Skeletal muscle signaling response to concurrent endurance and resistance exercise

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Abstract Concurrent training, which is a combination of resistance exercise (RE) and endurance exercise (EE) performed in succession, is used to improve both muscle strength and cardiovascular function. Although numerous studies have investigated the effects of concurrent training on muscle adaptation, no consensus has been reached. Skeletal muscle adaptation is induced by the cumulative effects of the repeated cellular and molecular responses to an acute bout of exercise. Divergent exercise modes induce different molecular signaling responses depending on the muscle contraction type. It is well known that RE induces the mammalian target of the rapamycin complex 1 (mTORC1) signaling pathway while EE activates AMP-activated protein kinase (AMPK) signaling, and the signaling pathways stimulated by each exercise could interfere with each other. Thus, the inconsistencies in the effects of concurrent training on muscle adaptation may be explained by the different signaling interactions occurring in response to RE and EE. This review article describes the signaling pathways induced by RE, EE, and concurrent training.

Keywords: resistance exercise, endurance exercise, concurrent training, mTORC1, AMPK

Introduction Concurrent training, which is a combination of endurance exercise (EE) and resistance exercise (RE) performed in succession, is used to improve both muscle strength and cardiovascular function. Many studies have investigated the effects of concurrent training on muscle adaptation, and it has been demonstrated that the combination of RE and EE attenuates muscle hypertrophic response and increases muscle strength induced by RE alone1-4. In contrast, various studies have shown that the increase in muscle cross-sectional area (CSA) induced by concurrent training is greater than by RE alone5,6.

Skeletal muscle, the largest organ in the body, is very responsive to various types of stimulation. Chronic exercise training alters the metabolic and morphological characteristics of skeletal muscle7. The adaptation of skeletal muscle is induced by cumulative effects from repeated cellular and molecular responses to an acute bout of exercise. Divergent exercise modes induce different molecular signaling responses depending on the muscle contraction type7,8. RE, which consists of high-intensity muscle contractions, induces an increase in muscle protein synthesis, and subsequent muscle hypertrophy is considered to be almost fully dependent on the activation of the mammalian target of the rapamycin complex 1 (mTORC1) signaling pathway. On the other hand, activation of AMP-activated protein kinase (AMPK) signaling is considered to be largely responsible for EE-induced improvements in oxidative capacity and glucose uptake9-14.

Several previous studies have demonstrated that AMPK has inhibitory effects on mTORC1 activation in muscle cells15,16. The stimulation of an AMPK agonist, 5-aminimidazole-4-carboxamide ribonucleoside (AICAR), has been shown to activate AMPK and simultaneously suppress mTORC1 activation and subsequent protein synthesis15,16. Furthermore, the activation of mTORC1 signaling in response to resistance exercise-like high-intensity muscle contraction is also suppressed by AICAR administration-induced AMPK activation17. Given that EE and RE preferentially activate AMPK and mTORC1, respectively, the signaling pathways stimulated by each exercise could interfere with each other18. Thus, the effect of concurrent training on muscle hypertrophic response may be explained by the different signaling interactions induced by RE and EE. For example, a discrepancy could be induced depending on the order of exercise and interval duration between each exercise. In this article, the signaling pathways induced by RE, EE, and concurrent training will be reviewed.

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mTORC1 signaling response to RE

The mTORC1 pathway, which consists of mTOR, mammalian lethal with Sec13 protein 8 (mLST8, also known as Gbl), regulatory associated protein of mTOR (Raptor), DEP domain-containing mTOR-interacting protein (DEPTOR), and the proline-rich AKT substrate of 40 kDa (PRAS40), plays an important role in regulating muscle mass in response to various types of stimuli, e.g., mechanical stimulation, nutrients, growth factors, and hormones. The activated mTORC1 upregulates mRNA translation initiation and then increases muscle protein synthesis. Numerous studies have demonstrated that RE activates mTORC1 signaling and muscle protein synthesis in both animal and human experiments. Furthermore, rapamycin, an mTORC1 inhibitor, suppresses muscle contraction-induced muscle protein synthesis and subsequent skeletal muscle hypertrophy, indicating that mTORC1 plays as a critical role in regulating muscle protein synthesis and subsequent skeletal muscle hypertrophy in response to RE.

