Strength Training with Blood Flow Restriction Diminishes Myostatin Gene Expression

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ABSTRACT

LAURENTINO, G. C., C. UGRINOWITSCH, H. ROSCHEL, M. S. AOKI, A. G. SOARES, M. NEVES JR, A. Y. AIHARA, A. DA ROCHA CORREA FERNANDES, and V. TRICOLI. Strength Training with Blood Flow Restriction Diminishes Myostatin Gene Expression. Med. Sci. Sports Exerc., Vol. 44, No. 3, pp. 406–412, 2012. Purpose: The aim of the study was to determine whether the similar muscle strength and hypertrophy responses observed after either low-intensity resistance exercise associated with moderate blood flow restriction or high-intensity resistance exercise are associated with similar changes in messenger RNA (mRNA) expression of selected genes involved in myostatin (MSTN) signaling. Methods: Twenty-nine physically active male subjects were divided into three groups: low-intensity (20% one-repetition maximum (1RM)) resistance training (LI) (n = 10), low-intensity resistance exercise associated with moderate blood flow restriction (LIR) (n = 10), and high-intensity (80% 1RM) resistance exercise (HI) (n = 9). All of the groups underwent an 8-wk training program. Maximal dynamic knee extension strength (1RM), quadriceps cross-sectional area (CSA), MSTN, follistatin-like related genes (follistatin (FLST), follistatin-like 3 (FLST-3)), activin Ibβ, growth and differentiation factor–associated serum protein 1 (GASP-1), and MAD-related protein (SMAD-7) mRNA gene expression were assessed before and after training. Results: Knee extension 1RM significantly increased in all groups (LI = 20.7%, LIR = 40.1%, and HI = 36.2%). CSA increased in both the LIR and HI groups (6.3% and 6.1%, respectively). MSTN mRNA expression decreased in the LIR and HI groups (45% and 41%, respectively). There were no significant changes in activin Ibβ (P > 0.05). FLST and FLST-3 mRNA expression increased in all groups from pre- to posttest (P < 0.001). FLST-3 expression was significantly greater in the HI when compared with the LIR and LI groups at posttest (P = 0.024 and P = 0.018, respectively). GASP-1 and SMAD-7 gene expression significantly increased in both the LIR and HI groups. Conclusions: We concluded that LIR was able to induce gains in 1RM and quadriceps CSA similar to those observed after traditional HI. These responses may be related to the concomitant decrease in MSTN and increase in FLST isoforms, GASP-1, and SMAD-7 mRNA gene expression. Key Words: RESISTANCE TRAINING, SKELETAL MUSCLE HYPERTROPHY, MUSCLE BIOPSY, VASCULAR OCCLUSION, HYPOXIA

Myostatin (MSTN), or growth and differentiation factor-8, is a transforming growth factor-β family member that functions as a regulator of muscle mass (15,20,21). Accordingly, MSTN overexpression has been shown to reduce muscle mass, fiber size, and myonuclei number. Its role as a negative regulator of hypertrophy has also been highlighted by the remarkable increase in muscle mass of MSTN-deficient animals and humans (20,23,28).

In this regard, Drummond et al. (9) demonstrated that muscle contraction (i.e., resistance exercise) reduces MSTN gene expression 3 h after an acute bout of exercise. These findings suggest that the downregulation of MSTN after exercise may result in greater muscle hypertrophy in a long-term resistance training program (25,27,30).

Indeed, Roth et al. (25) observed diminished MSTN messenger RNA (mRNA) gene expression after 9 wk of traditional high-intensity (i.e., 75%–85% one-repetition maximum (1RM)) resistance exercise (HI) with a concomitant increase in muscle strength and hypertrophy. On the other hand, assessing not only MSTN but also its regulatory genes (e.g., follistatin-like 3 (FLST-3), growth and differentiation factor–associated serum protein 1 (GASP-1), SMAD family genes, and activin Ibβ) is essential for a more comprehensive overview of the molecular responses to exercise training.

