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Effects of Concurrent Strength and Endurance Training on Genes Related to Myostatin Signaling Pathway and Muscle Fiber Responses

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Abstract

De Souza, EO, Tricoli, V, Aoki, MS, Roschel, H, Brum PC, Bacurau, AVN, Silva-Batista, C, Wilson, JM, Neves, M Jr, Soares, AG, Ugrinowitsch, C. Effects of concurrent strength and endurance training on genes related to myostatin signaling pathway and muscle fiber responses. *J Strength Cond Res* 28(11): 3215–3223, 2014–Concurrent training (CT) seems to impair training-induced muscle hypertrophy. This study compared the effects of CT, strength training (ST) and interval training (IT) on the muscle fiber cross-sectional area (CSA) response, and on the expression of selected genes involved in the myostatin (MSTN) signaling mRNA levels. Thirty-seven physically active men were randomly divided into 4 groups: CT ($n = 11$), ST ($n = 11$), IT ($n = 8$), and control group (C) ($n = 7$) and underwent an 8-week training period. Vastus lateralis biopsy muscle samples were obtained at baseline and 48 hours after the last training session. Muscle fiber CSA, selected genes expression, and maximum dynamic ST (1 repetition maximum) were evaluated before and after training. Type IIa and type I muscle fiber CSA increased from pre- to posttest only in the ST group (17.08 and 17.9%, respectively). The SMAD-7 gene expression significantly increased at the posttest in the ST (53.9%) and CT groups (39.3%). The MSTN and its regulatory genes ActIIb, FLST-3, FOXO-3a, and GASP-1 mRNA levels remained unchanged across time and groups. One repetition maximum increased from pre- to posttest in both the ST and CT groups (ST = 18.5%; CT = 17.6%). Our findings are suggestive that MSTN and their regulatory genes at transcript level cannot differentiate muscle fiber CSA responses between CT and ST regimens in humans.

Key Words concurrent training, muscle fiber CSA, MSTN pathway, 1RM

Introduction

Concurrent training (CT) is the combination of exercises aiming to simultaneously develop aerobic capacity/power and muscle strength throughout a training period (15,20). This training mode has been recommended for health promotion throughout the life span (33,38). However, some studies have demonstrated that CT can impair skeletal muscle hypertrophy when compared with strength training (ST) alone in both healthy individuals and highly trained athletes (3,20,29,30). Although several hypotheses have been proposed to explain the impaired changes in muscle phenotype following CT (11,23,24), the mechanisms responsible for the diminished responses are far from being elucidated. Some authors have suggested that the impairment might be triggered at the molecular level (7,14,28). According to these authors, CT may activate the AMPK-PGC-1α (i.e., enhances mitochondrial biogenesis) pathway, which inhibits the Akt/mTOR/$p70^{S6K1}$ (i.e., activates protein synthesis) pathway and, therefore diminishes protein accretion (2,4,28).

In addition to the AMPK-PGC-1α pathway, myostatin (MSTN), i.e., growth differentiation factor-8, is recognized as a skeletal muscle–specific negative regulator (27,39), which impairs muscle protein synthesis and, consequently, muscle fiber hypertrophy (36). Furthermore, MSTN regulatory genes (i.e., FLST-3, GASP-1, SMAD-7, and activin IIb) are also related to the mitigation of skeletal muscle hypertrophy response as they enhance MSTN activity (1,16,17,22).

Moreover, CT impairment has been suggested to be a fiber type–specific phenomenon. In fact, it has been previously

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demonstrated that although ST and CT produced similar gains in strength and whole muscle cross-sectional area (CSA) (26), CT impairs type I fibers hypertrophy. Thus, it is possible to speculate that the CT-induced negative interference on muscle fiber CSA might be mediated through an upregulation of MSTN signaling proteins. A recent study described the effects of CT on MSTN gene expression (25), but it did not assess the expression of other genes related to the MSTN pathway (i.e., FLST-3, GASP-1, SMAD-7, and activin IIb), requiring further scrutinization regarding the possible role of this pathway on CT hampering effect on muscle hypertrophy.

Therefore, we aimed to investigate if CT interference effect, at the muscle fiber level, may be triggered by an increase in the expression of selected genes involved in the MSTN signaling. We hypothesized that if in fact CT hampers type I fiber hypertrophy, gene expression of MSTN signaling proteins will be elevated after a training period.

