

The acute effects of strength, endurance and concurrent exercises on the Akt/mTOR/p70^{S6K1} and AMPK signaling pathway responses in rat skeletal muscle

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Abstract

The activation of competing intracellular pathways has been proposed to explain the reduced training adaptations after concurrent strength and endurance exercises (CE). The present study investigated the acute effects of CE, strength exercises (SE), and endurance exercises (EE) on phosphorylated/total ratios of selected AMPK and Akt/mTOR/p70^{S6K1} pathway proteins in rats. Six animals per exercise group were killed immediately (0 h) and 2 h after each exercise mode. In addition, 6 animals in a non-exercised condition (NE) were killed on the same day and under the same conditions. The levels of AMPK, phospho-Thr¹⁷²AMPK (p-AMPK), Akt, phospho-Ser⁴⁷³Akt (p-Akt), p70^{S6K1}, phospho-Thr³⁸⁹-p70^{S6K1} (p-p70^{S6K1}), mTOR, phospho-Ser²⁴⁴⁸mTOR (p-mTOR), and phospho-Thr¹⁴⁶²-TSC2 (p-TSC2) expression were evaluated by immunoblotting in total plantaris muscle extracts. The only significant difference detected was an increase (i.e., 87%) in Akt phosphorylated/total ratio in the CE group 2 h after exercise compared to the NE group ($P = 0.002$). There were no changes in AMPK, TSC2, mTOR, or p70^{S6K1} ratios when the exercise modes were compared to the NE condition ($P \geq 0.05$). In conclusion, our data suggest that low-intensity and low-volume CE might not blunt the training-induced adaptations, since it did not activate competing intracellular pathways in an acute bout of strength and endurance exercises in rat skeletal muscle.

Key words: Strength exercise; Concurrent training; Cell signaling; AMPK

Introduction

The combination of strength and endurance exercises throughout a training period is referred to as concurrent training or concurrent exercise (CE) (1,2). The CE mode is thought to hamper strength-training induced skeletal muscle adaptations (i.e., reduced muscle strength and muscle fiber hypertrophy) when compared to strength training alone. This diminished training adaptation is known as the interference effect (3,4). Among the several theories postulated to explain the interference effect (2,3,5), a molecular hypothesis has been suggested to explain the reduced training adaptations after CE. This hypothesis claims that CE may simultaneously activate competing intracellular pathways that may down-regulate the adaptive response of muscle growth (6,7).

In this regard, it has been demonstrated that strength exercise (SE) is capable of increasing the activation of the Akt/mTOR/p70^{S6K1} pathway, and, as a consequence, muscle protein synthesis and accretion (8,9). On the other hand, endurance exercise (EE) promotes changes in the mitochondrial biogenesis machinery through the activation of the AMPK-PGC-1 α pathway (10,11). Accordingly, Atherton et al. (12) mimicked both EE and SE by applying either low- (LFS) or high-frequency (HFS) electro-stimulation, respectively, to scrutinize the activation of the aforementioned signaling pathways in rat skeletal muscle. The most important findings of the cited study were that the activation of the Akt/mTOR/p70^{S6K1} pathway and myofibrillar protein synthesis rate increased after HFS, while the AMPK-PGC-1 α pathway was activated only after LFS.

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The phosphorylation of AMPK (p-AMPK) seems to trigger the interference effect at the molecular level (7). Once activated, p-AMPK inhibits the cell anabolic processes that demand ATP expenditure, such as protein synthesis, in order to replenish ATP stores (13,14). Lower protein synthesis could be mediated in part by an increase in p-AMPK and a concomitant decrease in the anabolic response downstream of mTOR signaling (13).

Therefore, it seems reasonable to suggest that CE may increase AMPK activity, thus possibly attenuating mTOR phosphorylation and its downstream targets such as p70^{S6K1} (13,14). Thus, the purpose of the present study was to investigate the acute effects of EE, SE, and CE on selected AMPK and Akt/mTOR/p70^{S6K1} pathway proteins.

Material and Methods

Experimental design

Initially, the animals were acquainted with the handling and exercise procedures and then performed two functional tests: a graded treadmill exercise test (GTET) and one repetition maximum test (1RM). After the functional tests, 42 male Wistar rats (345.3 ± 27.1 g and 14 weeks old) were randomly assigned to four experimental groups: no exercise (i.e., sedentary control, NE, $n = 6$); SE, $n = 12$; EE, $n = 12$, and CE, $n = 12$. Animals in the exercise groups were killed immediately (0 h, $n = 6$ for each exercised group) and 2 h ($n = 6$ for each exercised group) after the completion of the exercise protocol. The NE animals were killed on the same day in random order and at the same time and conditions as the animals of the experimental groups. The plantaris muscle was excised from the animals and stored at -80°C for further analysis.