In this regard, it was demonstrated that 12 wk of HI (85%–90% 1RM) conversely induced an increase in the skeletal muscle MSTN mRNA and in the serum MSTN content.
Importantly, the author also observed a significant increase in serum follistatin-like related gene levels, which act as an endogenous inhibitor of MSTN, hence diminishing proteolysis. In fact, increased myofibrillar protein content and fat-free mass were observed after the HI training period when compared with control (31). GASP-1 is also thought to play a critical role in controlling MSTN function in the skeletal muscle cell (13). A previous study has shown increased levels of serum GASP-1 after 8 wk of HI (27). In addition, SMAD family proteins are known to mediate the intracellular signaling of MSTN (10,12,17), whereas activin IIB functions as a receptor site for MSTN, thus mediating its action (22,24). Different types of mechanical stimuli have been shown to increase MAD-related protein (SMAD-7) gene expression (1) and decrease activin IIB skeletal muscle content (14,31).

Despite the growing body of evidence emphasizing the important role of MSTN and its regulatory genes in the HI-induced muscle hypertrophy response, it seems that low-intensity muscle contractions, when performed under conditions of blood flow restriction, may also have the potential to initiate the necessary cellular signals responsible for protein synthesis and cell growth. In fact, it has been recently proposed that low-intensity resistance exercise associated with blood flow restriction (LIR) is able to promote similar gains in strength and muscle mass when compared with conventional HI training (16,18). However, little is known regarding the mechanisms underlying the LIR-induced hypertrophy.

Therefore, the aim of the study was to determine whether the similar muscle strength and hypertrophy responses observed after either LIR or HI are associated with similar changes in mRNA expression of selected genes involved in the MSTN signaling.

**METHODS**

**Participants**

Twenty-nine physically active male college students volunteered to participate in this study. Participants were not engaged in any form of regular physical training and had no previous experience in strength training and were free from any musculoskeletal disorders or drug or nutritional supplements ingestion. For the composition of the experimental groups, the participants were ranked into quartiles according to their knee extensors maximum dynamic strength (1RM) and quadriceps cross-sectional area (CSA). Participants from each quartile were then randomly allocated into three groups: low-intensity resistance training (LI) \((n = 10, \text{age} = 20.3 \pm 4.2 \text{ yr, weight} = 75.3 \pm 15.4 \text{ kg, height} = 175.7 \pm 4.9 \text{ cm})\), LI combined with moderate blood flow restriction (LIR) \((n = 10, \text{age} = 20.0 \pm 4.5 \text{ yr, weight} = 72.1 \pm 11.9 \text{ kg, height} = 175.2 \pm 9.0 \text{ cm})\), and high-intensity resistance exercise (HI) \((n = 9, \text{age} = 23.6 \pm 6 \text{ yr, weight} = 73.8 \pm 12 \text{ kg, height} = 173.6 \pm 6 \text{ cm})\). A one-way ANOVA ensured the lack of differences in the knee extension 1RM and quadriceps CSA values between groups \((P > 0.05)\). The study was approved by the local ethics committee, and all of the participants were informed of the inherent risks and benefits before signing a written informed consent form.

**Study Design**

Before the experimental protocol, quadriceps CSA was obtained through magnetic resonance imaging (MRI). After the MRI, muscle tissue samples were obtained from the vastus lateralis muscle of the participants’ dominant leg using the percutaneous needle biopsy technique. Afterward, the participants engaged in two familiarization sessions for the resistance training protocol and knee extension 1RM testing. One week after the familiarization sessions, the participants were tested for their knee extension 1RM. To test the effects of resistance training associated with moderate blood flow restriction on muscle strength and hypertrophy and selected MSTN-related genes, participants were engaged in one of three training modes, as follows: high-intensity resistance training (HI), low-intensity with moderate blood flow restriction (LIR), and LI. Training was performed twice a week for 8 wk. The quadriceps CSA, knee extension 1RM, and muscle biopsy samples were obtained after the completion of the training protocol.

**Quadriceps CSA**

Quadriceps CSA was obtained through MRI (Signa LX 9.1; GE Healthcare, Milwaukee, WI). Participants were positioned on the device in a supine laid position with the knees extended and legs straight. A bandage was used to restrain leg movements during the test. An initial reference image was obtained to determine the perpendicular distance from the greater trochanter of the femur to the inferior border of the lateral epicondyle of the femur, which was defined as the segment length. Quadriceps CSA was measured at 50% of the segment length with 0.8-cm slices for 3 s. The pulse sequence was performed with a field of view between 400 and 420 mm, time of repetition of 350 ms, echo time from 9 to 11 ms, two signal acquisitions, and a matrix of reconstruction of 256 × 256 mm. The images were then transferred to a workstation (Advantage Workstation 4.3; GE Healthcare) to determine quadriceps CSA. The quadriceps images were traced in triplicates by a specialized researcher, and their mean values were used for further analysis. The coefficient of variation between measurements was less than 1%. The segment slice was divided into skeletal muscle, subcutaneous fat tissue, bone, and residual tissue. Then, muscle CSA was determined by subtracting the bone and subcutaneous fat area.

