

Molecular Adaptations to Concurrent Training

Authors

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Key words

- maximum dynamic strength
- muscle hypertrophy
- interval training

Abstract

This study investigated the chronic effects of concurrent training (CT) on morphological and molecular adaptations. 37 men (age=23.7±5.5 year) were divided into 4 groups: interval (IT), strength (ST) and concurrent (CT) training and a control group (C) and underwent 8 weeks of training. Maximum strength (1RM) and muscle cross-sectional area (CSA) were evaluated before and after training. Muscle samples were obtained before the training program and 48 h after the last training session. VO_{2max} improved in 5±0.95% and 15±1.3% (pre- to post-test) in groups CT and IT, respectively, when compared to C. Time to exhaustion (TE) improved from pre-

post-test when compared to C (CT=6.1±0.58%; IT=8.3±0.88%; ST=3.2±0.66%). 1RM increased from pre- to post-test only in ST and CT groups (ST=18.5±3.16%; CT=17.6±3.01%). Similarly, ST and CT groups increased quadriceps CSA from pre- to post-test (6.2±1.4%; 7.8±1.66%). The p70S6K1 total protein content increased after CT. The ST group showed increased Akt phosphorylation at Ser473 (45.0±3.3%) whereas AMPK phosphorylation at Thr172 increased only in IT group, (100±17.6%). In summary, our data suggest that despite the differences in molecular adaptations between training regimens, CT did not blunt muscle strength and hypertrophy increments when compared with ST.

Introduction

The usage of exercises aiming to develop aerobic power/capacity and muscle strength throughout a training period is referred to as concurrent training (CT) [24]. This training mode is thought to hamper some training adaptations, such as increases in muscle strength and muscle fiber hypertrophy, when compared to isolated strength training (ST) [19,24,34]. The reduced gains in muscle strength and muscle fiber hypertrophy after a CT period seem to be dependent on training variables such as frequency and volume [24] and the type of the endurance exercise (i.e., continuous or interval training) performed before the strength-training bout [24,27]. Among several proposed theories for the diminished training adaptations [12,28], a molecular hypothesis has been put forward to explain the reduced training adaptations after CT. This hypothesis suggests that CT may simultaneously activate competing intracellular pathways, resulting in interference between the strength and endurance training-induced adaptations [2,8,32].

Initially, Atherton et al. [2] mimicked endurance and strength exercises through low- and high-frequency electrical stimulation, respectively, to assess the activation of the AKt/mTOR/p70^{S6k1} and AMPK pathways in rats. The high-frequency stimulation increased the phosphorylation of selected proteins of the AKt/mTOR/p70^{S6k1} pathway, which is highly correlated to the increase in muscle protein synthesis and accretion. On the other hand, the low-frequency electrical stimulation produced a reduction in the muscle cell energy level (i.e., ATP/AMP ratio) [2]. A decrease in the cell energy stores activates 5'AMP-activated protein kinase (AMPK), which controls cell energy status and mitochondrial biogenesis [33]. Based on Atherton et al.'s [2] findings, some review studies have attributed the CT-induced interference effect to the molecular events described above. Endurance exercises decrease the ATP/AMP ratio producing the phosphorylation of AMPK (p-AMPK) [8,30,32] and inhibiting anabolic processes that demand ATP breakdown, such as protein synthesis [5]. In fact, it has been suggested that p-AMPK attenuates mTOR phos-

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phorylation, downregulating its downstream targets (e.g. p70^{S6K1}) [7,36]. In addition, Dreyer et al. [13] showed that the AMPK activity remained elevated not only after endurance exercise but also after strength exercise, thus hampering muscle protein synthesis.

Nevertheless, to the best of our knowledge, no study has examined these molecular responses in a CT regimen in humans. We hypothesized that a CT regimen may increase AMPK activation and possibly decrease muscle hypertrophy when compared to an isolated ST regimen. Furthermore, as CT may hamper muscle hypertrophy when compared to ST, it may also impair the maximum strength. Therefore, the purpose of this study was to investigate the chronic effects of CT on strength and skeletal muscle hypertrophy as well as phosphorylation of selected AMPK and Akt/mTOR/p70^{S6K1} pathway proteins.