Animal care

All animals were maintained on a 12:12-h dark-light cycle in a temperature-controlled environment (22°C) with free access to standard laboratory chow (Nuvital Nutrientes S/A, Brazil) and tap water. The study was conducted according to the guidelines of the Brazilian College of Animal Experimentation (www.cobea.org.br). In addition, all procedures were approved by the Ethics Review Board of the Escola de Educação Física e Esporte, Universidade de São Paulo.

Familiarization procedures

Before the allocation to their respective groups, all animals were handled in their cages for 5 days to minimize contact stress. In addition, they underwent three familiarization sessions, separated by 48-h intervals, to get acquainted with the exercise protocols (i.e., endurance and strength exercises).

Functional tests

GTET. Briefly, rats were placed in the treadmill and allowed to acclimatize for at least 30 min. The GTET was

then started at 0% grade and 6 m/min with increments of 3 m/min every 3 min until exhaustion. The maximum velocity (V_{max}) attained during the test was used to determine the training load (15).

1RM test. This test was performed in a strength training apparatus that was built exactly as described by Tamaki et al. (16). Animals were fitted with a canvas jacket able to limit twisting and flexion of their torsos and were suspended in the standard hindlimb position. Electrical stimulation (10-15 V, 0.3-s duration, 3-s intervals) was applied to the rat tail through a surface electrode. As a result, the rats extended their legs repeatedly, which lifted the weight-arm of the training apparatus. The load used to start the test was the animal's body mass. Then, animals were allowed up to five attempts to achieve the 1RM load with a 3-min interval between attempts. During the rest intervals, animals were returned to their cage. The maximum exercise load was taken to be when the animals were unable to move up the weight-arm of the training apparatus after an electrical stimulation.

Exercise protocols

Animals in the SE group performed five sets of 10 repetitions of the strength exercise with a load corresponding to 75% of the 1RM (i.e., 540.0 ± 56.6 g). Repetitions within sets were performed at 2-s intervals. A 2-min interval was allowed between sets. Animals were returned to their cage during the rest interval between sets. EE animals ran for 60 min at 60% V_{max} (i.e., 11.43 ± 1.53 m/min), achieved during the GTET. Finally, CE animals performed both SE and EE protocols (i.e., 530.0 ± 97.5 g and 12.6 ± 2.4 m/min, respectively). Animals in the CE group performed the EE protocol first. Immediately after the completion of the EE bout, they were placed in the strength-training apparatus to perform the SE protocol.

Sample analyses

Animals were killed by decapitation immediately (0 h) and 2 h after the exercise protocols. In addition, the animals in the NE group ($n = 6$) were killed on the same day and under the same conditions. The plantaris muscles were removed from both legs, weighed on an analytical balance, frozen in liquid nitrogen, and stored at -80°C for further analysis. To avoid differences in the time of death, the test order was counterbalanced among the groups.

Immunoblotting

Immunoblots of the plantaris muscle homogenates were performed as previously described (17). Briefly, tissues frozen in liquid nitrogen were homogenized in a buffer containing 1 mM EDTA, 1 mM EGTA, 2 mM MgCl_2 , 5 mM KCl, 25 mM HEPES, pH 7.5, 100 μM PMSF, 2 mM DTT, 1% Triton X-100, and a protease inhibitor cocktail (P8340; 1:100, Sigma-Aldrich, USA). The

samples were then subjected to SDS-PAGE in polyacrylamide gels (6-15%) depending upon protein molecular weight. After electrophoresis, proteins were electrotransferred to nitrocellulose membranes (BioRad Biosciences, USA). Equal gel loading and transfer efficiency were monitored using 0.5% Ponceau S staining of the blot membrane. The 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, phospho-Thr¹⁷²AMPK (p-AMPK), Akt, phospho-Ser⁴⁷³Akt (p-Akt), p70^{S6K1}, phospho-Thr³⁸⁹-p70^{S6K1} (p-p70^{S6K1}), mTOR, phospho-Ser²⁴⁴⁸mTOR (p-mTOR), and phospho-Thr¹⁴⁶²-TSC2 (p-TSC2) (Cell Signaling Technology, USA). Binding of one antibody per gel 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, USA) detected by autoradiography. Quantification analysis of the blots was performed using the Image J software (Image J based on NIH image), which measures pixel density of the autoradiographies. Targeted bands were normalized to α -tubulin antibody (1:1000; Santa Cruz Biotechnology, USA). The phosphorylated/total (P/T) ratios were calculated to assess changes in the activity of these proteins.

