REVIEW / SYNTHÈSE

Changes in muscle mass with mechanical load: possible cellular mechanisms[1]

Espen E. Spangenburg

Abstract: Understanding the mechanisms that regulate skeletal muscle mass has remained a focus of numerous researchers for many years. Recent investigations have begun to elucidate cellular signaling mechanisms that regulate skeletal muscle hypertrophy, with significant effort being focused on the Akt/mammalian target of rapamycin (mTOR) signaling pathway. The Akt/mTOR pathway plays a major role in regulating the initiation of protein synthesis after the onset of mechanical loading of skeletal muscle. Although a number of downstream substrates for Akt/mTOR have been elucidated, very little is known about the upstream mechanisms that mechanical load employs to activate the Akt/mTOR signaling pathway. Thus, the purpose of this review is to discuss potential mechanisms that may contribute to the activation of the Akt/mTOR signaling mechanism in mechanically loaded skeletal muscle.

Key words: mTOR, Akt/PKB, insulin-like growth factor, hypertrophy, stretch, overload.

Résumé : Depuis de nombreuses années, des chercheurs tentent d'élucider les mécanismes de contrôle de la masse des muscles squelettiques. Des études récentes commencent à clarifier les mécanismes de signalisation qui régule l'hypertrophie du muscle squelettique et pointent du côté de la voie de signalisation Akt/mTOR. La voie de signalisation Akt/mTOR joue un rôle important dans le contrôle du démarrage de la synthèse des protéines consécutive à la mise en charge du muscle squelettique. Même si on connaît un certain nombre de substrats en aval sur la voie Akt/mTOR, on sait très peu de choses au sujet des mécanismes utilisés en amont par la charge mécanique pour agir sur cette voie. Par conséquent, cet article-synthèse se propose d'analyser les mécanismes potentiels d'activation de la voie de signalisation Akt/mTOR dans le muscle squelettique soumis à une charge mécanique.

Mots-clés : mTOR, Akt/PKB, facteur de croissance insulinomimétique, hypertrophie, étirement, surcharge.

[Traduit par la Rédaction]

Mass is a dynamic property of skeletal muscle that changes with the loading pattern placed on the muscle. For example, increased loads placed on the muscle (i.e., strength training) can induce significant increases in muscle mass, while removal of mechanical loads (i.e., extended bedrest) results in the loss of muscle mass. Understanding the mechanisms that regulate muscle mass is necessary, since skeletal muscle plays critical roles in locomotion, breathing, and metabolism. Skeletal muscle mass is a critical factor in the maintenance of functional independence. The largest deficits of overall physical function occur in the range of low to moderate muscle strength output (Janssen et al. 2004). Thus, small changes in muscle mass can be critical, since there is a threshold for the loss of muscle mass that induces physical disability and loss of independence (Janssen et al. 2004). Therefore, defining mechanisms that regulate muscle growth or hypertrophy is critical for preventing long-term disability. The purpose of this review will be to discuss the cellular signaling mechanisms activated during mechanical loading and to review the potential upstream signals that mechanical loading may use to activate these mechanisms.

Common animal models that use mechanical load to induce muscle growth

Animal models are frequently used to examine molecular and cellular mechanisms that regulate muscle growth. The most commonly used model is functional overload of the plantaris muscle, which is also referred to as synergist ablation. This model involves the surgical removal of the soleus and the majority of the gastrocnemius muscle from the animal, thereby requiring the plantaris muscle to bear the load of the removed muscles. This mechanical overload of the plantaris muscle results in significant inductions of muscle

Received 28 January 2009. Accepted 29 January 2009. Published on the NRC Research Press Web site at apnm.nrc.ca on 25 April 2009.

E.E. Spangenburg. University of Maryland, School of Public Health, Department of Kinesiology, College Park, MD 20742, USA (e-mail: espen@umd.edu).

[1]This paper is one of a selection of papers published in this Special Issue, entitled 14th International Biochemistry of Exercise Conference – Muscles as Molecular and Metabolic Machines, and has undergone the Journal's usual peer review process.

growth of ~30% after 7 days and ~100% after 35 days (Spangenburg and Booth 2006; Spangenburg et al. 2008). With such significant and rapid growth of the muscle, there are large inductions of mechanisms necessary to induce muscle growth. The model is frequently used because it does not require any equipment to induce the load, other than the ability to properly anesthetize the animal. With that said, this model is also extreme, in that growth is very rapid and robust. The magnitude of hypertrophy is unlike the type of growth we typically see with resistance training.

Another commonly used model, developed by Ted Wong in Frank Booth's lab (Wong and Booth 1988), is a model of muscle hypertrophy induced by electrical stimulation. In this model, the sciatic nerve is stimulated with 1-ms pulses at 100 Hz, with a 2.5-s train duration, causing net plantar flexion of the lower limb, resulting in a shortening of the gastrocnemius-plantaris-soleus muscle group and a lengthening of the tibalis anterior–extensor digitorum longus muscle group (Wong and Booth 1990*b*). The end result of this protocol is significant hypertrophy of ~16%–30% of the tibalis anterior muscle, due to the repetitive lengthening contractions. Baar and Esser (1999) found that, since planatarflexors are stronger, the stimulation resulted in lengthening the contraction of the dorsiflexors and, ultimately, hypertrophy of the tibalis anterior–extensor digitorum longus muscle after 4 weeks of training. Advantages of this model include the ease with which one can perform time course measures, control over amount of the work done by each animal, and, with the proper equipment, the ability to measure simultaneous force production (Burry et al. 2007). Disadvantages include the fact that it is more time consuming to do long-term training studies, which may require the chronic insertion of electrodes on the sciatic nerve, and the requirement of access to an electrical stimulator.