**Knee Extension 1RM Test**

The procedures adopted for knee extension 1RM determination followed the recommendations described by Brown.
and Weir (4). In short, subjects ran for 5 min on a treadmill at 9 km h⁻¹, followed by lower limb light stretching exercises and two warm-up sets of knee extension exercise. In the first set, individuals performed eight repetitions with a load correspondent to 50% of their estimated 1RM obtained during the familiarization sessions. In the second set, they performed three repetitions with 70% of their estimated 1RM. A 2-min interval was allowed between warm-up sets. After the completion of the second set, participants rested for 3 min and then had up to five attempts to achieve their 1RM. A 3-min interval was enforced between attempts. Subjects’ settings on the knee extension machine were recorded and reproduced throughout the study. Tests were conducted by an experienced researcher, and strong verbal encouragement was provided during the attempts.

**Strength Training Protocol**

Strength training consisted of bilateral knee extensions using a conventional knee extension machine (SL 1030; Righetto®, Campinas, São Paulo, Brazil). Subjects were asked to sit comfortably on the machine with their backs fully supported against the backrest. Range of motion was set at 90°, and each repetition cycle started with the knees at a 90° flexion. Then, subjects were asked to extend their knees until their shanks were parallel to the floor and return to the starting position. During the first 4 wk of training, the HI group performed three sets of eight repetitions at 80% 1RM, whereas both of the low-intensity groups performed three sets of 15 repetitions at 20% 1RM in association (LIR) or not (LI) with the moderate blood flow restriction protocol. Exercise volume was then increased to four sets for all of the groups for the remainder of the training protocol. A 1-min rest interval was allowed between sets throughout the training protocol. The duration of each repetition cycle was established at 4 s (2 s for the concentric and 2 s for the eccentric muscle action). Subjects were instructed to consume a light meal before each training session.

The LIR group trained with an air cuff placed at the inguinal fold (175 mm (width) × 920 mm (length)). A moderate blood flow restriction (~50%) was sustained throughout the training session, including the rest intervals, and was released immediately after the end of the training session. No adverse effects from the blood flow restriction protocol (e.g., excessive fatigue or pain) were reported by any of the subjects.

**Determination of the Blood Flow Restriction Pressure**

Subjects were asked to lie on a supine position while resting comfortably. A vascular Doppler probe (DV-600; Marted, Ribeirão Preto, São Paulo, Brazil) was placed over the tibial artery to capture its auscultatory pulse. For the determination of blood pressure (mm Hg) necessary for a complete vascular restriction (pulse elimination pressure), a standard blood pressure cuff was attached to the participant’s thigh (inguinal fold region) and then inflated up to the point in which the auscultatory pulse was interrupted (19). The cuff pressure used during the training protocol was determined as 80% of the necessary pressure for complete blood flow restriction in a resting condition. The average pressure used throughout the training protocol was 94.8 ± 10.3 mm Hg.

We opted for a wider cuff because it has been previously demonstrated that the width of the tourniquet has a large effect on the pressure required to achieve full blood flow occlusion (8). The authors demonstrated that the wider the cuff, the lower the pressure required to occlude circulation (e.g., for an 18-cm-wide cuff, ~140 mm Hg was needed to occlude blood flow, whereas a 4.5-cm cuff required more than 360 mm Hg of pressure).

**Muscle Biopsy**

A unilateral muscle sample was obtained from the midpoint of the vastus lateralis of the subject’s dominant leg using the percutaneous biopsy technique with suction. All of the biopsies were performed in the morning (between 8:00 and 11:00 a.m.) after an overnight (8 h) fasting period. A standardized breakfast meal (~311 kcal; 63.5% CHO, 21.8% proteins, and 14.7% fat) was offered to all of the subjects 2 h before the biopsies. Immediately after the procedure, the muscle sample was removed from the needle and frozen in liquid nitrogen, for further storage at ~80°C. The pretest biopsy was performed a week before the familiarization sessions, whereas the posttest biopsy was performed through an incision adjacent to the pretest site (3 cm above the previous incision) 48 h after the last training session. The mRNA expression levels of MSTN, activin Ib, follistatin (FLST), FLST-3, GASP-1, and SMAD-7 were quantified using real-time polymerase chain reaction (PCR).

