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Convergence of Theories of Alcohol Administration Postanabolic Stimulation on mTOR Signaling: Lessons for Exercise Regimen

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The conscientiousness of health, exercise, and muscle development has been desired by humans, but so has the intoxicating and recreational usage of alcohol (ethanol [EtOH]) been prevalent. The recent study by Steiner and Lang (2015) analyzed the effects of EtOH administered postanabolic stimulation and its effect on muscle protein synthesis. The experimental decorum of EtOH research in animal models has adhered to EtOH insemination prior to anabolic stimulation of muscle and other tissue. An obvious dissolution of this theory lies in the high probability with which EtOH would be consumed by humans prior to exercise is low. Albeit animal models hope to replicate and study stimulations of signaling pathways in response to EtOH stimuli, observations made after EtOH administration would be counterproductive models. Postexercise regimens of intake of leucine-rich protein sources activate independent but complementary signaling responses that converge at the mechanistic target of rapamycin (mTOR) to stimulate protein translation by enhancing protein synthesis, increasing anabolic response of the skeletal muscles (Parr et al., 2014). However, the cultural environment surrounding team sports involves recreational drinking after training, with athletes in several team sports at risk for binge drinking practices (Parr et al., 2014). We are interested in bridging the gaps of thought on the role of mTOR in protein synthesis postexercise and the elements influencing the balance of compensated recovery versus decomposition as a result of EtOH use after exercise.

INVOLVEMENT OF mTOR SIGNALING IN MUSCLE REGENERATION AFTER EXERCISE

Exercise exerts mechanical loading that is a major regulator of skeletal muscle mass, wherein muscle hypertrophy develops with increase in mechanical load and muscle atrophy develops with a decrease (Adams and Bamman, 2012). The damaged muscle regeneration mimics the skeletal muscle remodeling processes by recapitulating embryonic myogenesis; in atrophied or injured muscles, quiescent precursor or satellite cells positioned between the basal lamina and the plasma membrane of muscle fibers are activated to proliferate and differentiate into new myofibers or repair existing myofibers. Within the last decade of

extensive research, it has become apparent that the protein kinase known as mammalian mechanistic target of rapamycin (mTOR) multiprotein complex plays a fundamental role in the regulation of skeletal muscle mass. The mTOR signaling is a key regulator of skeletal muscle differentiation and remodeling, while also regulating several biological processes of cellular growth, differentiation, and metabolism. These are indirect responses to nutrient bioavailability, energy insufficiency, stress, hormones, and mitogens (Ma and Blenis, 2009) that are altered during routine or strenuous exercise. There is a close association between skeletal muscle mass that integrates signals from nutrients and growth factors, and it is influenced by the rate of protein synthesis by mTOR signaling. Studies in mammals revealed the supporting evidences that adaptive muscle growth was dependent on activation of mTOR and its downstream targets (Bodine, 2006). However, the precise molecular mechanisms by which mechanical loading regulated protein synthesis and its resultant muscle mass are yet to be clearly determined.