Changes in the central dogma of biology during muscle hypertrophy

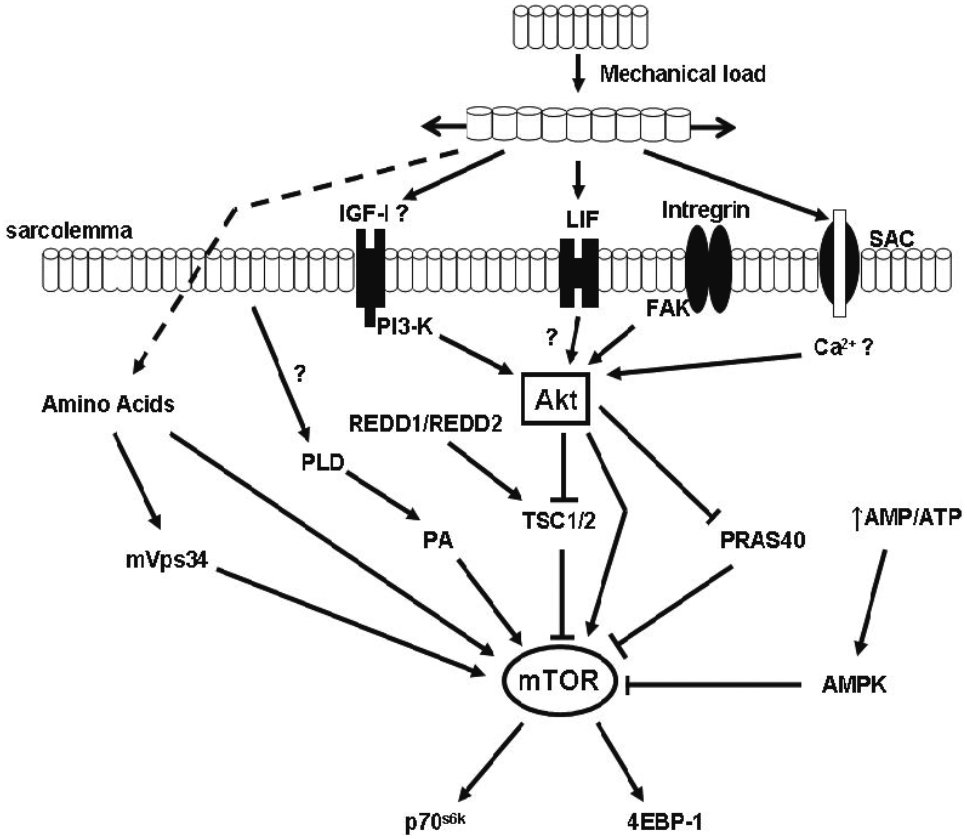
The initial or early phases of induction of skeletal muscle hypertrophy are characterized by significant increases in protein synthesis rates, followed by increases in total protein content in the muscle that are not matched by changes in total RNA content (Baar et al. 2006). However, if this stimulus that is inducing the muscle growth is continued, then increases in total RNA content will follow at later time points (Carson 1997). This suggests that the initial phases of mechanical loading of the muscle mechanisms that regulate protein synthesis are critical for induction of muscle hypertrophy, but as the changes in muscle mass continue, then it is thought that gene transcription contributes to the increases in RNA content. In addition, the contribution of satellite cell incorporation during muscle hypertrophy is thought to be critical, but remains a controversial area (O'Connor et al. 2007). Thus, what is clear is that the cellular mechanisms that regulate muscle hypertrophy are complex and work in a dynamic fashion. For the purposes of this review, the focus will be on mechanisms that regulate protein translation; for those interested in material on satellite cells and gene transcription during muscle hypertrophy, readers are referred to Bassel-Duby and Olson (2006) and Hawke and Garry (2001).

Cellular and molecular mechanisms that regulate protein synthesis

Protein synthesis is a physiological mechanism that is dictated by the genetic code within the messenger (m)RNA of each specific gene. Each mRNA contains a translation start site, whereby the ribosome can bind to the specific mRNA to translate the mRNA based on the codons present. In a seminal study, Baar and Esser (1999) demonstrated that acute mechanical loading of the muscle resulted in significant increases in the rate of initiation of protein synthesis, compared with the resting muscle. These data suggest that signaling events that control the rate of initiation of protein synthesis are key factors in the hypertrophic process. Initiation of protein synthesis appears to be regulated by a complex myriad of signaling proteins, with the Akt/mammalian target of rapamycin (mTOR) signaling proteins being tabbed as the key regulators (Fig. 1). With the onset of acute mechanical loading, significant increases in Akt/protein kinase B (PKB) phosphorylation at Thr308 and Ser473 have been detected immediately after the cessation of the load, and the phosphorylation typically returns to baseline within 3 h (Nader and Esser 2001). Using the functional overload model, significant increases in Akt/PKB phosphorylation can still be detected up to 7 days after the onset of the load, which may be, in part, due to the chronic nature of the load (Spangenburg et al. 2008). Bodine and colleagues (2001) demonstrated that overexpression of a myristoylated (i.e., active) form of Akt/PKB could induce muscle hypertrophy and prevent muscle atrophy, thus providing evidence that Akt/PKB may be a key contributor to the hypertrophic process.