Methods



Participants

43 active male physical education students, not undergoing regular strength and aerobic power/capacity training for 6 months before the experimental period, volunteered for this study. None of the individuals had strength training experience. Initially, participants were classified into quartiles according to their quadriceps cross-sectional area (CSA, mm²) and then participants from each quartile were randomly assigned to the experimental groups. 6 subjects did not complete the experimental protocol due to personal reasons, leading to the following groups. Strength training (ST n=11, age: 25.9±6.4 years, body mass: 73.5±16.1 kg, CSA: 8332.4±893.3 mm², height: 1.72±4.3 m); interval training (IT, n=8, age: 24.0±7.5 years, body mass: 71.5±7.7 kg, CSA: 8390.3±817.5 mm², height: 174.6±7.9 m); concurrent training (CT, n=11, age: 22.5±3.9 years, body mass: 72.9±9.8 kg, CSA: 8340.8±1000.0 mm², height: 176.0±8.1 m); and control (C, n=7, age: 22.1±2.4 years, body mass: 82.2±25.0 kg, CSA: 8347.3±1643.0 mm², height: 1.77±4.8 m) groups. There were no significant differences between groups for age, body mass, height, and CSA in the pre-test assessments ($P>0.05$). Participants were free from health problems and/or neuromuscular disorders that could affect their ability to complete the training protocol. The study was conducted according to the ethical standards of the International Journal of Sports Medicine, described by [18] and was approved by the Institution's Ethics Committee, and all subjects were informed of the inherent risks and benefits prior to signing an informed consent form.

Experimental design

The study was designed to investigate the molecular and functional adaptations of 8-wks of ST, IT and CT regimens performed twice a week. Muscle biopsy and leg press 1 RM test were performed before the experimental period. As the order of the strength and endurance exercises within training sessions may affect the occurrence of the interference effect [9,10] the order of the strength and endurance exercises was alternated between sessions (i.e. interval training routine followed by strength training routine, and vice-versa). In addition, the order of the exercises was balanced between the individuals of the concurrent training group (i.e. half of the participants performed the strength training routine first, and the other half performed the interval training routine first). Muscle biopsy (~48 h after the last training session) and leg press 1 RM test were also per-

formed after the experimental period. Participants were instructed to refrain from performing additional exercise during the experimental period. There was a partial control of the nutritional intake during the study. The participants were oriented to maintain their normal diet, as well as to refrain from taking nutritional supplements. Moreover, they were asked to record and reproduce their meals throughout a 48 h-period before each muscle biopsy. Participants were offered a standard breakfast approximately 2 h before the muscle biopsy. All tests and the muscle biopsies were performed at the same time of the day.

Familiarization sessions

All of the participants completed 4 familiarization sessions. In these sessions, participants got acquainted with the experimental procedures used in the present study. Initially, participants performed a general warm-up consisting of 5 min running at 9 km·h⁻¹ on a treadmill (Movement Technology®, Brudden, São Paulo, Brazil) followed by 3 min of light stretching exercises. After warming up, the participants were familiarized with the leg-press 1RM test protocol. First, they were seated in the machine and placed 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 both in the familiarization and testing sessions. Then the machine was unlocked and the platform was lowered up to a knee angle of 90°. The position of leg press platform at a 90° knee angle was annotated on 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 for each training and testing session. The repetition started at complete knee extension, participants lowered the platform until it touched the plastic device and then returned to full extension. Participants had 5 attempts to achieve an estimation of the leg press 1 RM. The inter-day variance was <5% between familiarization sessions 3 and 4.