It has been established that mTOR signaling is involved in protein synthesis through the phosphorylation of downstream targets, phosphorylated mitogen-activated 70-kDa ribosomal protein S6 kinase, p70^{S6K1} (which is also a regulator of substrates, ribosomal protein S6 and elongation factor 2 kinase, i.e., eEF2K), and 4E-BP1 (initiating cap binding complex), which finally leads to translation initiation, peptide elongation, and protein synthesis (Ma and Blenis, 2009). mTOR multiprotein complex was shown to associate with Raptor (the regulatory associated protein of mTOR), which was seen to directly interact with mTOR substrates, 4E-BP1 and p70^{S6K1}. One of the 2 types of mTOR complexes is the Raptor-associated rapamycin-sensitive mTOR complex 1 (mTORC1) that was sensitive to rapamycin in comparison with mTORC2 (Rictor-associated rapamycin-insensitive mTOR complex 2), which rendered essentially resistant to rapamycin. mTOR interaction with Raptor, mLST8/GβL, and PRAS40 forms the mTORC1 complex. Insulin-sensitive protein, PRAS40 (proline-rich Akt substrate of 40 kDa), is shown to be bound to Raptor and an inhibitor of mTORC1 kinase activity (Sancak et al., 2007; Vander Haar et al., 2007), and muscle activity increases insulin sensitivity (Vander Haar et al., 2007). Upon stimulation with insulin, Akt-induced phosphorylation of PRAS40 resulted in the dissociation from mTORC1 and an increase in mTORC1 signaling (Sancak et al., 2007; Vander Haar et al., 2007). Alternatively, the GβL, mSin1, and Rictor associate to form the mTORC2 complex. Despite the reported roles of regulation of mTOR signaling through the identified major targets of phosphorylation, p70^{S6K1} and 4E-BP1, required for increased protein synthesis in skeletal muscle, there remain many hypotheses that need validation. These include whether there is a need for mTORC1-mediated phosphorylation and quantifying the extent of responsiveness of mTOR signaling to different types of mechanical activation, to predict the corresponding increase in skeletal muscle mass (Goodman, 2014).

MODELS STUDYING mTOR SIGNALING FOR ADAPTIVE MUSCLE HYPERTROPHY

The first evidence of mTOR signaling pathway mediated by high-resistance exercise training came from studying the after effects of high-resistance lengthening contractions in rats; it was shown that p70^{S6K} activity increased and correlated with increase in muscle

mass, and identified as the first good biomarker of phenotypic changes characteristic of muscle hypertrophy (Baar and Esser, 1999). The role of Akt/mTOR pathway in regulating muscle atrophy was validated by studying key protein kinases and initiation factors in the plantaris muscle of young adult female Sprague-Dawley rats after a functional overload by chronic exposure to activity. The increased phosphorylation state of Akt was observed during the period of hypertrophy, had an overall increase of 4-fold more than controls, and a 9-fold increase in hypertrophied plantaris (Bodine et al., 2001). Downstream targets of mTOR, p70^{S6K} showed an increase in phosphorylation status and pHAS-1/4E-BP1 was hyperphosphorylated, resulting in an increase in eIF4E, subsequent binding of eIF4G, thereby establishing translation initiation complex. To determine whether the activation of mTOR was essential to produce muscle hypertrophy, rats subjected to functional overload were treated with rapamycin (Bodine et al., 2001); no phosphorylation of Akt or mTOR-independent target GSK-3 β was observed, but daily treatment of rapamycin did specifically block the activation of downstream targets of mTOR (Bodine et al., 2001). These data indicated that Akt and mTOR and targets p70^{S6K} and pHAS-1/4E-BP1 were activated through phosphorylation, and necessary for load-induced adaptive muscle hypertrophy (Bodine, 2006). A study by Ge and colleagues (2009) demonstrated that mTOR regulates muscle regeneration through kinase-independent and kinase-dependent mechanisms at the stages of nascent myofiber formation and growth, comparatively, the characteristic repetitions of low to moderate force contractions over a prolonged period of time are defined as acute endurance training, for which evidence suggests that protein synthesis is depressed during acute exercise, but increased for many hours postexercise (Mascher et al., 2011). This increase in protein synthesis postexercise during recovery may be due to an increased synthesis of mitochondrial protein and/or the turnover proteins damaged during the exercise bout (e.g., calcium-activated protease- or oxidative stress-induced damage; Seene et al., 2011).