Downstream of Akt/PKB lies the tuberous sclerosis complex (TSC1/TSC2), which is inhibited by active Akt/PKB. TSC1/TSC2 is thought to negatively influence the activation of mTOR through the inhibition of Rheb (Miyazaki and Esser 2008). However, at this time, few data exist examining the effect of mechanical loading on the regulation of the TSC1/TSC2 complex or Rheb. Using the functional overload model, increases in Ser1345 phosphorylation of TSC2 have been detected, which is the 5-AMP activated protein kinase (AMPK) phosphorylation site of TSC2 (McGee et al. 2008). Overexpression of TSC1 induces muscle atrophy; thus, one might predict that inhibition of TSC1 would be critical to muscle hypertrophy (Wan et al. 2006). Finally, changes in Rheb mRNA expression have been detected with mechanical loading (Drummond et al. 2008). Downstream of TSC1/TSC2 is mTOR, which has been implicated as a key regulator of muscle hypertrophy. The role of mTOR in hypertrophy is based on experiments in which animals received rapamycin, resulting in an inhibition of muscle hypertrophy during mechanical loading (Bodine et al. 2001). Rapamycin binds to FKBP12, removing FKBP12 from the FRB domain in mTOR, resulting in the inhibition of mTOR activity (Lorenz and Heitman 1995). Hornberger et al. (2007) have eloquently demonstrated that rapamycin specifically inhibits stretch-induced activation of mTOR, resulting in the reduced activation of downstream signaling proteins. Due to the complexity of

Fig. 1. A depiction of how mechanical loading of the muscle membrane may induce activation of the Akt/mammalian target of rapamycin (mTOR) signaling pathway in skeletal muscle. FAK, focal adhesion kinase; IGF-1, insulin-like growth factor; LIF, leukemia inhibitory factor, PA, phosphatidic acid; PLD, phospholipase D; SAC, stretch-activated channels, TSC, tuberous sclerosis complex.



mTOR regulation, delineating mechanisms that affect mTOR activity during mechanical loading has been hampered. Specifically, mTOR is bound directly or indirectly by a number of different proteins that can regulate activity and can be phosphorylated at Ser-2448 and Ser-2481 residues, all of which can affect mTOR activity (Peterson et al. 2000). Recent data have shown that mechanical loading appears to affect the phosphorylation status of mTOR on Ser-2448, resulting in increased phosphorylation of known mTOR substrates (Reynolds et al. 2002); however, less is known about the effects of mechanical load on the status of phosphorylation status of Ser-2481. mTOR activity is also affected by direct or indirect interactions with REDD2 (Miyazaki and Esser 2009), PRAS40 (Sancak et al. 2007), rictor (Bentzinger et al. 2008), and raptor (Bentzinger et al. 2008). For example, Miyazaki and Esser (2008) recently demonstrated that overexpression of REDD2 reduced activation of mTOR in response to stretch and amino acid stimulation. Interestingly, the mechanism by which REDD2 affects mTOR appears to occur independent of Akt, but works through some complex interaction with TSC1/TSC2 (Miyazaki and Esser 2008). Another interesting signaling molecule, PRAS40, appears to inhibit mTOR indirectly by interacting with raptor (Sancak et al. 2007). Akt/PKB can alleviate the PRAS40 inhibition of mTOR by phosphorylating PRAS40 at the Thr-246 residue, resulting in release of PRAS40 from the mTOR complex, which leads to increased mTOR activity (Sancak et al. 2007). My lab recently found that lengthening contractions of the tibalis anterior muscle results in increased Thr-246 phosphorylation of PRAS40, which is associated with increased phosphorylation of $p70^{s6k}$ on the Thr-389 residue. The fact that the Thr-389 residue on $p70^{s6k}$ is the mTOR phosphorylation residue on $p70^{s6k}$ suggests that PRAS40 is another mechanism that may affect mTOR activation with mechanical loading (S. Witkowski and E.E. Spangenburg 2008, unpublished data). Unfortunately, at this time, very few data exist concerning the regulation of rictor and raptor with mechanical loading of skeletal muscle, although genetic ablation of raptor, specifically in skeletal muscle, results in mice that exhibit poor postnatal muscle growth and a dystrophy pathology (Bentzinger et al. 2008). Raptor binds to mTOR substrates, including 4E-BP1 and $p70^{s6k}$, through their TOR signaling motifs, and is required for mTOR-mediated phosphorylation of these substrates. This suggests that at least raptor plays a critical role in skeletal muscle; the role for rictor is unclear, since the genetic ablation resulted in no obvious muscle phenotype (Bentzinger et al. 2008).

Direct interaction is one means to regulate mTOR; however, recent exciting evidence has found that mechanical loading of muscle can affect mTOR activity by modulating the production of phosphatidic acid or increasing the amino acid concentrations. Hornberger et al. (2006) found that mechanical stretch of muscle induced activation of phospholipase D, resulting in the production of phosphatidic acid, which increased mTOR activity through the binding of phosphatidic acid to the FRB domain in mTOR. Further, recent data from Keith Baar's lab (Mackenzie et al. 2009) has

found that mechanical loading of the muscle increases leucine levels in the muscle activating the class 3 PI3-K, vacuolar protein sorting mutant 34 (mVps34), which is predicted to prolong the activation of mTOR. Another form of regulation of mTOR can occur through changes in AMPK activity (Gordon et al. 2008). AMPK has the ability to negatively regulate mTOR activity by affecting the phosphorylation status of mTOR or through phosphorylation of TSC2 (Chan and Dyck 2005). Acute mechanical loading results in very little activation of AMPK, while in the functional overload model, there are significant increases in AMPK phosphorylation (McGee et al. 2008; Thomson et al. 2008; Thomson and Gordon 2005). However, it is clear that AMPK is detrimental to the hypertrophic process, in that, if animals are pretreated with AICAR (an AMPK agonist), there is an attenuation of $p70^{s6k}$ and eIF4E activation in response to mechanical loading, suggesting that AMPK activation suppresses protein synthesis in skeletal muscle (Thomson et al. 2008). In aged rats, an attenuated hypertrophic response to the functional overload model is associated with increased activation of AMPK, suggesting that AMPK is a negative regulator of mTOR (Thomson and Gordon 2005). Thus, it is clear that cellular regulation of mTOR is complex, and it will necessitate further research to fully delineate how mechanical loading induces the activation of mTOR.