**Gene Expression Analysis**

**Reverse transcription.** Total cellular RNA was isolated from the muscle sample by using the TRIzol reagent (Invitrogen®, Carlsbad, CA). Total RNA (1 μg) was typically used in a reaction containing oligo dT (500 μg·mL⁻¹), deoxyribonucleoside triphosphates (10 mM each), 5× first-strand buffer, 0.1 M of dithiothreitol, and 200 U of reverse transcriptase (SuperScript II; Invitrogen®). Reverse transcription was performed at 70°C for 10 min followed by 42°C for 60 min and 95°C for 10 min.

**Primer design.** Primer sets were designed using Primer Express version 2.0 software (Applied Biosystems®, Foster City, CA) using sequences accessed through the GenBank and were checked for specificity using the Nucleotide-Nucleotide BLAST search (Table 1).

**Real-time PCR.** All of the samples were analyzed in duplicate, and the reaction fluorescence was quantified with an ABI Prism 7300 sequence detector (Applied Biosystems®) on the basis of current methodology (5). The amplification analysis was performed with a sequence detection software.
(Applied Biosystems®). Results were expressed by using the comparative cycle threshold (Ct) method described in the manufacturer’s User Bulletin no. 2 (Applied Biosystems®).

The Ct represents the PCR cycle at which an increase in reporter gene fluorescence above a baseline signal can be detected. For each gene of interest, ΔCt values were calculated in all of the samples as follows: Ct (gene of interest) – Ct (internal control gene). The ribosomal protein large P0 gene was used as a housekeeping gene, and as expected, no change was observed (5).

The calculation of the relative changes in the expression levels of one specific gene was performed by subtracting pre- to posttest ΔCt values for each experimental group. The values and ranges given were determined as follows: $2^{-\Delta \Delta Ct}$ with ΔΔCt ± SEM (SEM is the SE of the mean ΔΔCt value; User Bulletin no. 2; Applied Biosystems®). The final values were reported as a fold difference relative to the expression of the pretest values (calculated as $2^{-\Delta \Delta Ct}$), with the pretest values arbitrarily set to 1.

Statistical Analysis

Results are presented as means and SD. Data normality and variance equality were assessed through the Shapiro–Wilk and Levene tests. A mixed model was performed for each dependent variable, having group (LI, LIR, and HI) and time (pre- and posttest) as fixed factors and subjects as a random factor. Whenever a significant F value was obtained, a post hoc test with a Tukey adjustment was performed for multiple comparison purposes. The significance level was set at $P \leq 0.05$. All of the statistical tests were performed using SAS 9.0 for Windows software (SAS Institute Inc., Cary, NC). An initial analysis revealed no differences between groups in baseline values for all of the dependent variables.

RESULTS

Knee Extension 1RM

All of the groups showed significantly greater 1RM values in the posttest (LI = 20.7%, LIR = 40.1%, and HI = 36.2%) when compared with baseline (main effect for time, $P < 0.001$). However, no significant differences were detected between groups in the posttest (Fig. 1A). Importantly, the LI group demonstrated a significantly lower delta change in 1RM values (20.7%) when compared with both the LIR and HI groups (36.2% and 40.1%, $P = 0.04$ and $P = 0.0078$, respectively).

**Quadriceps CSA**

Both the LIR and the HI groups significantly increased CSA from pre- to posttest (6.3%, $P = 0.0007$ and 6.1%, $P = 0.0004$, respectively). No significant difference was observed in CSA from pre- to posttest in the LI group (2.0%, $P = 0.9653$). However, no significant differences in quadriceps CSA values were detected between groups at posttest (Fig. 1B).

**mRNA Gene Expression**

**MSTN.** MSTN mRNA expression was significantly decreased in the LIR and HI groups (45%, $P < 0.0001$ and 41%, $P = 0.0004$, respectively). No significant differences were observed in MSTN mRNA expression between the LIR and HI groups at posttest ($P = 0.99$). However, LIR presented significantly lower MSTN mRNA expression when compared with LI in the posttest ($P = 0.002$). In addition, LI and HI MSTN mRNA expression were similar at the posttest ($P = 0.10$) (Fig. 2A).