Methods

Experimental Approach to the Problem

Part of the data, such as muscle strength, whole muscle hypertrophy, and aerobic fitness has been published elsewhere (10). The current study investigated the effects of 8 weeks of CT, ST, and interval training (IT) regimens on muscle fiber hypertrophy. We also sought to investigate the effects of these training regimens on MSTN signaling pathway–related gene expression. Muscle biopsies were performed at baseline and 48 hours after the last training session. Additionally, as the order of the strength and endurance exercises within the CT training regimen may affect the occurrence of the acute interference effect because of the residual fatigue induced by the exercise mode performed first in the training session (8,9), the order of the exercises was alternated every session for the CT group.

Subjects

Forty-three active male physical education students, undergoing neither strength nor aerobic training for at least 6 months before the experimental period, volunteered for this study. However, after the commencement of the current study, 6 participants withdrew because of personal reasons not related to training procedures. None of the individuals had previous ST experience. Initially, participants were classified into quartiles according to their quadriceps CSA (mm^2). Then, participants from each quartile were randomly assigned to the following experimental groups: ST ($n = 11$, age: 25.9 ± 6.4 years, body mass: 73.5 ± 16.1 kg, height: 172.0 ± 4.3 cm); IT ($n = 8$, age: 24.0 ± 7.5 years, body mass: 71.5 ± 7.7 kg, height: 174.6 ± 7.9 cm); CT ($n = 11$, age: 22.5 ± 3.9 years, body mass: 72.9 ± 9.8 kg, height: 176.0 ± 8.1 cm); and control (C; $n = 7$, age: 22.1 ± 2.4 years, body mass: 82.2 ± 25.0 kg, height: 177.0 ± 4.8 cm). On average, participants from each group completed 95.8% of the training sessions (e.g., 15.3 sessions per group). There were no significant differences between groups for age, body mass, height, and quadriceps CSA in the pretest assessments (1-way analysis of variance [ANOVA], $p > 0.05$). Participants had neither any health issues nor neuromuscular disorders that could affect their ability to complete the training protocols. In addition, participants were asked to refrain from any additional exercise during the experimental period. The current study was approved by the Institution's Ethics Committee, and all the subjects were informed of the inherent risks and benefits before signing an informed consent form.

Familiarization Sessions

Participants completed 4 familiarization sessions before the commencement of the experimental protocols. Initially, they performed a general warm-up consisting of a 5-minute run at 9 $km \cdot h^{-1}$ on a treadmill (Movement Technology; Brudden, São Paulo, Brazil) followed by 3 minutes of light stretching exercises. After warming-up, the participants were familiarized with the leg-press maximum dynamic strength (1 repetition maximum [1RM]) test protocol. First, they were seated in a standard 45° leg-press machine (Nakagym, São Paulo, Brazil) and asked to place both feet in a self-selected position. The area of the leg-press platform was divided into 10 cm squares to keep record of the feet location. Feet position was reproduced during all the familiarization and testing sessions. Then, the machine was unlocked and the platform was lowered until a knee angle of 90°. The position of the leg-press platform at a 90° knee angle was annotated in a measuring tape fixed on the side of the sliding track. A plastic device was then fixed at the recorded centimeter to assure a correct range of motion on each repetition, on each training and testing sessions. The repetition started at complete knee extension; participants then lowered the platform until it touched the plastic device, returning to full extension. Participants had 5 attempts to achieve an estimation of the leg-press 1RM. The within-subject variance in the leg-press 1RM values was <5% between familiarization sessions 3 and 4.

Pre- and Posttests

Maximum Dynamic Strength (1RM). The leg-press 1RM test was performed as follows, participants ran for 5 minutes on a treadmill (Movement Technology; Brudden) at 9 $km \cdot h^{-1}$, followed by 3 minutes of lower limb light stretching exercises and 2 warm-up sets of the leg-press exercise. In the first set, the participants performed 8 repetitions with a load correspondent to 50% of their estimated 1RM obtained during the familiarization sessions. In the second set, they performed 3 repetitions with 70% of their estimated 1RM. A 2-minute interval was allowed between warm-up sets. After the completion of the second set, participants rested for 3 minutes and then had up to 5 attempts to achieve their leg-press 1RM. A 3-minute interval was adopted between attempts. All the tests were conducted by an experienced

researcher, and strong verbal encouragement was provided during the test.