Any alterations in mTOR activation would result in changes in $p70^{s6k}$ activation and 4EBP-1 inactivation, ultimately affecting the initiation of protein synthesis with mechanical load. Phosphorylation of $p70^{s6k}$ at Thr-389 by mTOR promotes the increased translation of mRNAs with a 5′-tract of pyrimidine. The 5′-tract of pyrimidine is a series of cytosine or thymine repeats at the 5′-gene terminus, which is contained in all known ribosomal proteins. Thus, increased activation of $p70^{s6k}$ by mechanical loading is thought to result in increased ribogenesis. The critical nature of $p70^{s6k}$ in muscle was demonstrated when $p70^{s6k}$ was genetically ablated, and it was found that the cross sectional area of the individual fibers was significantly smaller than that of fibers from the wild-type animals (Ohanna et al. 2005). Unfortunately, the muscles from these knockout mice have not yet been challenged with mechanical loading, so it is unclear what the responses would be to a hypertrophic stimulus. 4E-BP1 regulates the initiation of protein synthesis by sequestering eIF4E. In order for initiation of protein synthesis to occur, eIF4E must be released by 4E-BP1. When 4E-BP1 is hyperphosphorylated by mTOR, eIF4E detaches from 4E-BP1, allowing eIF4E to complex with eIF4G, resulting in the initiation of translation (Rennie et al. 2004). Ultimately, it is clear that the Akt/mTOR pathway is an important contributor to the initiation of protein synthesis during mechanical loading in skeletal muscle; however, at this time, the exact mechanisms that regulate mTOR remain elusive. In particular, we have very little understanding of how physical loading of the cell membrane is translated into a signal to induce the necessary activation of the Akt/mTOR signaling pathway.

How does mechanical load induce activation of signals that increase muscle growth?

As the key signaling proteins have become elucidated over the years, the tools to measure the activation status of these proteins have become more available. Thus, we are beginning to gain a better understanding of the time frame and the means by which these various proteins are activated and deactivated. However, what has remained elusive is the signal induced by the mechanical load that activates these signaling proteins.

Insulin-like growth factor

Insulin-like growth factor (IGF-I) remains the most prescribed signal by researchers for activating the signal transduction necessary for the initiation of protein translation, which is initiated during mechanical loading of the muscle (Adams 2002; Glass 2005). IGF-I was first suggested as the autocine–paracrine anabolic factor produced by the muscle, when the functional overload model was applied to hypophysectomized animals (Goldberg 1967). It was demonstrated that muscle hypertrophied normally in response to the mechanical load, even with reduced levels of circulating IGF-I; however, what was uniquely demonstrated was that IGF-I mRNA levels within the muscle increased in response to the load (Goldberg 1967). This finding has been confirmed by other investigators (Adams and Haddad 1996; DeVol et al. 1990). Adams and Haddad (1996) produced a detailed analysis of changes in IGF-I production by the muscle after mechanical loading, in which they demonstrated that IGF-I mRNA and protein levels increased significantly after 3 days. IGF-I is a potent activator of the Akt/mTOR signaling pathway and, through the use of various gain of function models, IGF-I overexpression has been shown to increase muscle mass with concurrent activation of the Akt/mTOR signaling pathway (Barton 2006). Further, exposure of myotubes or muscle to recombinant IGF-I increases the total protein content of the muscle, making IGF-I an attractive target as the potential upstream regulator of initiation of protein synthesis (Adams and McCue 1998; Vyas et al. 2002). Thus, numerous investigations have indicated that IGF-I is one of the primary upstream signals involved in the regulation of the Akt/mTOR pathway during mechanical load-induced muscle hypertrophy.

However, recent publications have begun to question the necessity of IGF-I for induction of muscle hypertrophy through mechanical loading. Specifically, data from my lab has found, using transgenic mice that express a skeletal-muscle-specific dominant negative IGF-I receptor (MKR), that these mice retain the ability to undergo muscle hypertrophy after exposure to the functional overload model (Spangenburg et al. 2008). Further, the Akt/mTOR signaling pathway is activated to the same extent in the MKR and wild-type mice after the functional overload surgery. These animals do not have a deficiency in IGF-I receptor expression, as previously suggested (Goldspink et al. 2008); instead, the skeletal muscle does not have the ability to respond to IGF-I or insulin exposure, owing to a mutation in the IGF-I receptor (Spangenburg et al. 2008). Indirect evidence also indicates that there is a poor correlation between load-induced increases in IGF-I concentrations, Akt/mTOR activation, and increases in protein synthesis. Specifically, mechanical load-induced IGF-I production by the skeletal muscle occurs between 24 and 72 h after the onset of the

load (Adams and Haddad 1996; Adams et al. 1999), while activation of the Akt/mTOR signaling pathway has been documented to occur a few hours after the onset of the mechanical load (Baar and Esser 1999; Nader and Esser 2001). Further, in rats, 25%–50% increases in protein synthesis have been detected 12–17 h after the addition of the mechanical load (Wong and Booth 1990*a*, 1990*b*). This finding has been confirmed in humans, in that significant increases in fractional protein synthesis rates have been detected 4 h after a bout of resistance exercise (Chesley et al. 1992). Thus, based on these data, there must be other signals for activation of protein synthesis, because endogenous IGF-I production by the muscle occurs at a much later time frame than activation of the Akt/mTOR pathway and initiation of protein synthesis.

