical analysis

The variables which were not influenced by acute RE (muscle weight, index score, joint edema, food intake and CSA) were analyzed with the animals divided in two groups: without rheumatoid arthritis (CT + Ex, n = 13) and a group with rheumatoid arthritis (RA + RAEx, n = 13). The data were tested for normal distribution using the Shapiro–Wilk test and for variance homogeneity using the Levene test. The body weight, food intake, joint thickness and score variables were compared (intra-groups) at different moments by repeated measures ANOVA followed by Greenhouse-Geisser sphericity test and between groups (inter-groups) by independent test-t. The muscle weight, CSA of the fibers and expression of genes were compared between groups (inter-groups) by Kruskal-Wallis or Mann Whitney test (nonparametric data). When appropriate ($p < 0.05$), a post hoc rank comparison test of subgroups was made. Spearman's coefficient of rank correlation was used to associate the variables. In order to standardize the results, the data are expressed as median ± 25^{th} – 75^{th} percentiles. Statistical procedures were performed with a statistical software. $P < 0.05$ was considered to be significant.

Results

One day after intra-articular Met-BSA injection, the arthritic rats showed external signs of the illness.

Arthritis index score strongly increased after intra-articular Met-BSA injection, reaching the highest level after day 1. All arthritic rats showed several ankylosis of the limb (total loss of movement) after intra-articular Met-BSA injection. This high score value was accompanied by an increase of 90,8% ($p < 0,05$) in the ankle thickness. A Joint thickness decrease from day 1 to day 8 (-4,8%) and 15 (-20%) was noted. A moderate ankylosis (partial loss of movement) was observed at the days 8 and 15. However, the joint thickness and score values remained higher than the control group's up to day 15 ($p < 0,05$) (date of inflammation score are showed in Figure 1).

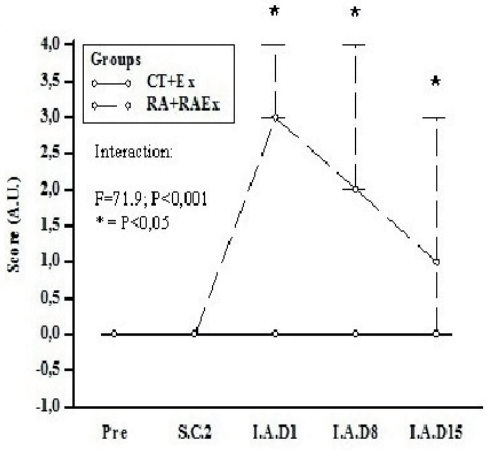

Figure 1. Changes of inflammation score. Inflamation score in control (CT+Ex) and arthritis rats (RA+RAEx) at subcutaneous injection 1 (S.C.1), subcutaneous injection 2 (S.C.2), one day after intra-articular injection (I.A.D1), eigth days after intra-articular injection (I.A.D8) and fifteen days after intra-articular injection (I.A.D15). Data are expressed as median and 25 – 75 percentiles.

The food intake value was calculated by arithmetic average of seven days. The mean of food intake corrected by body weight was 0,86g/g (both groups) and there was no change in the food intake during the study. However, at week 4 the food intake in arthritic rats was 11,9% higher ($p = 0,001$) than in the control group. This change in food intake in arthritic rats did not seem to result in body weight change. Progressive increase in the body weight ($p < 0,05$) was observed in the 5 weeks following the pre-induction moment. However, there was no difference in the body weight between the groups.

The gastrocnemius weight (-17%) as well as fiber CSA of gastrocnemius (-15%) were lower in arthritic rats (Figure 2) than in rats without arthritis. The arthritis increased myostatin (~4 fold) and artrogin-1 (~3 fold) mRNA in the gastrocnemius muscle, but not MuRF-1 mRNA (Figure 3). The arthritic rats that performed exercise showed no increase in myostatin and atrogin-1 mRNA. Similarly, the arthritis induced an increase in IGF-1 (~2.0 fold), MyoD (~2.5 fold) and myogenin (~4 fold) mRNA (Figure 4). The rats without arthritis that performed RE also increased IGF-1 and myogenin mRNA (~3.5 fold). The arthritics rats that performed exercise showed increase in IGF-1 mRNA. However, this increase was not different from the one found in arthritics rats. In contrast, arthritic rats that performed exercise showed no increase in expression of MyoD and myogenin.