The p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding protein 1 (eEF-BP1) are well-known downstream signaling proteins of mTORC1. Specifically, the phosphorylation of p70S6K, a serine/threonine kinase, is widely used as the indicator of mTORC1 activity. Previous studies have reported that acute changes in the phosphorylation of p70S6K are correlated with an increase in muscle mass following repeated muscle contractions in both rodents and humans. The phosphorylation of p70S6K gradually increased until 3 h after RE and remained at high levels until 24 h after the initiation of RE when compared to basal levels in untrained animals. In untrained human subjects, an acute bout of RE increased p70S6K phosphorylation immediately and at 2-4 h after RE.

AMPK signaling response to EE

AMPK is a well-known sensor of cellular energy status and is activated in response to EE and high intensity interval exercise. AMPK has subunits, including α1, α2, β1, β2, γ1, γ2, and γ3. AMPKα1 and α2 subunits play an important role in regulating AMPK activity after muscle contraction. AMPK is widely known to be responsible for EE training-induced muscle adaptations, such as enhancement of glucose uptake and increases in mitochondrial enzymes. EE-induced AMPK activation is observed during and immediately after exercise, and then rapidly returns to the basal level.

Signaling interaction between RE-induced mTORC1 and EE-induced AMPK

Although numerous studies have examined the magnitude of muscle hypertrophy or increase in muscle strength after chronic concurrent training, few studies have investigated the effects of a single bout of concurrent exercise on the mTORC1 signaling pathways in human subjects. In previous studies, EE was conducted before or after RE, and mTORC1 activation in response to RE was evaluated. In one study, subjects performed unilateral-leg cycle ergometer exercise before RE, and the magnitude of p70S6K phosphorylation was the same as when RE was performed alone. In addition, more recent studies have demonstrated a lack of inhibitory effects of EE after RE on mTORC1 activation induced by RE. In contrast, a group found that sprint and aerobic cycling exercise, performed prior to RE, hampered p70S6K phosphorylation in response to RE. Furthermore, sprint cycling exercise, but not aerobic cycling exercise, performed after RE, decreased p70S6K phosphorylation induced by RE.

Therefore, there is conflicting evidence surrounding the effect of EE on the RE-induced activation of mTORC1. However, some previous studies described a failed attempt to elicit mTORC1 activation in response to RE or concurrent training, or did not observe significant AMPK activation after EE alone or in combination with RE. These study design flaws may have contributed to the conflicting effects of EE on mTORC1 activation in response to RE.

Recently, we performed an animal study in which animals underwent an acute bout of EE prior to or after RE in order to modify the timing and interaction between AMPK and mTORC1 activation. We used electrical stimulation-induced maximal muscle contraction (5 sets of ten 3-s contractions, with a 7-s interval between contractions and 3-min rest intervals between sets) as an animal RE model and treadmill running (25 m/min for 60 min) as an EE model. We observed that AMPK activation overlapped mTORC1 activation only when EE was performed after RE, and this overlap also coincided with the attenuation of RE-induced mTORC1 activation and subsequent muscle protein synthesis. Furthermore, we observed that phosphorylation of AMPK and Raptor, the downstream pathway of AMPK and suppressor of mTORC1 signaling, respectively, was elevated when EE was performed after RE, while EE before RE did not alter the phosphorylation levels of the proteins. Thus, the attenuation of the RE-induced mTORC1 upregulation might be mediated by AMPK signaling pathways, and the order of exercise may be an important factor in determining the effect of concurrent training on muscle hypertrophy.

Some studies have reported that RE enhances or does not disrupt the improvement in oxidative capacity in response to EE training, while EE could attenuate RE-induced muscle hypertrophy. The previously discussed studies investigated the effect of a single bout of RE on EE-induced PGC-1α expression, which is a positive regulator of mitochondrial biogenesis. PGC-1α activates nuclear respiratory factors (NRFs) 1 and 2, which promote mitochondrial transcription factor A (TFAM),
Previous studies have shown that PGC-1α expression is increased by EE and is important for mitochondrial biogenesis. Lundberg et al. reported that RE after EE did not inhibit the increase in EE-induced PGC-1α mRNA expression. Similarly, RE before EE also did not inhibit the increase in PGC-1α in response to EE. In studies focused on exercise order, there were no significant effects of exercise order on EE-induced PGC-1α expression. In summary, RE performed either before or after EE may affect EE-induced upregulation of PGC-1α expression.

**Conclusion**

In summary, despite numerous studies, there is yet to be a consensus on the effect of concurrent exercise on muscle hypertrophic adaptation. RE and EE each induce different molecular signaling, and the order of exercise could contribute to mTORC1 activation. Further studies are needed to investigate whether the exercise sequence used when performing concurrent exercise affects long-term training adaptations such as muscle mass or oxidative capacity.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

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