Aerobic Fitness. The maximal oxygen uptake ($\dot{V}O_2max$) of the subjects was measured using a gas analyzer ($Quark_{b2}$; Cosmed, Rome, Italy) while running in a motorized treadmill (Sper ATL, Inbrasport; Porto Alegre, Brazil). Before each test, the gas analyzer was calibrated using ambient air and a gas of a known composition (20.9% O_2 and 5% CO_2). The turbine flowmeter was calibrated using a 3-L syringe (Quinton Instruments, Seattle, WA, USA). The test started at 6 $km \cdot h^{-1}$ with increments of 1.2 $km \cdot h^{-1} \cdot min^{-1}$, until exhaustion. Heart rate was monitored during the test with a heart rate transmitter (model S810; Polar Electro Oy, Kempele, Finland) coupled with the gas analyzer. Throughout the test, the participants wore a mask (Hans Rudolph, Kansas City, MO, USA) connected to the gas analyzer for breath-by-breath measurements of gaseous exchange. $\dot{V}O_2max$ was defined when 2 or more of the following criteria were met: (a) an increase in $\dot{V}O_2$ of less than 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ between 2 consecutive stages, (b) a respiratory exchange ratio greater than 1.1, (c) a blood lactate concentration higher than 8.0 $mmol \cdot L^{-1}$, and (d) ± 10 $b \cdot min^{-1}$ of the predicted maximal heart rate (i.e., 220-age). $\dot{V}O_2$ data were smoothed by averaging 10 seconds intervals and $\dot{V}O_2max$ was obtained from the average of the 3 highest values obtained during the test. In addition, the time taken to exhaustion was recorded as an endurance performance variable. Verbal encouragement was provided to ensure a maximal effort.

Diet Control. Participants were instructed to maintain their normal diet and to refrain from taking nutritional supplements. Moreover, they were asked to record and reproduce their meals throughout a 48-hour period before the pre- and posttraining muscle biopsies. All the subjects were offered a standard breakfast approximately 2 hours before the muscle biopsy (approximately 311 kcal; 63.5% carbohydrates, 21.8% proteins, and 14.7% fat). All the tests and muscle biopsies were performed at the same time of the day.

Muscle Biopsies. Pre- and posttraining muscle samples were taken from the midportion of the vastus lateralis of the participants' dominant leg using the percutaneous biopsy technique with suction. Muscle specimens were dissected free from blood and connective tissue and washed in deionized water and divided in 2 samples. The muscle sample used for fiber analysis was oriented in tragacanth gum, frozen in isopentane cooled by liquid nitrogen and then frozen in liquid nitrogen and stored at $-80°$ C for further analysis. The other sample was stored at $-80°$ C for mRNA analysis. The pre- and posttraining biopsies were taken, respectively, 4 days before the start of training and 48 hours after the last training session. The posttraining sample was obtained from a site 2 cm proximal to the pretraining scar.

Muscle Fiber Type and CSA. Frozen muscle samples were cut into 10-μm cross sections using a cryostat (Criostat Mícron HM505E, Walldorf, Germany). Muscle sections were then incubated for myofibrillar ATPase activity after acid preincubation (mATPase, pH 4.6) as previously described (5). The myosin ATPase reaction was used to identify the muscle fiber type. Type I fibers reacted deeply after acid preincubation at pH 4.6, and the inverse occurred with type II muscle fibers. A single researcher blinded to the training condition evaluated fiber typing and fiber cross-sectional area in the whole muscle tissue sample at $\times 100$ magnification and further analyzed on a digitizing unit connected to a computer (Image Pro-plus; Media Cybernetic, Silver Spring, MD, USA). The total number of each fiber type was counted to calculate the fiber type distribution (percentage of I, IIa, and IIx fiber types). The average muscle fiber number per participant was 284.9 $\pm$ 139.9. Cross-sectional areas of muscle fiber types I, IIa, and IIx were determined using computerized planimetry (Image Pro-plus; Media Cybernetics).