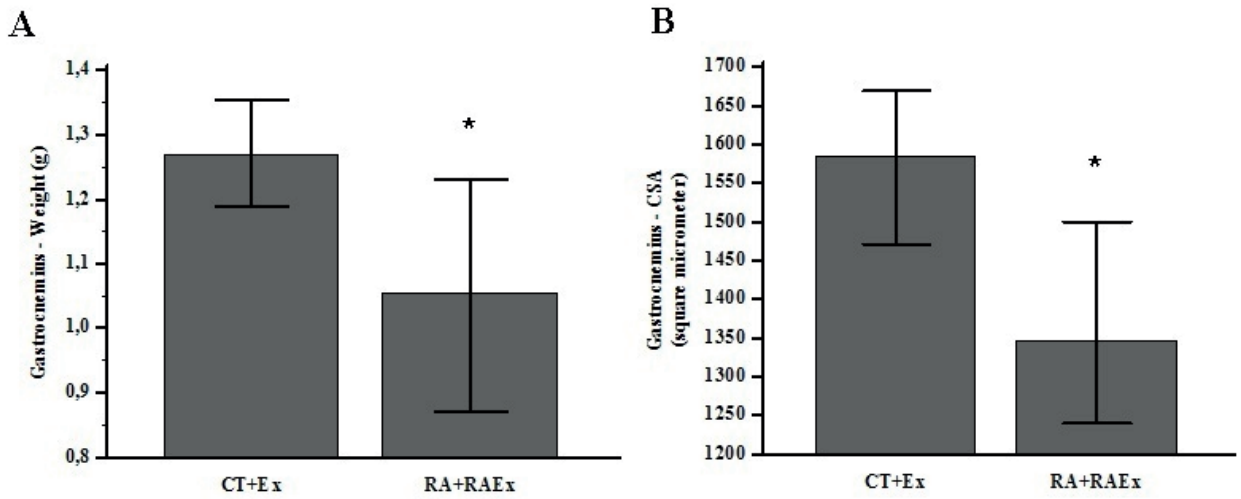

Figure 2. Gastrocnemius weight and cross-sectional area of muscle fiber. Absolute gastrocnemius weight (A) and cross-sectional area of muscle fiber (B) in control (CT+Ex) and arthritic rats (RA + RAEx). Data are expressed as median and 25 – 75 percentiles. *P < 0.05 vs. CT+Ex.