Performance tests

Strength test: In order to determine the maximum dynamic strength (1RM test) in the 45° leg press exercise, the 1RM test was performed as follows, participants ran for 5-min on a treadmill (Movement Technology®, Brudden, São Paulo, Brazil) at 9 km·h⁻¹, followed by 5 min of lower limb stretching exercises. Then they performed 2 warm-up sets of leg press exercise. In the first set, participants performed 5 repetitions with 50% of the estimated 1RM and in the second set performed 3 repetitions with 70% of the estimated 1RM. A 3-min resting interval was granted between sets. 3 min after the warm-up, participants were requested to execute up to 5 trials to obtain the 1RM load (e.g. maximum weight that could be lifted once with a proper technique), with a 3-min interval between attempts [6]. Strong verbal encouragement was given throughout the test.

Maximal Incremental Test: maximal oxygen uptake (VO_{2max}) was measured on a motorized treadmill (Sper ATL, Inbrasport®, Porto Alegre, Brazil), using a gas analyzer (Quark_{b2}, Cosmed®, Rome, Italy). Before each test, the device was calibrated using ambient air and a gas of a known composition containing 20.9% O₂ and 5% CO₂. The turbine flowmeter was calibrated using a 3-L syringe (Quinton Instruments, Seattle, WA, USA). The test started at 6 km·h⁻¹ with increments of 1.2 km·h⁻¹ per minute, 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)

Table 1 Strength and interval training progressions throughout 8 weeks.

Strength Training				
weeks	1–2	3–4	5–6	7–8
intensity	12 RM	8–10 RM	6–8 RM	10–12 RM
sets	3	4	5	3
total volume	36	32–40	30–40	30–36
rest interval	90 s	120 s	120 s	120 s
Interval training				
intensity	80%	85–90%	95%	95–100%
	vVO _{2max}	vVO _{2max}	vVO _{2max}	vVO _{2max}
bouts	20	20	20	15
bout time	60 s	60 s	60 s	60 s
rest interval	45 s	60 s	60 s	90 s

connected to the gas analyzer for breath-by-breath measurements of gaseous exchange. VO_{2max} was defined when 2 or more of the following criteria were met: an increase in VO₂ of less than 2.1 ml·kg⁻¹·min⁻¹ between 2 consecutive stages, a respiratory exchange ratio greater than 1.1, a blood lactate concentration higher than 8.0 mmol·l⁻¹, and a ± 10 bpm of the predicted maximal heart rate (i.e. 220-age) [21]. The data was smoothed averaging the data over 10-s intervals and VO_{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 that maximal values were reached.

Muscle cross-sectional area

Quadriceps cross-sectional area was obtained through magnetic resonance imaging (MRI) (Signa LX 9.1, GE Healthcare, Milwaukee, WI, USA). Subjects lay in the device in a supine position with straight legs. A bandage was used to restrain leg movements during image acquisition. All images were captured from both legs. An initial image was captured to determine the perpendicular distance from the greater trochanter to the inferior border of the lateral epicondyle of the femur, which was defined as thigh length. Quadriceps cross-sectional image was acquired at 50% of the segment length with 0.8-cm slices for 3 s. The pulse sequence was performed with a view field between 400 and 420 mm, time repetition of 350 milliseconds, echo time from 9 to 11 milliseconds, 2 signal acquisitions, and matrix of reconstruction of 256×256. The images were transferred to a workstation (Advantage Workstation 4.3, GE Healthcare, Milwaukee, WI, USA) to determine quadriceps cross-sectional area. In short, the segment slice was divided into the following components: skeletal muscle, subcutaneous fat tissue, bone and residual tissue. Then the cross-sectional area of the quadriceps muscle was assessed by computerized planimetry by a blinded researcher.

Diet control and standard breakfast

Participants were oriented to maintain their normal diet and refrain from taking nutritional supplements during the experimental protocol. In addition, they were asked to record their food intake for 2 days prior to the pre-training muscle biopsy. Then they were instructed to duplicate the same food intake for the 2 days prior to the post-training biopsy. Additionally, a standardized breakfast meal (~311 kcal; 63.5% carbohydrates, 21.8% proteins, and 14.7% fat) was offered to all of the subjects 2 h prior to the biopsies in order to minimize any effects of the nutrition on the proteins evaluated.