**Activin Ib.** No changes were found after the training protocol (Fig. 2B).

**FLST.** A main time effect was observed for this gene. All of the groups increased from before to after training ($P < 0.0001$) (Fig. 2C).

**FLST-3.** Significant increases in FLST-3 mRNA expression were observed from before to after training for all groups ($P < 0.001$). No significant differences were observed between LI and LIR at posttest ($P > 0.05$). However, the increase in FLST-3 mRNA expression was significantly greater in the HI when compared with both the LIR and the LI groups at posttest ($P = 0.024$ and $P = 0.018$, respectively) (Fig. 2D).

**GASP-1.** GASP-1 mRNA expression increased significantly in the LIR (82%, $P < 0.0001$) and HI (79%, $P < 0.0001$) groups, when compared with baseline (main effect for time, $P < 0.001$). Results were expressed by using the comparative cycle threshold (Ct) method described in the manufacturer’s User Bulletin no. 2 (Applied Biosystems®).
groups from before to after training. Also, no significant difference between groups were observed at posttest ($P = 0.99$). No change was observed over time for the LI group (23%, $P > 0.05$). In addition, GASP-1 mRNA expression posttest values were significantly lower in the LI group when compared with both HI ($P < 0.0001$) and LIR ($P < 0.0001$) (Fig. 2E).

**SMAD-7.** There was a significant increase in the LIR (86%, $P < 0.0001$) and HI (66%, $P = 0.002$) groups from pre- to posttest. The LI group did not present any changes in SMAD-7 mRNA expression over time ($P = 0.356$). The LIR group presented significantly higher mRNA expression than LI ($P = 0.016$), whereas the HI group showed only a trend toward a higher posttest mRNA expression when compared with LI ($P = 0.067$) (Fig. 2F).

**DISCUSSION**

The aim of the study was to determine whether the similar muscle strength and hypertrophy responses observed after either low-intensity resistance exercise associated with moderate blood flow restriction (LIR) or high-intensity resistance exercise (HI) are associated with similar changes in mRNA expression of selected genes involved in the MSTN signaling. The main and novel finding of the present study was that LIR and HI produced similar changes in selected MSTN-related genes mRNA expression and equivalent increases in muscle mass and strength.

Regarding the muscle strength and CSA data, previous studies have reported similar increases in muscle strength and CSA between LIR and HI groups (18,29). For instance, Kubo et al. (18) demonstrated significant and similar increases in CSA after either HI or LIR regimens (7.5% and 6.1%, respectively). The training loads used in our study were similar to those of Kubo et al. (18). In our study, we found that not only the HI but also both the LI and LIR groups significantly increased knee extension 1RM. Despite the lack of between-group differences at the posttest, it is important to note that the delta change in 1RM values was significantly lower in the LI group (20.7%) when compared with both LIR and HI (36.2% and 40.1%, $P = 0.04$ and $P = 0.0078$, respectively). Despite previous reports indicating that low-load and high-volume strength training regimens are able to induce strength increments in previously untrained individuals (6), one may speculate that a longer intervention period could have revealed significant differences in 1RM between LIR and HI when compared with LI. In fact, the early adaptations to strength training (i.e., neural adaptations) rather than muscle hypertrophy may have accounted for the strength gains in the LI group. Data from the present study support the latter because CSA increments were significant only in the LIR and HI groups.

During the past years, the underlying mechanisms responsible for muscle mass catabolism have been extensively investigated (2,11). In this regard, MSTN has been suggested to play a key role in controlling muscle remodeling/wasting (15,24,26). It has been previously demonstrated that an acute bout of resistance exercise decreases MSTN gene expression (9), which may be related to long-term training-induced muscle growth. However, it should be emphasized that acute responses to an exercise bout are not necessarily observed in a long-term training model. In fact, several authors have reported an attenuation of acute exercise responses after several weeks of training (7). Thus, assessing chronic changes to blood flow restriction training programs may provide a better picture of the physiological processes involved in muscle adaptations. In the present study, MSTN mRNA gene expression was significantly decreased after 8 wk of training in both the LIR and HI groups. However, MSTN gene response after a period of resistance training is still controversial. For example, Roth et al. (25) observed a decrease in MSTN gene expression after 9 wk of HI. Conversely, Willoughby (31) demonstrated that 12 wk of HI resulted in increased MSTN mRNA expression, MSTN protein content, and MSTN serum levels. It may be speculated that these dissonant findings are related to the timing of the biopsy after the last training session. Roth et al. (25) performed posttraining biopsies 48–72 h after the last training session, whereas Willoughby (31) collected the samples only 15 min
after it. Thus, it is conceivable that Willoughby’s findings may reflect an acute response to the last exercise session of the training program rather than an actual long-term effect.