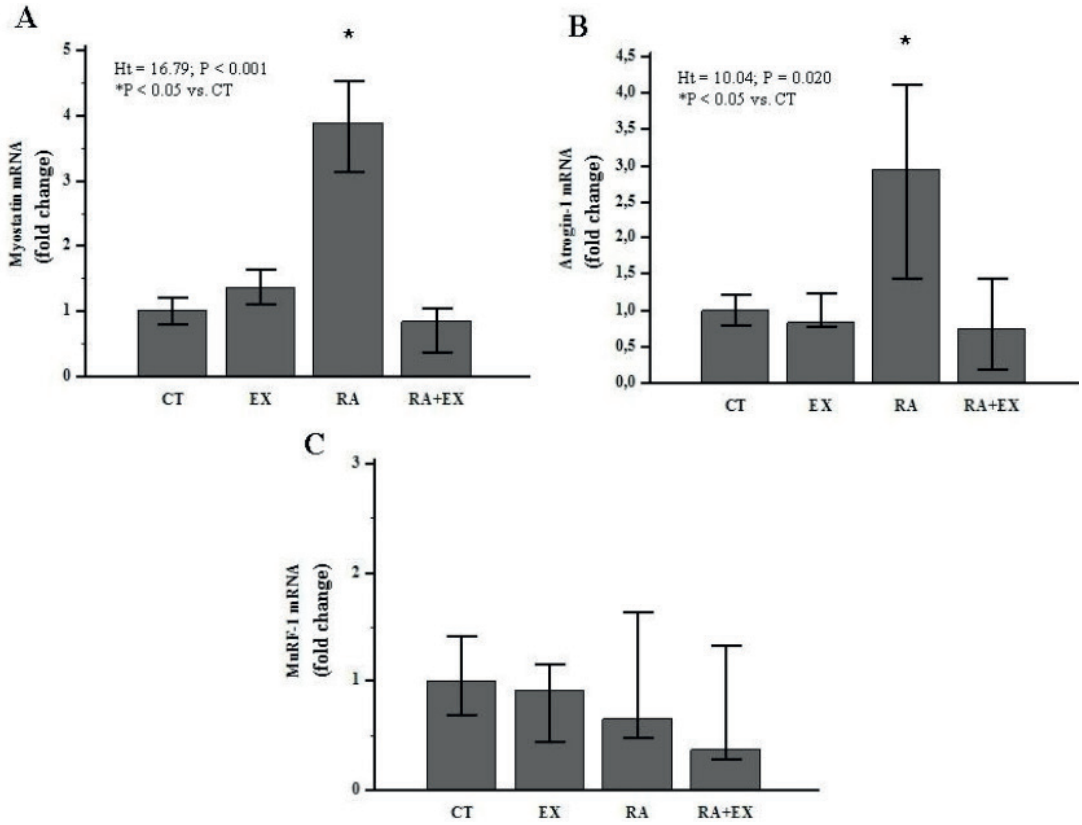

Figure 3. Myostatin mRNA, Atrogina mRNA and MuRF-1 mRNA.Effect of RE on myostatin mRNA (A), atrogin-1 mRNA (B) and MuRF-1 (C) in control (CT and Ex) and arthritic rats (RA an RAEx). Data are expressed as median and 25 – 75 percentiles. 8P < 0.05 vs. CT.

There were significant correlations of atrogin-1 mRNA with myostain mRNA (r = 0.70, p = 0.0001), MyoD mRNA (r = 0.89, p < 0.0001) and myogenin mRNA (r = 0.64, p = 0.0004). However, there no significant correlation of atrogin-1mRNA with IGF-1 mRNA (r = -0.034, p = 0.867). The MuRF-1 mRNA was significantly correlated with IGF-1 mRNA (r = 0.57, p = 0.002). However, there were no significant correlations of MuRF-1 with myostain (r = -0.02, p = 0.940), MyoD (r = 0.26, p = 0.201) and myogenin (r = 0.31 p = 0.126).

Discussion

Experimental arthritis studies have been of critic importance for defining regulatory pathways that could be manipulated therapeutically for improved treatment options of muscle wasting. Muscle disuse/unloading as a result of joint pain and stiffness has also been suggested as a mechanism involved in muscle atrophy of rheumatoid arthritis in human and animal model[14-16]. Experimental arthritis and disuse/unloading models have been shown to simultaneously upregulate the anabolic and catabolic gene expressions in skeletal muscle[5,6,10,17,22]. In disuse model, a few days of reload has been sufficient to restore myostatin, atrogin-1 and MuRF-1 mRNA levels back to control and to increase IGF-1 and MyoD in skeletal muscle atrophy[17,18,19,20,22]. Hence, in this paper we investigated the acute effects of "muscle reusing/ reloading" through RE on the mRNA levels of MyoD, myogenin, IGF-1, atrogin-1, MuRF-1, and myostatin in muscle atrophy of an experimental arthritis (adjuvant-induced arthritis in rats). The results showed that arthritis induces disuse (↑ joint thickness and arthritis index score) and muscular mass loss concomitantly with the increase of IGF-1, myogenin, MyoD, myostatin e atrogin-1 gene expressions. However, an acute RE bout (muscle loading) was sufficient to restore myogenin, MyoD, myostatin e atrogin-1 mRNA levels back to control, but not IGF-1, in muscle of arthritic rats. The current study strengthens the role of disuse on muscle genes expression acting in myogenic and proteolytic pathway. Especially, our results provide compelling evidence that RE bout restores experimental arthritis-increased gene expression back to control in muscle atrophy and also suggest that resistance training could be effective in counteracting muscle wasting in disuse conditions such rheumatoid arthritis.