Muscle biopsy

Pre- and post-training muscle samples were taken from the midportion of the vastus lateralis of the participants' dominant legs using the percutaneous biopsy technique with suction. Muscle specimens were dissected free from blood and connective tissue and washed in deionized water, then frozen in liquid nitrogen and stored at -80 °C for protein extraction. The pre-training and post-training biopsies were taken, respectively, 4 days before the start of training and 48 h after the last training session. The post-training sample was obtained from a site 3 cm proximal to the pre-training incision.

Muscle analyses

Immunoblotting

AMPK, phospho-Thr¹⁷²AMPK (p-AMPK), Akt, phospho-Ser⁴⁷³Akt (p-Akt), p70^{S6K1} and phospho-Thr³⁸⁹-p70^{S6K1} (p-p70^{S6K1}) expression levels were evaluated by immunoblotting in total vastus lateralis extracts. Briefly, samples were subjected to SDS-PAGE in polyacrylamide gels (6–15%) depending upon protein molecular weight. After electrophoresis, proteins were electrotransferred to nitrocellulose membranes (BioRad Biosciences; Piscataway, NJ, USA). Equal gel loading and transfer efficiency were monitored using 0.5% Ponceau S staining of blot membrane. Blotted membrane was then blocked (5% BSA, 10 mM Tris-HCl (pH=7.6), 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated overnight at 4 °C with specific antibodies against AMPK, p-AMPK, Akt, pAkt, p70^{S6K1} and p-p70^{S6K1} (Cell Signaling Tech., MA, USA) and GAPDH (Advanced Immunochemical, CA, USA). Binding of the primary antibody was detected with the use of peroxidase-conjugated secondary antibodies (rabbit or mouse, depending on the protein, for 2 h at room temperature) and developed using enhanced chemiluminescence (Amersham Biosciences, NJ, USA) detected by autoradiography. Quantification analysis of blots was performed with the use of Image J software (Image J based on NIH image). Samples were normalized to relative changes in GAPDH. In addition, AMPK, Akt, and p70^{S6K1} phosphorylated/total ratios were calculated to assess changes in the activity of these proteins.

Training program

The ST group trained aiming at producing muscle hypertrophy. The target strength training intensity ranged from 12 to 6 maximal repetitions (RM) for the leg-press 45°, knee extension and knee flexion exercises throughout 8 weeks, 2 sessions per week (• Table 1). All exercises were performed with constant speed, eccentric and concentric muscle actions, and a 90° range of motion at the knee joint.

The subjects in the IT group performed a high-intensity interval training on a treadmill. The targeted training intensity was 80–100% of the maximal speed where VO_{2MAX} occurs (vVO_{2MAX}). Training took place for 8 weeks, 2 sessions per week (• Table 1). CT group performed the same strength and IT training protocols described above on the same day (• Table 1), for 8 weeks, twice a week. The order in which the participants performed the strength and aerobic exercises within sessions 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 min.

Statistical analysis

After normality (i.e. Shapiro Wilk) and variance assurance (i.e., Levene), a mixed model was performed for each dependent variable, having group and time as fixed factors, and subjects as a random factor [37] (SAS® 9.2). 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 towards significant values, the percentage change from pre- to post-training was calculated for each participant and a one way-ANOVA was used to compare the groups (i.e., VO_{2max} and time to exhaustion). A Tukey post-hoc test was used for the multiple comparisons when necessary. The significance level was set at p<0.05. Results are expressed as mean±standard error (SE).

Results

Maximal strength

The ST and CT groups increased leg-press 1RM similarly from pre- to post-test (p≤0.001) and presented greater maximum strength values than the C group in the post-test (p≤0.001). There were no training effects in leg press 1RM for the IT and C groups (p≥0.93) (Table 2).

Aerobic fitness

Aerobic fitness was significantly improved in the interval-trained groups after training. VO_{2max} was improved in 5±0.95% and 15±1.3% (pre- to post-test) in groups CT and IT, respectively,

(p=0.003 and p=0.003 when compared to the C group). There were no significant differences in maximal aerobic power increments between CT and IT (p≥0.05). All of the training groups presented similar and significant percentage increase in time to exhaustion (TE) when compared to C (CT=6.1±0.58%, p=0.04; IT=8.3±0.88%, p=0.04; ST=3.2±0.66%, p=0.04) (Fig. 1).