Stretch-activated channels

Stretch-activated channels (SACs) create a unique stimulus to connect the events at the membrane with signaling structure within the cell. SACs were initially described in 1990 in muscle, based on findings in the *mdx* mouse line and in cultured muscle cells (Franco and Lansman, 1990*a*, 1990*b*). SACs are calcium and sodium permeable channels that increase their open probability in response to mechanical loading of the membrane. Numerous findings have suggested that SACs may play a major role in the induction of cardiac hypertrophy; however, the majority of the findings in skeletal muscle have been confined to muscle injury. Initially, the role of SACs in mechanical load-induced signaling was tested in a multiaxial C2C12 myotube stretch model, and no effect of SAC inhibition was found on stretch-induced $p70^{s6k}$ phosphorylation (Hornberger et al. 2005). Conversely, we found that in vivo treatment of animals with 2 different SAC inhibitors reduced skeletal muscle hypertrophy and attenuated activation of the Akt/mTOR signaling pathway in response to mechanical loading (Spangenburg and McBride 2006). Butterfield and Best (2009) recently demonstrated that the inhibition of SAC activity reduced expected adaptations to eccentric contractions. Specifically, they found inhibition of SACs attenuated increases in muscle mass and prevented improvements in muscle force production response to the exercise training. Yeung et al. (2005) demonstrated in single muscle fibers that SACs appear to allow the influx of Ca^{2+} across the sarcolemma and into the muscle cell. It has been suggested that the Akt/mTOR signaling pathway is sensitive to changes in intracellular calcium levels, owing to amino acid exposure via Vps34 (Gulati et al. 2008); however, it is unclear if Akt/mTOR signaling is affected by calcium during mechanical loading of the muscle. Currently, SACs are identified based on patch-clamping experiments, and modulation of channel activity is performed using various pharmacological treatments. Until the SAC protein structure or gene is discovered, it will be difficult to mechanistically address this pathway in more detail. Unfortunately, most of the studies are reliant upon pharmacological inhibitors of SACs and, therefore, are at the mercy of any nonspecific actions that may occur with the drugs. SACs represent an exciting possibility for the transduction of cellular signals from the sarcolemma, but significantly more understanding of the SAC itself will be needed to confirm the contribution of the SACs in this area.

Integrin and FAK signaling

Another intriguing hypothesis suggests that skeletal muscle maintains the ability to sense mechanical loading of the tissue, potentially through the extracellular matrix. The conversion of strain to signal may be mediated by focal adhesion complexes (FACs). FACs are thought to transmit force across the cell membrane using a complicated assembly of structural and signaling proteins (Burridge and Chrzanowska-Wodnicka 1996). FACs are thought to be important for the maintenance of cell tensegrity, a model in which FACs serve as anchor points for connecting cytoskeletal proteins to the extracellular matrix (Burridge and Chrzanowska-Wodnicka 1996). FACs in skeletal muscle express β1-integrins, which are bound by focal adhesion kinase (FAK) and paxillin (Carson and Wei 2000; Fluck et al. 1999*a*). Gordon et al. (2001) have demonstrated that mechanical loading of skeletal muscle will induce activation of FAC signaling, as determined by changes in the phosphorylation status of FAK or paxillin. With respect to mechanical loading, much of the data has suggested that FAK can affect activation of the transcription factor serum response factor, which targets skeletal α-actin (Carson et al. 1996; Fluck et al. 1999*b*). However, recent findings in other tissues have indicated that FAK activation can affect activation of mTOR by affecting TSC2 activation (Gan et al. 2006). FAK can also affect activation of Akt/mTOR by increasing PI3-K activity (Xia et al. 2004). However, the role of PI3-K in mechanical loading-induced activation of the Akt/mTOR pathway is unclear, since it has been demonstrated that mechanical loading can activate Akt/mTOR independent of PI3-K (Hornberger et al. 2007). Thus, if the integrin-FAK is affecting protein synthesis, it would be expected that it is doing so independent of PI3-K. At this time, there are few data examining the role of FAK-induced activation of protein synthesis in skeletal muscle.

Leukemia inhibitory factor

It has been suggested that a number of cytokines have the ability to contribute to muscle hypertrophy. Specifically, leukemia inhibitory factor (LIF) appears to have a promising role in the hypertrophic process. Sakuma et al. (2000) found that animals that actively undergo mechanical loading increase the expression levels of LIF in their skeletal muscle. Further, the delivery of recombinant LIF to mice after an acute muscle injury resulted in larger muscle fibers, compared with those in nontreated animals (Kurek et al. 1996). Also, LIF contributes to cardiac hypertrophy by inducing activation of the Akt/mTOR pathway and enhancing protein synthesis (Oh et al. 1998). Thus, to test the role of LIF in mechanical load-induced muscle hypertrophy, mice that had LIF genetically ablated were used. The loss of LIF resulted in a failed hypertrophic response to 7 and 28 days of mechanical loading (Spangenburg and Booth 2006). The delivery of recombinant LIF back to $LIF^{-/-}$ fully restored the hypertrophic response to $LIF^{-/-}$ animals, suggesting that LIF is a critical factor in the hypertrophic process (Spangenburg and Booth 2006). Unfortunately, at this time, the mechanism

by which LIF is affecting muscle hypertrophy remains elusive; however, there is evidence to suggest that LIF can affect mechanisms that regulate both satellite proliferation and protein synthesis (Spangenburg and Booth 2002).