Activin Ib acts as an MSTN receptor, thus eliciting its biological function. Despite the lack of significant differences in activin Ib responses after training, it is plausible to suggest a trend toward a decreased activin Ib mRNA gene expression ($P = 0.0602$). This trend could be advantageous for muscle growth because it would blunt muscle wasting. Accordingly, Hulmi et al. (14) showed diminished activin Ib mRNA gene expression after 21 wk of resistance training. FLST isoforms are important physiological regulators of activin (1,20) and other transforming growth factor-β superfamily members including MSTN (1,15). It has been previously demonstrated that FLST isoforms are responsive to HI (31). Our results expanded this concept showing that FLST and FLST-3 are responsive to a mechanical stimulus independently of the training load.

To our knowledge, this is the first study to report significant increases in GASP-1 and SMAD-7 gene expression after both HI and LIR training regimens. GASP-1 has been suggested to play an important role in controlling the MSTN function. It blocks MSTN protein processing through the inhibition of proteases. SMAD-7, an intracellular inhibitor of MSTN signaling, has also been shown to increase in response to a mechanical stimulus (1). Taken together, these findings suggest that both LIR and HI resulted in a diminished proteolysis rate, which may at least partially explain the significant muscle mass increment observed in these groups.

Despite the interesting findings presented herein, caution should be exercised when interpreting and extrapolating these data. For instance, additional biopsy time points could provide further insight into the molecular response to blood flow restriction of the selected genes. Previous studies (32,33) have demonstrated that different genes have specific time course dynamics in response to resistance exercise. Thus, it is feasible that distinct biopsy time points could affect mRNA gene expression. Also, it is conceivable that both the width and pressure of the cuff as well as changes in the training intensity, volume, and type of exercise may produce different morphological and functional adaptations.

In summary, we confirm the previous findings that LIR is able to induce gains in muscle strength (1RM) and muscle mass (CSA) similar to those observed after traditional HI. Furthermore, our data indicate that LIR-induced increase in muscle mass may be related to the concomitant decrease in the mRNA gene expression of MSTN and increase in the mRNA gene expression of FLST isoforms, GASP-1, and SMAD-7. Nevertheless, other intracellular signaling pathways are known to be involved in the HI-induced muscle hypertrophy (2,3,22), warranting further studies regarding the molecular responses to blood flow restriction in association or not with low-intensity resistance exercise.

The following are the contributions of the authors: Gilberto Candido Laurentino, Hamilton Roschel, Valmor Tricoli, and Carlos Ugrinowitsch are significant article writers; Manoel Neves, Jr., Marcelo Saldanha Aoki, André Yui Ahara, and Artur da Rocha Correa Fernandes are significant article reviewers/revisers; Gilberto Candido Laurentino, Carlos Ugrinowitsch, Hamilton Roschel, Valmor Tricoli, and Marcelo Saldanha Aoki contributed the concept and design; Gilberto Candido Laurentino, Manoel Neves, Jr., André Yui Ahara, Artur da Rocha Correa Fernandes, and Antonio Garcia Soares performed data acquisition; Gilberto Candido Laurentino, Hamilton Roschel, Valmor Tricoli, Carlos Ugrinowitsch, and Antonio Garcia Soares performed data analysis and interpretation; and Carlos Ugrinowitsch, Gilberto Candido Laurentino, and Hamilton Roschel contributed statistical expertise. The authors are supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (2009/09820-5 for V.T. and 2010/51428-2 for H.R.), C.U. is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (470207/2008-6, 303162/2008-2). The authors thank Diagnósticos das Américas S/A for the MRI images. The authors declare no conflict of interests. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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