Real-Time Polymerase Chain Reaction. Total cellular RNA was isolated from pre- and posttraining muscle samples (approximately 25 mg) by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA (1 μg) was used in a reaction containing oligo-DT (0.5 $\mu g \cdot \mu L^{-1}$), 10 mM each dNTP, 5 $\times$ first-strand buffer, 0.1 M dithiothreitol and 200 U reverse transcriptase (SuperScript II; Invitrogen). Primer sets were designed by Applied Biosystems (Foster City, CA, USA) (Table 1). The results were expressed by using the comparative cycle threshold (C_T) method described in the manufacturer's user bulletin no.2 (Applied Biosystems). The C_T represented the polymerase chain reaction cycle at which an increase in reporter gene fluorescence above a baseline signal could first be detected. The ΔC_T values were calculated in every sample for each gene of interest as follows: ($C_{T\ Target}$ $C_{T\ Endogenous\ control}$). The RPLP0 (ribosomal protein large P0) was used as the endogenous control gene. This gene has been previously used as endogenous control in exercising humans (34). The calculations of the relative changes in the expression levels of 1 specific gene were performed by subtracting ΔC_T of the pretest condition (used as the calibrator) from the corresponding ΔC_Ts from the 2 experimental groups. The values and ranges given were determined as follows: $2^{-\Delta\Delta CT}$ with $\Delta\Delta C_T \pm SD = (C_{T\ Target}\ C_{T\ Endogenous\ control})_{Posttest}$ $(C_{T\ Target}\ C_{T\ Endogenous\ control})_{Pretest}$ (*SD* is the standard deviation of the mean $\Delta\Delta C_T$ value; user bulletin no.2, Applied Biosystems). Expression values were shown as mean plus *SD* as fold differences relative to the pretest condition, which was arbitrarily set to 1.

Training Programs

Details regarding the training programs have been published elsewhere (10). In brief, the ST group training aimed at producing muscle hypertrophy. The target training intensity ranged from 6 to 12 RM, and from 3 to 5 sets per exercise,

Table 1. Sequence of primers used in real-time polymerase chain reaction.

Genes	Forward	Reverse
ActIIb	GTACGAGCCACCCCCGACAGC	AGCGCCCCCGAGCCTTGAT
FOXO-3a	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
FLST-3	CCAGGCTGGGAACTGCTGGC	TCTCTGCTCCCCACCTCTAAGT
GASP-1	GGATTTCTGGAGGCCTGCTT	TCCAGAGGTGTGAGCCAGTCT
MSTN	GACCAGGAGAAGATGGGCTGAATCCGTT	GCTCATCACAGTCAAGACCAAAATCCCTT
SMAD-7	CAGATACCCGATGGATTTTCTCA	CCCTGTTTCAGCGGAGGAA

for the leg-press 45°, knee extension, and knee flexion exercises. All the exercises were performed at constant-speed eccentric and concentric muscle actions, and through a 90° range of motion at the knee joint. The subjects in the IT group performed high-intensity IT on a treadmill. The targeted training intensity was 80–100% of the speed needed to elicit $\dot{V}O_2max$ ($_v\dot{V}O_2max$). Concurrent training group performed the same ST and IT training protocols described