Statistical analysis

After normality (i.e., Shapiro Wilk test) and variance assurance (i.e., Levene test), treatments were compared by two-way analysis of variance, assuming time (i.e., 0 and 2 h) and group (i.e., exercise conditions) as factors. Whenever a significant F-value was obtained, the Tukey *post hoc* test was applied for multiple comparison purposes. The level of significance was set at $P < 0.05$. Data are reported as means \pm SE.

Results

Functional tests

There were no differences in the 1RM test ($F = 1.74$; $P \geq 0.21$) or graded treadmill exercise test ($F = 1.08$; $P \geq 0.38$) between groups at any time.

Molecular responses

Since the total protein content remained unchanged between groups and times, data are presented as P/T ratios to assess changes in the phosphorylation of the selected proteins due to the exercise protocols.

The Akt P/T ratio was significantly higher (i.e., 87%) in the CE group at the 2-h time point when compared to the NE group ($P = 0.002$). There were no differences in the AMPK, TSC2, mTOR, or p70^{S6K1} P/T ratios between groups ($P \geq 0.05$; Figure 1), indicating no effect of the exercise bouts (i.e., EE, SE and CE) on the activity of the

selected proteins across time.

Discussion

The purpose of this study was to investigate the acute effects of EE, SE, and CE on selected AMPK and Akt/mTOR/p70^{S6K1} pathway proteins. We did not obtain any data to support the notion that CE might activate the AMPK pathway and, as a consequence, inhibit the Akt/mTOR/p70^{S6K1} signaling pathway. The only significant difference detected was the increase (i.e., 87%) in the Akt P/T ratio in the CE group 2 h after exercise ($P = 0.002$).

The strength exercise used in the present study was developed by Tamaki et al. (16), and has been shown to increase skeletal muscle mass in rats. Tamaki et al. reported a 31.4% increase in muscle weight/body mass ratio after 12 weeks of training. However, Tamaki's exercise mode does not optimize the eccentric phase of the lifts. In humans, maximal eccentric contractions are reported to be effective in stimulating mTOR and p70^{S6K1} via an Akt-independent pathway (18,19). This effect may be mediated by a combination of greater tension and stretching of the skeletal muscles during the descending phase of the lift. In the current study, the animal does not need to control the descending part of the squatting movement, possibly decreasing the mechanical load generated during the eccentric phase. Therefore, in an acute condition, this strength exercise may not produce sufficient mechanical stress to activate the Akt/mTOR/p70^{S6K1} signaling pathway. Moreover, the absence of activation of the selected proteins might be associated with the exercise bout intensity. In fact, the animals in the present study exercised with lower intensity protocols compared with previous studies with the same weight-lifting model (16,20). Importantly, although one may speculate that this was due to the low number of familiarization sessions employed (three), we chose this approach in order to avoid any training effects that could have occurred due to a longer familiarization period, which could have potentially blunted the muscle response to the exercise protocol.

Furthermore, we attempted to reproduce a typical CE bout used by humans in order to investigate the acute responses of the aforementioned pathways. AMPK is an important protein kinase involved in the control of the cell energy status that seems to be activated by either a strength or endurance exercise bout (10,21). The activation of AMPK reduces skeletal muscle protein synthesis by inhibiting the mTOR signaling pathway via activation of tuberous sclerosis complex 2 (TSC2) (13). We found neither a significant activation of AMPK and TSC2 nor a down-regulation of mTOR and p70^{S6K1} after CE. Predominantly, controlled animal conditions have shown AMPK activation followed by a down-regulation in proteins involved in mRNA translation (13,14). For example, Thomson et al. (14) reported an acute attenuation in p-p70^{S6K1/Thr389}, 4E-BP1 and eEF2^{Thr56} after AMPK activation. However, they applied electrically