Overall conclusions

Understanding the mechanisms that regulate muscle hypertrophy is critical for gaining insight into the therapeutic means for preventing physical disability due to lost muscle mass. If larger muscle masses can be maintained for longer periods time, it could extend various individuals' functional independence for a greater span of their life. At this time, we are making significant strides in understanding mechanisms activated during mechanical loading of the muscle; however, we still appear to have a limited understanding of how the strain on the muscle is translated into a signal that induces muscle hypertrophy.

Acknowledgements

The author wishes to thank past and present members of the lab and current collaborators that contributed to this work. In particular, Dr. Sarah Witkowski is thanked for her contribution to unpublished work mentioned in this manuscript. This work was supported by National Institutes of Health Grant AR051396 (E.E.S.).

References

Adams, G.R. 2002. Invited Review: Autocrine/paracrine IGF-I and skeletal muscle adaptation. J. Appl. Physiol. **93**: 1159–1167. PMID:12183514.

Adams, G.R., and Haddad, F. 1996. The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. J. Appl. Physiol. **81**: 2509–2516. PMID: 9018499.

Adams, G.R., and McCue, S.A. 1998. Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats. J. Appl. Physiol. **84**: 1716–1722. PMID:9572822.

Adams, G.R., Haddad, F., and Baldwin, K.M. 1999. Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. J. Appl. Physiol. **87**: 1705–1712. PMID:10562612.

Baar, K., and Esser, K. 1999. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. Am. J. Physiol. **276**: C120–C127. PMID:9886927.

Baar, K., Nader, G., and Bodine, S. 2006. Resistance exercise, muscle loading/unloading and the control of muscle mass. Essays Biochem. **42**: 61–74. doi:10.1042/bse0420061. PMID: 17144880.

Barton, E.R. 2006. Viral expression of insulin-like growth factor-I isoforms promotes different responses in skeletal muscle. J. Appl. Physiol. **100**: 1778–1784. doi:10.1152/japplphysiol.01405.2005. PMID:16439513.

Bassel-Duby, R., and Olson, E.N. 2006. Signaling pathways in skeletal muscle remodeling. Annu. Rev. Biochem. **75**: 19–37. doi:10.1146/annurev.biochem.75.103004.142622. PMID:16756483.

Bentzinger, C.F., Romanino, K., Cloetta, D., Lin, S., Mascarenhas, J.B., Oliveri, F., et al. 2008. Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. Cell Metab. **8**: 411–424. doi:10.1016/j.cmet.2008.10.002. PMID:19046572.

Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., et al. 2001. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat. Cell Biol. **3**: 1014–1019. doi:10.1038/ncb1101-1014. PMID:11715023.

Burridge, K., and Chrzanowska-Wodnicka, M. 1996. Focal adhesions, contractility, and signaling. Annu. Rev. Cell Dev. Biol. **12**: 463–518. doi:10.1146/annurev.cellbio.12.1.463. PMID:8970735.

Burry, M., Hawkins, D., and Spangenburg, E.E. 2007. Lengthening contractions differentially affect p70s6k phosphorylation compared to isometric contractions in rat skeletal muscle. Eur. J. Appl. Physiol. **100**: 409–415. doi:10.1007/s00421-007-0444-5. PMID:17401575.

Butterfield, T.A., and Best, T.M. 2009. Stretch-activated ion channel blockade attenuates adaptations to eccentric exercise. Med. Sci. Sports Exerc. **41**: 351–356. PMID:19127190.

Carson, J.A. 1997. The regulation of gene expression in hypertrophying skeletal muscle. Exerc. Sport Sci. Rev. **25**: 301–320. doi:10.1249/00003677-199700250-00013. PMID:9213096.

Carson, J.A., and Wei, L. 2000. Integrin signaling's potential for mediating gene expression in hypertrophying skeletal muscle. J. Appl. Physiol. **88**: 337–343. PMID:10642399.

Carson, J.A., Schwartz, R.J., and Booth, F.W. 1996. SRF and TEF-1 control of chicken skeletal alpha-actin gene during slow-muscle hypertrophy. Am. J. Physiol. **270**: C1624–C1633. PMID: 8764144.

Chan, A.Y., and Dyck, J.R. 2005. Activation of AMP-activated protein kinase (AMPK) inhibits protein synthesis: a potential strategy to prevent the development of cardiac hypertrophy. Can. J. Physiol. Pharmacol. **83**: 24–28. doi:10.1139/y04-107. PMID:15759047.

Chesley, A., MacDougall, J.D., Tarnopolsky, M.A., Atkinson, S.A., and Smith, K. 1992. Changes in human muscle protein synthesis after resistance exercise. J. Appl. Physiol. **73**: 1383–1388. PMID:1280254.

DeVol, D.L., Rotwein, P., Sadow, J.L., Novakofski, J., and Bechtel, P.J. 1990. Activation of insulin-like growth factor gene expression during work-induced skeletal muscle growth. Am. J. Physiol. **259**: E89–E95. PMID:2372054.

Drummond, M.J., Miyazaki, M., Dreyer, H.C., Pennings, B., Dhanani, S., Volpi, E., Esser, K.A., Rasmussen, B.B. 2008. Expression of growth-related genes in young and old human skeletal muscle following an acute stimulation of protein synthesis. J. Appl. Physiol. [Epub ahead of print]. PMID:18787087.