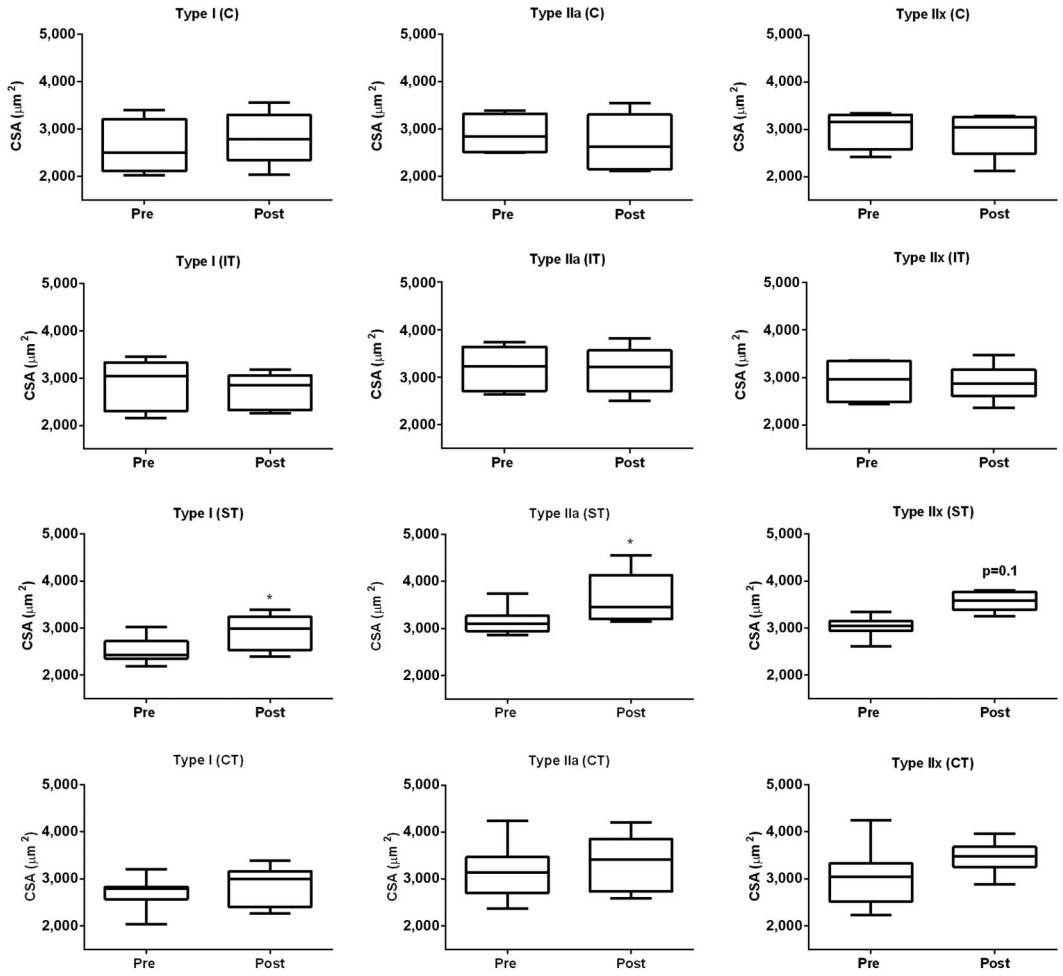

Figure 1. Muscle fibers types cross-sectional area responses before (Pre) and after (Post) the training regimens. *$p \leq 0.05$ for within-group comparisons (Pre vs. Post). C = control; CSA = cross-sectional area; CT = concurrent training; IT = interval training; ST = strength training.

above, at the same session. The order that the participants performed the strength and aerobic exercises within a given session was balanced (i.e., half of the participants performed the strength exercises first and the other half performed the aerobic exercise first) and altered during the training period to avoid any order effect in the muscle tissue molecular analyses. The time interval between the strength and the aerobic exercises within a session was no more than 5 minutes. All the experimental groups trained twice a week for 8 weeks.

Statistical Analyses

After normality (i.e., Shapiro-Wilk) and variance assurance (i.e., Levene), a mixed model was performed for each dependent variable, assuming group and time as fixed factors, and subjects as a random factor (SAS 9.2, SAS Institute Inc., Cary, NC, USA) (35). Whenever a significant F value was obtained, a post hoc test with a Tukey's adjustment was performed for multiple comparison purposes. Whenever p-values of the F tests indicated a trend toward significant values, the relative change from pre- to posttraining was calculated for each participant and a 1-way ANOVA was used to compare the groups (i.e., $\dot{V}O_2max$ and time to exhaustion). A Tukey's post hoc test was used for the multiple comparisons when necessary. The significance level was set at $p \leq 0.05$. Results are expressed as mean ± *SD*.

Results

Muscle Fiber CSA and Distribution

Only the ST group significantly increased vastus lateralis muscle fiber CSA. The type IIa and type I muscle fiber area increased by 17.08% ($p = 0.03$) and 17.9% ($p = 0.05$), respectively. The increase in type IIx muscle fiber area (19.3%) did not reach significance from pre- to posttest assessments ($p = 0.10$). Importantly, the changes in muscle fiber CSA in the CT group did not reach statistical significance (IIx = 13.1%; IIa = 6.9%; I = 6.3%, $p \geq 0.26$). No significant changes in muscle fibers CSA were detected for the C and IT groups ($p \geq 0.35$) (Figure 1). There were no differences in fiber type distribution ($p \geq 0.35$) (Table 2).

Gene Expression

The MSTN, FLST-3, ActIIb, GASP-1, and FOXO-3a mRNA levels remained unchanged across conditions and time after the training period ($p \geq 0.10$) (Figures 2A–D, F). A significant increase in the SMAD-7 mRNA gene expression was observed at the posttest in the ST (53.9%; $p = 0.007$) and CT (39.3%; $p = 0.05$), whereas no differences were observed in the SMAD-7 for IT ($p = 0.99$) and C ($p = 1.00$) groups (Figure 2E).

Muscle Strength

The ST and CT groups increased leg-press 1RM similarly from pre- to posttest by 17.6% ($p = 0.001$) and 18.5% ($p = 0.001$), respectively. In addition, ST and CT showed greater maximum dynamic strength values than C group in the posttest ($p = 0.001$ and $p = 0.001$, respectively). There were no

Table 2. Muscle fibers types distribution before (pre) and after (post) the training regimens.