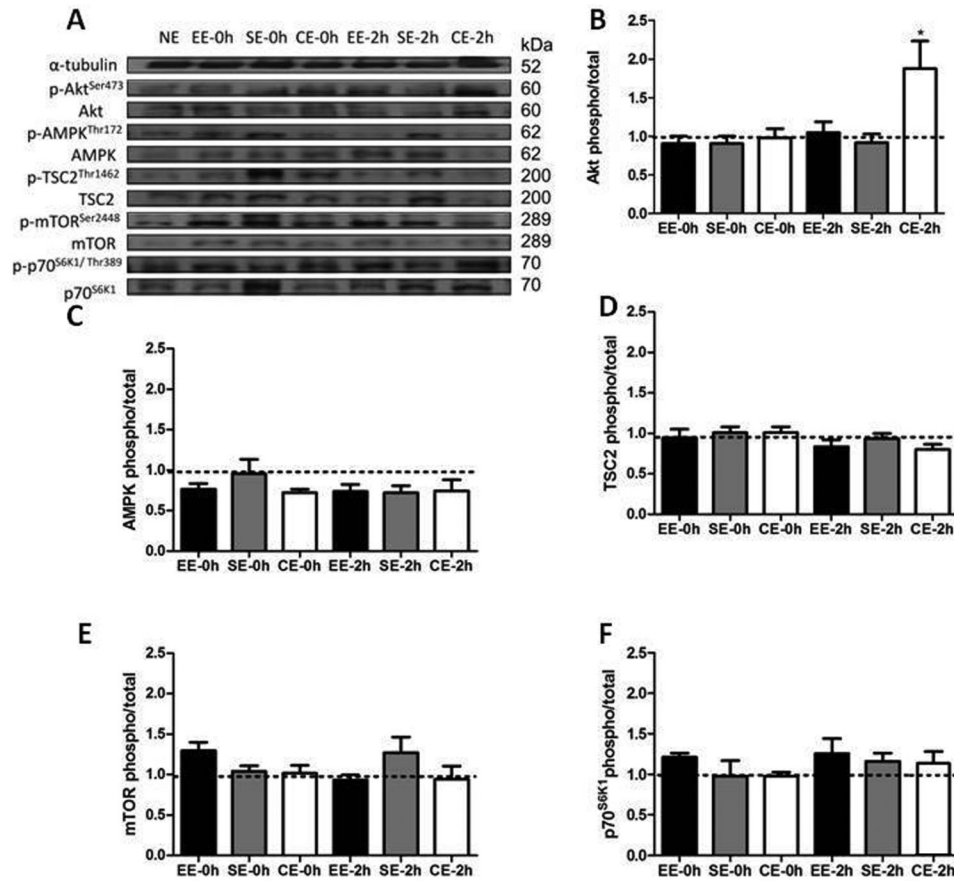


Figure 1. Phosphorylated/total ratio Akt, AMPK, TSC2, mTOR, and p70^{S6K1} protein expression for endurance exercise (EE; n = 6), strength exercise (SE; n = 6), and concurrent exercise (CE; n = 6) groups immediately after (0 h) and 2 h after exercise protocols. The traced line refers to the no exercise control group (NE; n = 6) (means \pm SE). *P = 0.002 compared to the NE group [two-way ANOVA (F test = 4.406)].

stimulated lengthening muscle contractions, which were produced through high-frequency stimulation of the sciatic nerve. In addition, 40 min before the muscle stimulation, rats were subcutaneously injected with AICAR, an AMPK agonist. On the other hand, our exercise protocols were devoid of pharmacological interventions or electrically elicited muscle contractions. Furthermore, an increase in p-AMPK via exercise only (i.e., non-pharmacologically induced or induced by electrical stimulation) seems to be dependent on chronic stimulation at greater exercise intensities. In this regard, Durante et al. (10) found detectable differences in AMPK activity in male Sprague-Dawley rats after a 4-week endurance training protocol ranging from 16 to 31 m/min at a 15% grade. Thus, our data suggest that it might be difficult to mimic an acute CE condition that is adequate to investigate the proposed hypothesis of an interference effect between AMPK and Akt/mTOR/p70^{S6K1} pathways in animals.

Regarding the significant difference detected in the Akt

P/T ratio in the CE group 2 h after exercise, it is important to note that Akt activity has been demonstrated to be up-regulated (7), down-regulated (22), or unchanged (23) in response to strength exercise bouts. As Akt might suffer a cross-talk from different cell signaling pathways and several stimuli (24), we suggest that the increased effect observed on Akt P/T after the CE protocol might be an overlap of both the endurance and the strength exercise stimuli as neither of the isolated exercise protocols produced a significant activation in the selected proteins.

We did not confirm that CE may simultaneously activate competing intracellular pathways in an acute bout of strength and endurance exercises in rat skeletal muscle. In addition, our data suggest that it might be difficult to create a concurrent training condition similar to a real-world training routine in an animal exercise mode. Future studies in human and animal models are required to confirm the molecular hypothesis that has been proposed to explain the CE-induced impaired training adaptations.

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