Muscle hypertrophy

Left and right legs quadriceps CSA were significantly increased in both the ST (6.2±1.4% and 5.5±1.42%, p≤0.0005) and CT groups (7.8±1.66% and 7.5±1.96%, p<0.0001) in the post-test. Quadriceps CSA was greater in the ST and CT groups compared to the C group in the post-test (p≤0.05). No differences were observed in both the C and IT groups (p≥0.75. and p≥0.18, respectively) (Table 2).

Molecular responses

AMPK total protein content remained unchanged across time in the ST, CT, IT, and C groups (p=0.90). Significantly greater Akt protein content was observed at the post-test in the ST group when compared with the C and IT groups (p≤0.03). The CT group presented a significant pre- to post-training increment in p70^{S6K1} protein content (p=0.04). Additionally, ST and CT groups showed greater p70^{S6K1} protein content when compared with both the C (p≤0.03) and IT (p≤0.01) groups at post-test. The IT group showed increased AMPK phosphorylation from the pre- to the post-training assessment and greater activity when compared with C, CT and ST groups (p=0.01) at the post-testing. The ST group presented a significantly increased Akt phosphorylation

Table 2 Leg press (LP) 1 RM, left (LT) and right thigh (RT) cross-sectional area for the control (C), interval training (IT), strength training (ST), and concurrent training (CT) groups pre- and post-training (mean ± SE).

		C	IT	ST	CT
LP-1RM (kg)	Pre	261.2±56.1	255.4±56.4	270.3±45.5	268.4±47.6
	Post	262.8±60.6	263.8±51.5	320.3±57.0 [†]	315.7±63.5 [†]
LT-CSA (mm ²)	Pre	8347.3±1643.1	8390.3±817.5	8332.4±893.3	8340.8±1000.0
	Post	8556.3±1579.7	8658.2±922.3	8849.5±893.3 [†]	8996.8±919.5 [†]
RT-CSA (mm ²)	Pre	8332.7±1511.6	8483.5±920.9	8215.4±898.8	8261.4±1002.0
	Post	8508.3±1467.4	8756.1±1001.6	8668.0±952.4 [†]	8882.7±868.4 [†]

*- Post-test values greater than pre-test values (p<0.001)

†- Post-test values for the ST and CT groups greater than the C and IT groups (p<0.001)

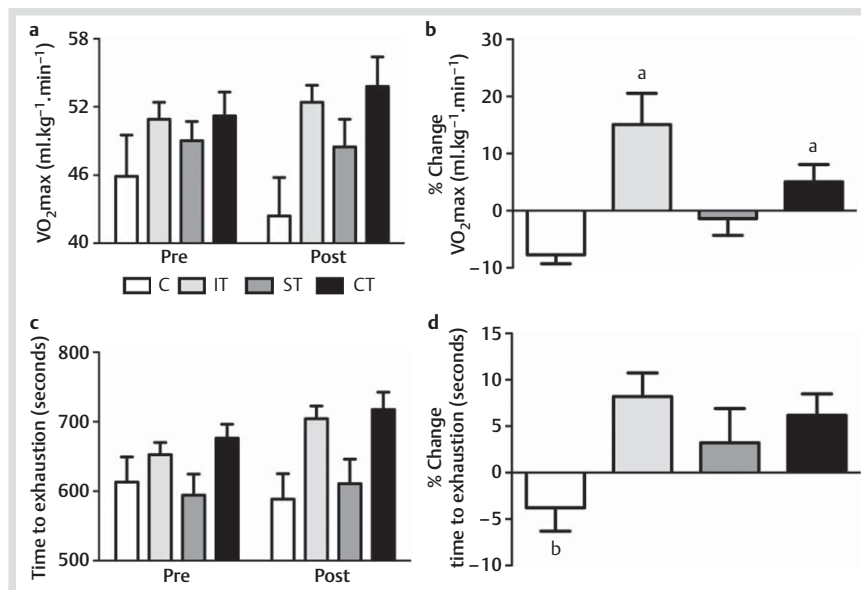


Fig. 1 Aerobic fitness for the control (C), interval training (IT), strength training (ST), and concurrent training (CT) groups. Pre- and Post-training (mean ± SE). **a** – greater than C and ST groups (p<0.05). **b** – lower than IT, ST and CT groups (p<0.05).