Variable	Pre to post			
	C	IT	ST	CT
Type I, %	47.3 ± 12.2 to 49.9 ± 9.1	44.1 ± 4.1 to 42.6 ± 6.6	46.5 ± 7.8 to 46.2 ± 8.8	51.6 ± 12.4 to 44.9 ± 13.3
Type IIa, %	51.3 ± 12.4 to 46.1 ± 10.6	43.2 ± 7.3 to 47.1 ± 6.7	47.06 ± 7.0 to 9.6 ± 8.2	44.0 ± 10.5 to 50.0 ± 12.6
Type IIx, %	1.3 ± 2.5 to 5.2 ± 2.9	12.5 ± 7.8 to 9.4 ± 8.3	6.3 ± 7.8 to 4.1 ± 3.6	4.3 ± 3.2 to 5.7 ± 6.4

C = control; CT = concurrent training; IT = interval training; ST = strength training.

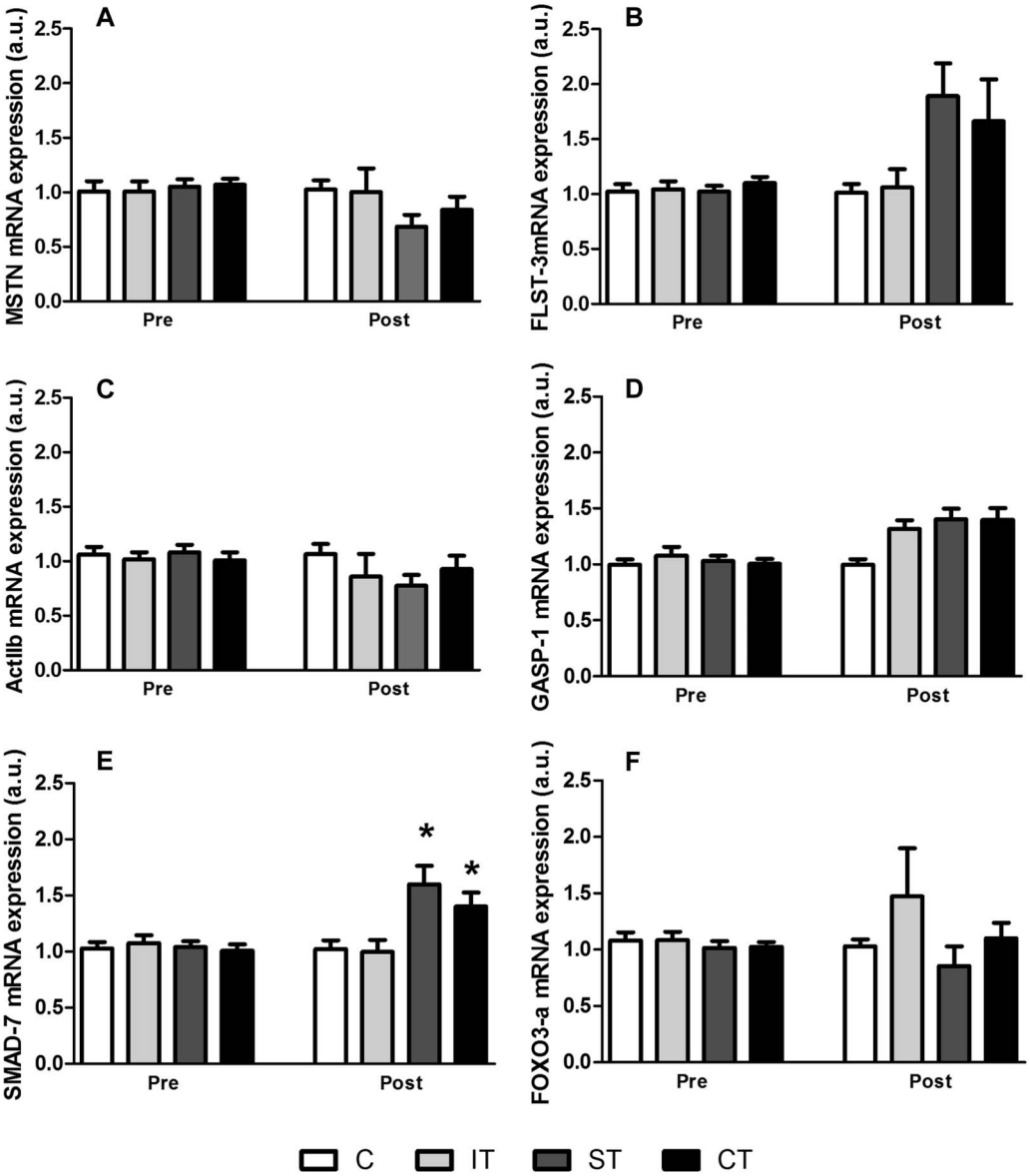

Figure 2. mRNA expression before (Pre) and after (Post) the training regimens. MSTN mRNA gene expression (A), FLST-3 mRNA gene expression (B), ActIIb mRNA gene expression (C), GASP-1 mRNA gene expression (D), SMAD-7 mRNA gene expression (E), FOXO-3a mRNA gene expression (F). *$p \leq 0.05$ for within-group comparisons (Pre vs. Post). a.u. = arbitrary units; C = Control; CT = concurrent training; IT = interval training; ST = strength training.

significant changes from pre- to postassessments in the leg-press 1RM in the IT ($p = 0.93$) and C groups ($p = 0.99$).

Aerobic Fitness

$\dot{V}O_2$max improved by 5.0% ($p = 0.003$) and 15.0% ($p = 0.003$) (pre- to posttest difference) in groups IT and CT, respectively. There were no significant differences in maximal aerobic power between IT and CT ($p \geq 0.26$). Groups ST and C did not increase maximal aerobic power (−1.3 and −7.0%), respectively ($p \geq 0.62$).

Discussion

The current study investigated the effects of CT, ST, and IT regimens on the muscle fiber hypertrophic response and on the gene expression of components of the MSTN pathway. We hypothesized that if negative interference in type I muscle fiber CSA was observed in the CT, then the MSTN signaling pathway would be involved in differences on CSA responses between CT and ST groups. Our findings do not support the proposed hypothesis. Muscle fiber type IIa and

type I CSA increased from pre- to posttest only in the ST group (17.08 and 17.9%, respectively). Although there was no significant hypertrophy at the muscle fiber level in CT, muscle strength increments did occur. The novel finding of the present study was that despite the differences in muscle fiber CSA increments between ST and CT groups, there were similar MSTN pathway responses in both groups. In addition, contrary to other studies, the interference phenomenon did not