EFFECT OF EtOH ADMINISTRATION ON mTOR SIGNALING

The recent paper by Steiner and Lang (2015) addresses an important approach to studying the effects of EtOH on mTOR signaling by identifying the high propensity with which EtOH is consumed postexercise and not preexercise. EtOH was studied and identified for its suppressive effects on mTORC1-mediated rates of protein synthesis in skeletal muscle, but upon muscle contraction, it was yet to be determined whether the EtOH could reverse these effects by abrogation of mTORC1 or MAPK signaling. They showed that indeed, as hypothesized, EtOH completely eliminated contraction-induced increase in skeletal muscle protein synthesis in addition to suppressing basal rates. This suppression in protein synthesis was due to the inhibition of translocation of the tRNA within the ribosome, thereby causing mRNA elongation termination by increased phosphorylation of eEF2, while still maintaining global increase in MAPK and mTORC1 signaling. They documented evidences that suggest the consequence of high levels of repeated EtOH consumption after exercise to interfere and be counterproductive to the benefits of resistance exercise and muscle contraction in increasing muscle protein synthesis and muscle repair. Another paper published recently also demonstrated novel data that EtOH consumption postexercise resulted in reduced rates of myofibrillar protein synthesis following strenuous exercise, even when co-ingested with

whey protein and carbohydrate sources, indicative of suppression of anabolic response and impairment of recovery by EtOH (Parr et al., 2014).

Further, mTORC1 signaling is positively or negatively regulated by changes in cellular redox state depending on exercise conditions (Corradetti and Guan, 2006). Mechanical stimulation of skeletal muscle also results in increases in muscular Ca^{2+} content, reactive oxygen species, and reactive nitrogen species (Goodman, 2014), which play a role in mTORC1 signaling and muscle hypertrophy (Ito et al., 2013a,b). The recent studies by Ito and colleagues (2013a,b) showed that the rapid/transient increase in nNOS-derived NO reacts with NADPH-4 (Nox4)-derived O_2^- to form ONOO⁻, which in turn induces an increase in intracellular Ca^{2+} via the activation of the sarcoplasmic reticulum (SR)-located transient receptor potential cation channel subfamily (TrpV1), which then leads to mTORC1 signaling activation and hypertrophic response for up to 7 days. It is yet unclear of the mechanism by which this intracellular Ca^{2+} might activate mTORC1 signaling and is contrary to previous work that suggested the Ca^{2+} did not play a role in mechanically induced activation of mTORC1 signaling (Goodman, 2014; Hornberger et al., 2006). The SR in the intracellular membranes of muscle cells regulated the muscle contraction by releasing Ca^{2+} through the ryanodine receptors into the cytosol, while also facilitating active re-uptake of Ca^{2+} by the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) (Periasamy and Kalyanasundaram, 2007). The dual role of the SERCA pump is to participate in muscle relaxation by lowering cytosolic calcium and restoring SR calcium reserves for muscle contraction. Strenuous exercise, such as running long distances, was shown to cause increased Ca^{2+} accumulation, which altered calcium homeostasis and muscle damage (Overgaard et al., 2004). In that regard, it has been shown that at high concentrations, EtOH inhibits calcium uptake in the SR by uncoupling the SERCA1 ATPase catalytic subunit, but there was no effect at low doses (Mitidieri and de Meis, 1995). Also, chronic increase in active loading on the fast-twitch skeletal muscle, as in tonic exercise, induces a shift in the gene expression from fast SERCA1 (high density) to slow SERCA2 (low density) isoform, with reduction in total SERCA expression and activity (Kandarian et al., 1994). However, there is no report in the literature on the effects of EtOH on SERCA1 with exercise (prior or post). On another note, however, the physiological effects on skeletal vasculatures when EtOH is administered after exercise and its relationship with Ca^{2+} handling are yet to be determined.

In conclusion, the evidence as presented in Steiner and Lang's (2015) recent work indicates that EtOH reverses the anabolic effects of muscle contraction and exercise on protein synthesis, potentially by the inhibition of peptide chain elongation. These theories converge to suggest that high levels of EtOH, particularly binge drinking episodes in immediately postexercise, can impair recovery, subsequent performance, and long-term adaptation of skeletal muscle and muscle mass.

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