over time ($p=0.03$). Moreover, Akt activity was significantly greater in the ST group when compared with both the C and IT groups ($p<0.03$) at the post-test. The ST group presented a trend toward higher p70^{S6K1} phosphorylation ($p=0.06$). The ST group presented significantly greater p70^{S6K1} phosphorylation when compared with the C and the IT groups ($p<0.02$) at the post-test. Finally, there were no changes in AMPK, Akt, and p70^{S6K1} phosphorylated/total ratios from pre- to post-training assessments ($p\geq 0.61$) (○ Fig. 2).

Discussion

The purpose of this study was to investigate the chronic effects of CT on skeletal muscle hypertrophy as well as phosphorylation of selected AMPK and Akt/mTOR/p70^{S6K1} proteins. We hypothesized that in a CT regimen, the activation of the AMPK pathway produced by the IT component would blunt the strength exercise-induced muscle hypertrophy and impair increases in muscle strength. Our findings do not support the proposed hypothesis (○ Fig. 2). Conversely, the novel finding of the present study was that, in humans, the muscle hypertrophy stimuli produced by CT seem to override the AMPK hypertrophy-blunting effect observed in the IT regimen. This data is further supported by the similar muscle strength and hypertrophy gains after CT and ST regimens.

The interval training regimen used in the present study was effective in increasing aerobic fitness in both groups that performed the IT (○ Fig. 1). The participants from the IT and CT

groups covered approximately 5065 m (± 371.5) per training session. The IT protocol used in the present study was based on previous findings that demonstrate: a) a significant superiority of intermittent vs. continuous training regimens in increasing aerobic fitness [16]; and b) the acute interference of the present IT protocol in muscle force production capacity [10]. Despite the expected increase in time to exhaustion in the IT group, the increase in this variable observed in the ST group is in accordance with previous studies demonstrating that maximal strength training may positively affect the TE [35].

Regarding the muscle strength, some studies have reported reduced gains after CT regimens [19,34]. For instance, Hickson [19] and Kraemer et al. [24] presented significant differences in strength gains from pre- to post-training for the ST and CT groups (30% and 19.5%, and 35% and 24%, respectively). However, it should be emphasized that it might be difficult to compare CT studies [19,24] due to some confounding factors, such as the type and the intensity of endurance training. Hickson [19] and Kraemer et al. [24] employed a constant workload and traditional endurance exercise (i.e. continuous running) as a greater part of their endurance regimens. In addition, these authors used a longer duration than the current study (i.e., 10–12 weeks of training) and a very high training volume (5 and 4 sessions of each training mode per week, respectively) [19,24]. Such an unusual high strength training volume may have hampered recovery between training sessions, causing the reduction in strength gains. On the other hand, the low-volume strength training protocol used in our CT regimen produced similar gains