Clinical signs. The external signs of the illness measured by joint thickness and arthritis index score (Figure 1) showed success in Met-BSA-induced arthritis in our experiment. Arthritic rats showed reduction in muscle weight

(↓17%) and muscle CSA (↓15%) (Figure 2) and it may not be attributed to the decrease in food intake, since in arthritic rats the food intake reached higher levels than in the control group in week 4[5]. The magnitude of the muscle reduction observed in the current study is similar to the findings of other animal studies that measure the same muscle[5,9,16].

Myostain mRNA response to experimental arthritis. Myostain has been associated with muscle wasting in different conditions[5,7,8,10,20,26]. In muscle atrophy of the arthritic rats there were substantial increases in the myostatin gene expression (Figure 3A). However, our study contrasts with Castillero *et al.* and Ramires *et al.* studies. Castillero *et al*. found no increased myostatin mRNA in muscle after 15 days from administration of adjuvant injection[27]. Ramires *et al.* found increased myostatin mRNA in muscle after two days of I–carrageenan injection. However, in Ramires *et al.* study the myostatin mRNA returned to baseline levels after seven days[10]. These two studies have suggested that after induction of rheumatoid arthritis there is an early myostatin increase, which returns to basal levels after a few days. However, these two studies used male rats while we used female rats. Haddad *et al.* have previously shown a high (4 fold) upregulation of myostatin mRNA in female rat muscle in response to five days of disuse[20]. De Naeyer *et al.* have shown that myostatin mRNA in EDL muscle returns to baseline levels after seven days of testosterone administration, but not with estrogen administration, in castrated rats[28]. These and our results suggest that testosterone and estrogen may have different roles in regulation of myostatin mRNA. Thus, the sex/hormonal differences between studies may, at least partially, explain the difference in myostatin response and have important implications of female muscle wasting.

Atrogin-1 and MuRF-1 mRNA response to experimental arthritis. The atrogin-1 and MuRF-1 have also been associated with muscle wasting in different atrophy models[5,10,19,20,29,30]. Myostatin association with the activin IIB receptor has been reported to increase Smad2/3 (mothers against decapentaplegic homolog 2/3)-mediated up-regulation of atrogin-1 and MuRF-1[7,26,31,32]. However, in the current study, there was a substantial increase in atrogin-1 gene expression, but not in MuRF-1 mRNA, in muscle atrophy of the arthritic rats (Figure 3; B and C). Moreover, we found strong and significant positive correlations of myostatin mRNA with atrogin-1 mRNA, but not with MuRF-1 mRNA. These data suggest that MuRF-1 and Atrogin-1 do not function similarly under all atrophy models. Our argument is consistent with other studies[10,28,29]. For instance, in dexamethasone-induced muscle atrophy, MuRF-1 seems to be more important for muscle atrophy than atrogin-1[29]. In inflammation- and immobilization-induced muscle atrophy, MuRF-1 and atrogin-1 mRNA have shown different time-course changes[10,19,20]. Moreover, testosterone and estrogen may play different roles in regulation of mRNA MuRF-1 and atrogin-1, which may also in part explain the difference of gene expressions between studies[28].

We also found strong and significant positive correlations of atrogin-1 mRNA with myogenin mRNA and MyoD mRNA, but not of MuRF-1 mRNA with myogenin mRNA and MyoD mRNA. Conversely, there was significant correlation of MuRF-1 mRNA with IGF-1 mRNA, but not between atrogin-1 mRNA and IGF-1 mRNA. These data imply that atrogin-1 and MuRF1 might have a specific role in the degradation of muscle proteins. Previous studies have suggested that atrogin-1 causes the ubiquitinization of MyoD and myogenin, which leads to its degradation in the proteasome, while other proteins are actually degraded by MuRF1-mediated UPS[33-35].