affect type I fibers only (20,29), as the low-volume CT protocol used herein blunted muscle fiber hypertrophy across the whole spectrum of fiber types. Finally, a collateral and somewhat unexpected finding of the present study was the lack of support to the suggestion that the exercise order within training sessions would be responsible for the CT negative interference effect. Accordingly, we did not observe significant muscle fiber hypertrophy in the CT group, although we attempted to mitigate the interference effect by alternating the order of the strength and aerobic exercises within training sessions.

In the current study, we hypothesized that MSTN might be considered as a potential target in the assessment of the CT-induced negative interference on muscle fiber CSA. The only significant increase was in the SMAD-7 mRNA observed at the posttest in the ST (53.9%; $p = 0.007$) and the CT (39.3%; $p = 0.05$), which negatively regulates the MSTN gene expression. In this regard, our results indicate that gene expression of selected regulatory genes involved in the MSTN signaling (e.g., FLST-3, ActIIb, GASP-1, and FOXO-3a) was not significantly altered across time in any of the training groups. These findings are interesting as the MSTN gene expression and their regulatory genes have demonstrated conflicting behavior in response to different ST protocols (18,22,32,37). For instance, Willoughby (37) found an increase in the MSTN gene expression (approximately 32%) after a 12-week ST period performed at 85–90% 1RM. In addition, the authors have also found an increase in the follistatin-like related gene (FLRG) and a decrease in the ActIIb, which might have inhibited MSTN from binding to its receptor, as muscle volume gain was not impaired. Conversely, Laurentino et al. (22) showed decreases in MSTN mRNA without changes in the ActIIb gene expression after 8 weeks of high-intensity ST at 80% 1RM, as well as a 6.3% increase in whole muscle CSA. Furthermore, similar to our findings, Jespersen et al. (18) showed increases in muscle fiber CSA with no significant changes in MSTN gene expression after 12 weeks of ST with the training load varying from 6 to 15RM. Importantly, the studies that reported decreases in MSTN mRNA used higher training intensities (at least constant high intensity) than the ones that did not found a similar response.