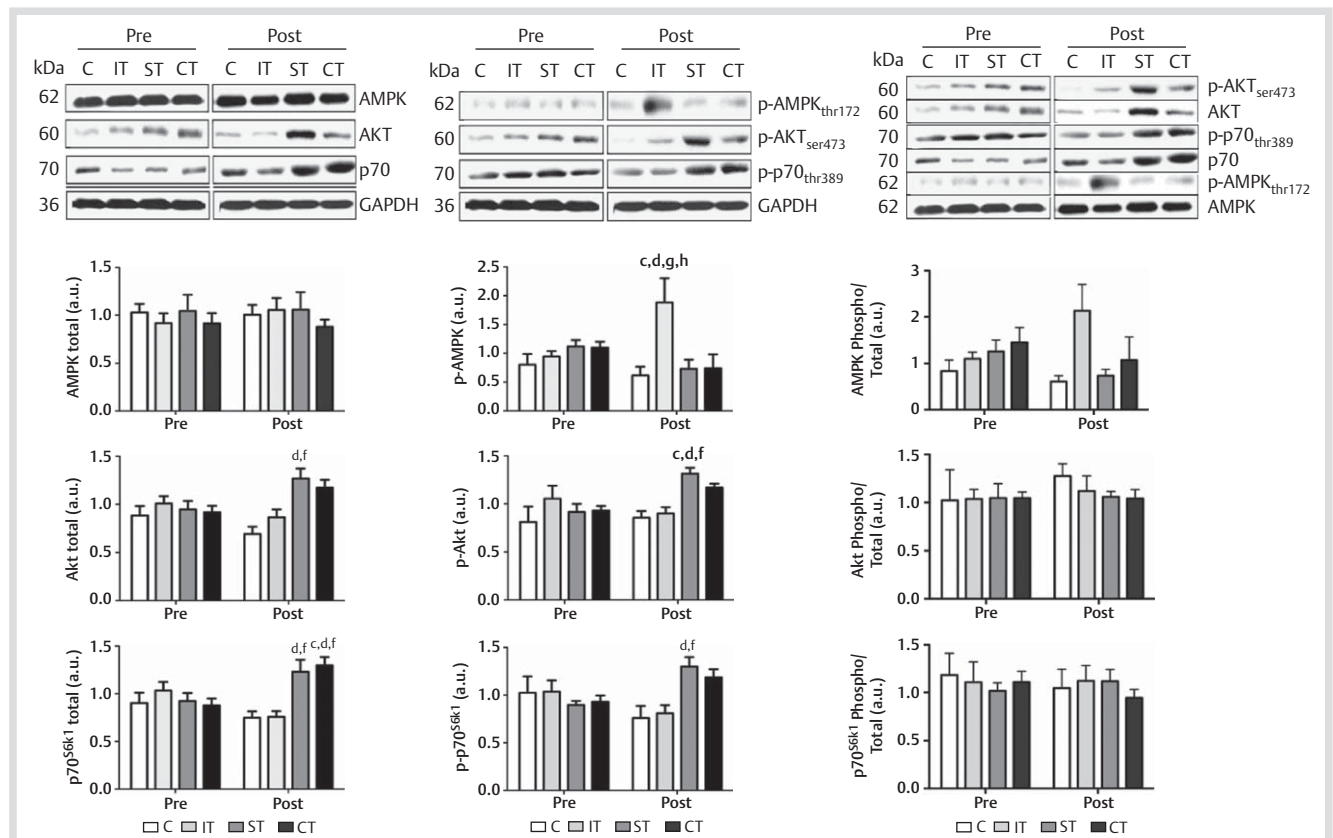


Fig. 2 Total, phosphorylated and phosphorylated/total ratio AMPK, Akt, and p70^{S6K1} protein expression for the control (C), interval training (IT), strength training (ST), and concurrent training (CT) groups. Pre- and Post-training (mean \pm SE). Protein expression was normalized by GAPDH. c – post-training values greater than pre-training values ($p<0.05$). d – post-training values greater than the C group, at the same time point ($p<0.05$). f – post-training values for the ST group greater than for the IT group, at the same time point ($p<0.05$). g – post-training values for the IT group greater than for the ST group, at the same time point ($p<0.05$). h – post-training values for the IT group greater than for the CT group, at the same time point ($p<0.05$).

in strength when compared to the ST, suggesting that training volume/frequency may play a role in the CT-induced impairment on maximal-strength gains. Supporting this concept, Glowacki et al. [15] and McCarthy et al. [31] did not find significant differences in strength increments between groups after a CT training regimen of similar volume/frequency (i.e., between 2 and 3 sessions of each training mode per week) [15,31]. Further, the strength gains presented in both of our groups (i.e. $18.5 \pm 3.16\%$ and $17.6 \pm 3.01\%$ for the CT and ST groups, respectively) (► **Table 2**) are within the range of those reported in the literature.