Myostatin, atrogin-1 and MuRF-1 mRNA response to acute RE. A lot of effort has been made to block the increase in muscle atrogin-1, MuRF-1 and myostatin during rheumatoid arthritis[6,27,36,37,38]. So far, to the best of our knowledge, this is the first study that has investigated the effects of acute "muscle reusing/reloading" (RE) on molecular signaling pathways associated with muscle wasting in rheumatoid arthritis. Interestingly, we observed that the RE repressed experimental arthritis-increased myostatin and atrogin-1 mRNA (Figure 3; A and B). These results suggest that changes in expression of genes involved in protein degradation during experimental arthritis are restored as a response to acute reusing/reloading. Similarly, the atrogin-1 and myostatin mRNA restorations after RE have been shown previously in dexamethasone- and unload-induced muscle atrophy studies[19,20,30,29]. Moreover, others studies have previously shown that acute RE reduces myostatin and atrogin-1 mRNA in muscle[30,39,40].

IGF-1, MyoD and myogenin mRNA response to experimental arthritis. In the current study, the IGF-1, MyoD and myogenin gene expressions were increased in the muscle of the arthritic rats after 15 days from administration of adjuvant injection (Figure 4; A, B and C). These data are consistent with previous reports[5,9,27]. As rheumatoid arthritis is an inflammatory disease, the action of inflammatory mediators appears to play an important role in the activation of myogenic factors[10-12]. Chronic joint inflammation has been reported to stimulate the cytokines pathways, which may interact with myogenic regulatory factors[10-12]. However, atrophy models induced by disuse have also been shown to

increase regenerative activity in skeletal muscle[17,18,21]. Thus, these studies with disuse/unloading model have questioned if rheumatoid arthritis necessarily change the expression of IGF-1, MyoD and myogenin genes due only to inflammation.

IGF-1, MyoD and myogenin mRNA response to acute RE. The stimulatory effect of exercise on muscle MyoD and myogenin is well known. MyoD is predominantly upregulated during satelite cell proliferation immediately post exercise, while myogenin is later (six hours) upregulated during myoblast differentiation[25,41,42]. Hence, our results showed an increase in mRNA levels of myogenin in muscles of rats without arthritis inducion, but not MyoD, six hours after the RE protocol (Figure 4B). Surprisingly, there was no increase in MyoD or myogenin in muscle of arthritic rats after RE (Figure 4; A and B). Similar to the atrogenes results, the MyoD and myogenin results suggest that changes in expression of genes involved in proliferation and differentiation of satellite cells during experimental arthritis are restored as a response to acute RE.

We observed that the IGF-1 mRNA increased in muscle after acute RE (Figure 4C). Increased IGF-1 mRNA after RE has been shown in humans and animals[25,41]. However, acute RE did not change the IGF-1 response in muscle of arthritic rats. In contrast with our study, IGF-1 expression has been shown to be increased in disuse-induced muscle atrophy after reloading[17,18,20]. However, these studies investigated the chronic response to resistance training after disuse. These data suggest that increased IGF-1 mRNA in disuse-induced muscle atrophy after RE appears to be an adaptation to successive reloading stimuli.

Therefore, we conclude that experimental arthritis induces muscle atrophy with concomitant increases in mRNA levels of IGF-1, myogenin, MyoD, myostatin and atrogin. These changes in the expression of genes (myostatin, atrogin-1, MyoD and myogenin) during experimental arthritis are restored back to control, but not IGF-1, as a response to acute RE. The current study reveals a new insight in the role of "reusing/reloding" on muscle genes expression acting in myogenic and proteolytic pathway during muscle wasting induced by experimental arthritis. Thus, our results provide compelling evidence that RE bout restores experimental arthritis-increased gene expression back to control in muscle atrophy and also suggest that resistance training could be effective in counteracting cachexia in disease condition such rheumatoid arthritis.

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Competing interests

The authors declare that they have no conflict of interest.

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