Fluck, M., Carson, J.A., Gordon, S.E., Ziemiecki, A., and Booth, F.W. 1999*a*. Focal adhesion proteins FAK and paxillin increase in hypertrophied skeletal muscle. Am. J. Physiol. **277**: C152–C162. PMID:10409118.

Fluck, M., Carson, J.A., Schwartz, R.J., and Booth, F.W. 1999*b*. SRF protein is upregulated during stretch-induced hypertrophy of rooster ALD muscle. J. Appl. Physiol. **86**: 1793–1799. PMID:10368339.

Franco, A., Jr., and Lansman, J.B. 1990*a*. Calcium entry through stretch-inactivated ion channels in mdx myotubes. Nature, **344**: 670–673. doi:10.1038/344670a0. PMID:1691450.

Franco, A., Jr., and Lansman, J.B. 1990*b*. Stretch-sensitive channels in developing muscle cells from a mouse cell line. J. Physiol. **427**: 361–380. PMID:2170636.

Gan, B., Yoo, Y., and Guan, J.L. 2006. Association of focal adhesion kinase with tuberous sclerosis complex 2 in the regulation of s6 kinase activation and cell growth. J. Biol. Chem. **281**: 37321–37329. doi:10.1074/jbc.M605241200. PMID:17043358.

Glass, D.J. 2005. Skeletal muscle hypertrophy and atrophy signaling pathways. Int. J. Biochem. Cell Biol. **37**: 1974–1984. PMID:16087388.

Goldberg, A.L. 1967. Work-induced growth of skeletal muscle in

normal and hypophysectomized rats. Am. J. Physiol. **213**: 1193–1198. PMID:6054866.

Goldspink, G., Wessner, B., and Bachl, N. 2008. Growth factors, muscle function and doping. Curr. Opin. Pharmacol. **8**: 352–357. doi:10.1016/j.coph.2008.02.002. PMID:18353722.

Gordon, S.E., Fluck, M., and Booth, F.W. 2001. Selected contribution: skeletal muscle focal adhesion kinase, paxillin, and serum response factor are loading dependent. J. Appl. Physiol. **90**: 1174–1183. [Discussion 1165.] PMID:11181634.

Gordon, S.E., Lake, J.A., Westerkamp, C.M., and Thomson, D.M. 2008. Does AMP-activated protein kinase negatively mediate aged fast-twitch skeletal muscle mass? Exerc. Sport Sci. Rev. **36**: 179–186. doi:10.1097/JES.0b013e3181877e13. PMID: 18815486.

Gulati, P., Gaspers, L.D., Dann, S.G., Joaquin, M., Nobukuni, T., Natt, F., et al. 2008. Amino acids activate mTOR complex 1 via Ca2+/CaM signaling to hVps34. Cell Metab. **7**: 456–465. doi:10.1016/j.cmet.2008.03.002. PMID:18460336.

Hawke, T.J., and Garry, D.J. 2001. Myogenic satellite cells: physiology to molecular biology. J. Appl. Physiol. **91**: 534–551. PMID:11457764.

Hornberger, T.A., Armstrong, D.D., Koh, T.J., Burkholder, T.J., and Esser, K.A. 2005. Intracellular signaling specificity in response to uniaxial vs. multiaxial stretch: implications for mechanotransduction. Am. J. Physiol. Cell Physiol. **288**: C185–C194. PMID:15371259.

Hornberger, T.A., Chu, W.K., Mak, Y.W., Hsiung, J.W., Huang, S.A., and Chien, S. 2006. The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. Proc. Natl. Acad. Sci. U.S.A. **103**: 4741–4746. doi:10.1073/pnas.0600678103. PMID:16537399.

Hornberger, T.A., Sukhija, K.B., Wang, X.R., and Chien, S. 2007. mTOR is the rapamycin-sensitive kinase that confers mechanically-induced phosphorylation of the hydrophobic motif site Thr(389) in p70(S6k). FEBS Lett. **581**: 4562–4566. doi:10.1016/j.febslet.2007.08.045. PMID:17825298.

Janssen, I., Baumgartner, R.N., Ross, R., Rosenberg, I.H., and Roubenoff, R. 2004. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. Am. J. Epidemiol. **159**: 413–421. doi:10.1093/aje/kwh058. PMID: 14769646.

Kurek, J., Bower, J., Romanella, M., and Austin, L. 1996. Leukaemia inhibitory factor treatment stimulates muscle regeneration in the mdx mouse. Neurosci. Lett. **212**: 167–170. doi:10.1016/0304-3940(96)12802-0. PMID:8843099.

Lorenz, M.C., and Heitman, J. 1995. TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. J. Biol. Chem. **270**: 27531–27537. doi:10.1074/jbc.270.10.5483. PMID:7499212.

Mackenzie, M.G., Hamilton, D.L., Murray, J.T., Taylor, P.M., and Baar, K. 2009. mVps34 is activated following high-resistance contractions. J. Physiol. **587**: 253–260. doi:10.1113/jphysiol.2008.159830. PMID:19015198.

McGee, S.L., Mustard, K.J., Hardie, D.G., and Baar, K. 2008. Normal hypertrophy accompanied by phosphoryation and activation of AMP-activated protein kinase alpha1 following overload in LKB1 knockout mice. J. Physiol. **586**: 1731–1741. doi:10.1113/jphysiol.2007.143685. PMID:18202101.

Miyazaki, M., and Esser, K.A. 2008. Cellular mechanisms regulating protein synthesis and skeletal muscle hypertrophy in animals. J. Appl. Physiol. [Epub ahead of print.] PMID:11299288.