It has been suggested that CT may also impair skeletal muscle hypertrophy [24,34]. In this regard, the increase in CSA observed in both ST and CT groups (i.e. $6.2 \pm 1.4\%$ and $7.8 \pm 1.66\%$, respectively, ► **Table 2**) corroborates previous findings presented in the literature. For instance, Holm et al. [29] and Laurentino et al. [25] reported increases in quadriceps CSA of $\sim 7.5\%$ after 10 and 8 weeks of ST, respectively. However, caution should be exercised when interpreting our findings because interference at the muscle fiber level cannot be discarded. In fact, McCarthy et al. [31] reported similar gains in strength and whole muscle CSA between the ST and CT groups, but at the muscle fiber level, no changes were observed in slow-twitch muscle fiber area in the CT group whilst an increase in the ST group was detected.

Greater CSA is a result of an integrative response of the activation of intracellular pathways leading to protein synthesis. In this respect, the Akt/mTOR/p70^{S6K1} pathway has been associated with mechanical overload-induced skeletal muscle hypertrophy through a cascade of intracellular events [4,11]. The mTOR is known to phosphorylate both the 4E-BP1 and p70^{S6K1} proteins, stimulating translation initiation [3,17]. Indeed, the ST showed increased Akt phosphorylation at Ser⁴⁷³ and a trend ($p=0.06$) towards increased p70^{S6K1} phosphorylation at Thr³⁸⁹. Leger et al. [26] observed significant muscle hypertrophy ($\sim 10\%$) after 8 weeks of ST with a concomitant increase in Akt phosphorylation with no changes in p70^{S6K1} phosphorylation at the same residues observed in our study [26]. It is interesting to note that, in the present study, similar muscle adaptations were observed in both the ST and CT groups, despite the lack of significant differences in the phosphorylation of proteins involved with muscle growth in the CT group.

Frosig et al. [14] demonstrated an increase in AMPK phosphorylation both 15 and 55 h after the last session of a 3-week endurance training regimen. AMPK activation has been demonstrated to activate tuberous sclerosis complex 2 (TSC2), which inhibits mTOR-p70^{S6K1} phosphorylation, protein synthesis rate and skeletal muscle hypertrophy [1,2,5]. However, it should be emphasized that even a strength exercise bout may increase AMPK phosphorylation [13]. During a strength exercise bout, this AMPK activation seems to be transitory (i.e. up to 1 h after the strength exercise bout). After this time window, there is an increase in mTOR-p70^{S6K1} phosphorylation, overriding AMPK activation, which enhances muscle fractional protein synthetic rate. Taken these findings together, it seems reasonable to speculate that the strength exercise overrides the endurance exercise stimulus in the proposed CT regimen, favoring skeletal muscle growth. Additionally, it is also plausible to suggest that other intracellular mechanisms may respond differently to CT, allowing similar muscle growth when compared to ST alone. Further research is required to provide additional insight on the effect of CT on skeletal muscle hypertrophy/atrophy regulatory mechanisms.

Further, AMPK is known to mediate the expression of genes associated with mitochondrial biogenesis in response to IT [22,23]. Accordingly, IT group had a robust increase of $100 \pm 17.6\%$ in AMPK phosphorylation at Thr¹⁷² (► **Fig. 2**), this response provoked by endurance exercises has been associated with activating the mitochondrial biogenesis machinery [20].

In summary, our data demonstrated that despite the differences in the molecular adaptations between training regimens, low-volume CT produced similar muscle strength and hypertrophy increments when compared with low-volume ST alone. The present study suggests that in these conditions the strength exercise stimulus might prevail over the endurance exercise-induced inhibition in muscle mass augmentation in the CT regimen, at least when using short-term training designs that produce modest gains in muscle size (5–7%). Nonetheless, caution should be exercised when interpreting this data as other CT regimens may differently modulate the molecular, functional and morphological responses.

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Conflict of Interest: The authors declare no conflict of interests.

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