GASP-1 and SMAD-7 genes also play important roles in regulating skeletal muscle hypertrophy. For instance, inhibiting MSTN activity through GASP-1 may play an important role in increasing muscle fiber CSA (16,31). Although no significant differences were found in GASP-1 at posttest, Laurentino et al. (22) demonstrated a significant increase in GASP-1 gene expression (i.e., 79%) after 8 weeks of high-intensity ST. Conversely, our results showed increased SMAD-7 gene expression in both the ST and CT groups (53.9 and 39.3%, respectively). The downregulation of SMAD-7, induced by small-interfering RNA, seems to increase MSTN mRNA, which suggests that SMAD-7 hampers MSTN gene expression (13). Our results are in accordance with Laurentino et al. (22) who showed an increase in SMAD-7 (i.e., 86%) after 8 weeks of high-intensity ST. Curiously, despite increases in SMAD-7 in the ST and CT regimens, muscle fiber hypertrophy was impaired only in the last.

We are the first to show MSTN pathway responses after CT (i.e., strength and endurance). Although the consensus that MSTN is a potent downregulator of muscle mass, the role of MSTN and their regulatory genes on muscle accretion, after ST, CT, and endurance training (ET) is still under scrutiny. For instance, Schiffer et al. (32) investigated the effects of 12 weeks of either ST with the training load at 70–80% 1RM or continuous ET at 80% of the heart rate. Similarly to the present study, no differences were observed on MSTN gene expression between ST, ET, and CT regimens. In addition, MSTN downregulation after ST cannot differentiate the hypertrophic responses in humans. For example, Kim et al. (19) demonstrated large variability in muscle fiber CSA after 16 weeks of ST despite similar MTSN gene expression among individuals. Collectively, the aforementioned data are suggestive that MSTN and their regulatory genes at transcript level cannot differentiate muscle fiber hypertrophic responses between CT and ST regimens in humans.

Muscle fiber hypertrophy is a common adaptation after ST (6,12,21). Accordingly, we observed a significant increase in type IIa and type I muscle fiber CSA after 8 weeks of ST (6,18). However, muscle fibers CSA did not significantly change in the CT group. In respect to CT, our findings are in accordance with Bell et al. (3) who found that resistance training coupled with 3 $d \cdot wk^{-1}$ of ET (2 continuous and 1 interval) blunted muscle fiber hypertrophy in both type I and type II fibers after 6 weeks of CT training when compared with a ST regimen. Kraemer et al. (20) also investigated the effects of CT vs. ST on muscle fiber adaptations. They found that ST concurrently combined with 2 $d \cdot wk^{-1}$ of continuous high-volume running, blunted hypertrophy in type I and IIc muscle fibers when compared with 4 days of ST alone. Despite the results by Bell et al. (3), the findings have been extended to type I fibers in response to a 3-$d \cdot wk^{-1}$ continuous ET combined with ST, as compared with ST alone (26,29). Currently, our results demonstrated that short-term CT regimen performed at low volume and frequency (i.e., 2-$d \cdot wk^{-1}$) blunted muscle fiber hypertrophy across the whole spectrum of fibers in active men.

In addition, our previous findings (10) are in agreement with those of McCarthy et al. (26) showing that despite the

fact that no whole muscle hypertrophy interference was observed in CT, type I muscle fiber CSA did not change when compared with pretraining values (26). Collectively, the current results along with those of McCarthy et al. (26) and de Souza et al. (10) reveal that, regardless of the interference at the fiber level, the whole muscle hypertrophy and the functional adaptations (i.e., muscle strength) demonstrate similar responses after short-term and low-volume CT and ST regimens. A possible explanation for such findings may be the increase in Akt and trend toward higher $p70^{S6K1}$ ($p = 0.06$) activity reported after ST in our previous study (10). However, the mechanism responsible for the Akt and $p70^{S6K1}$ blunted response after CT should be further addressed.

In conclusion, our data demonstrated that despite similar strength and MSTN pathway gene expression responses, low-volume CT blunted myofiber CSA adaptations when compared with low-volume ST. Our findings are suggestive that MSTN and their regulatory genes at transcript level cannot differentiate muscle fiber CSA responses between CT and ST regimens in humans. Nonetheless, a number of questions regarding what causes the interference phenomenon still remain. For instance, at what point will the interference effects at muscle fiber level reflect at the whole muscle hypertrophy? It is conceivable that either greater training periods or volumes are needed to realize these effects. In addition, we cannot exclude that myofiber adaptation anticipates a long-term CT reduction in total muscle strength, warranting further studies on the topic.

Practical Applications

Collectively, our results demonstrate that in athletes and conditioning practitioners who need to develop strength and aerobic fitness simultaneously, short-term CT may be used without blunting functional adaptations. However, coaches and athletes should keep in mind that the long-term effects of low-volume CT on functional capacity remain anecdotal.

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