Miyazaki, M., and Esser, K.A. 2009. REDD2 is enriched in skeletal muscle and inhibits mTOR signaling in response to leucine and stretch. Am. J. Physiol. Cell. Physiol. [Epub ahead of print.] PMID:11299288.

Nader, G.A., and Esser, K.A. 2001. Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. J. Appl. Physiol. **90**: 1936–1942. PMID:11299288.

O'Connor, R.S., Pavlath, G.K., McCarthy, J.J., and Esser, K.A. 2007. Last Word on Point:Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. J. Appl. Physiol. **103**: 1107. doi:10.1152/japplphysiol.00502.2007. PMID: 17724310.

Oh, H., Fujio, Y., Kunisada, K., Hirota, H., Matsui, H., Kishimoto, T., and Yamauchi-Takihara, K. 1998. Activation of phosphatidylinositol 3-kinase through glycoprotein 130 induces protein kinase B and p70 S6 kinase phosphorylation in cardiac myocytes. J. Biol. Chem. **273**: 9703–9710. doi:10.1074/jbc.273.16.9703. PMID:9545305.

Ohanna, M., Sobering, A.K., Lapointe, T., Lorenzo, L., Praud, C., Petroulakis, E., et al. 2005. Atrophy of S6K1$^{-/-}$ skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. Nat. Cell Biol. **7**: 286–294. doi:10.1038/ncb1231. PMID: 15723049.

Peterson, R.T., Beal, P.A., Comb, M.J., and Schreiber, S.L. 2000. FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. J. Biol. Chem. **275**: 7416–7423. doi:10.1074/jbc.275.10.7416. PMID:10702316.

Rennie, M.J., Wackerhage, H., Spangenburg, E.E., and Booth, F.W. 2004. Control of the size of the human muscle mass. Annu. Rev. Physiol. **66**: 799–828. doi:10.1146/annurev.physiol.66.052102.134444. PMID:14977422.

Reynolds, T.H. 4th., Bodine, S.C., Lawrence, J.C. Jr. 2002. Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. J. Biol. Chem. **277**: 17657–17662. doi:10.1074/jbc.M201142200. PMID:11884412.

Sakuma, K., Watanabe, K., Sano, M., Uramoto, I., and Totsuka, T. 2000. Differential adaptation of growth and differentiation factor 8/myostatin, fibroblast growth factor 6 and leukemia inhibitory factor in overloaded, regenerating and denervated rat muscles. Biochim. Biophys. Acta, **1497**: 77–88. doi:10.1016/S0167-4889(00)00044-6. PMID:10838161.

Sancak, Y., Thoreen, C.C., Peterson, T.R., Lindquist, R.A., Kang, S.A., Spooner, E., et al. 2007. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol. Cell, **25**: 903–915. doi:10.1016/j.molcel.2007.03.003. PMID:17386266.

Spangenburg, E.E., and Booth, F.W. 2002. Multiple signaling pathways mediate LIF-induced skeletal muscle satellite cell proliferation. Am. J. Physiol. Cell Physiol. **283**: C204–C211. PMID: 12055089.

Spangenburg, E.E., and Booth, F.W. 2006. Leukemia inhibitory factor restores the hypertrophic response to increased loading in the LIF(–/–) mouse. Cytokine, **34**: 125–130. doi:10.1016/j.cyto.2006.05.001. PMID:16781162.

Spangenburg, E.E., and McBride, T.A. 2006. Inhibition of stretch-activated channels during eccentric muscle contraction attenuates p70S6K activation. J. Appl. Physiol. **100**: 129–135. doi:10.1152/japplphysiol.00619.2005. PMID:16179399.

Spangenburg, E.E., Le Roith, D., Ward, C.W., and Bodine, S.C. 2008. A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. J. Physiol. **586**: 283–291. doi:10.1113/jphysiol.2007.141507. PMID:17974583.

Thomson, D.M., and Gordon, S.E. 2005. Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. J. Appl. Physiol. **98**: 557–564. doi:10.1152/japplphysiol.00811.2004. PMID: 15465886.

Thomson, D.M., Fick, C.A., and Gordon, S.E. 2008. AMPK activa-

tion attenuates S6K1, 4E–BP1, and eEF2 signaling responses to high-frequency electrically stimulated skeletal muscle contractions. J. Appl. Physiol. **104**: 625–632. doi:10.1152/japplphysiol.00915.2007. PMID:18187610.

Vyas, D.R., Spangenburg, E.E., Abraha, T.W., Childs, T.E., and Booth, F.W. 2002. GSK-3beta negatively regulates skeletal myotube hypertrophy. Am. J. Physiol. Cell Physiol. **283**: C545–C551. PMID:12107064.

Wan, M., Wu, X., Guan, K.L., Han, M., Zhuang, Y., and Xu, T. 2006. Muscle atrophy in transgenic mice expressing a human TSC1 transgene. FEBS Lett. **580**: 5621–5627. doi:10.1016/j.febslet.2006.09.008. PMID:16996505.

Wong, T.S., and Booth, F.W. 1988. Skeletal muscle enlargement with weight-lifting exercise by rats. J. Appl. Physiol. **65**: 950–954. PMID:2459101.

Wong, T.S., and Booth, F.W. 1990*a*. Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. J. Appl. Physiol. **69**: 1709–1717. PMID:1703145.

Wong, T.S., and Booth, F.W. 1990*b*. Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. J. Appl. Physiol. **69**: 1718–1724. PMID:1703146.

Xia, H., Nho, R.S., Kahm, J., Kleidon, J., and Henke, C.A. 2004. Focal adhesion kinase is upstream of phosphatidylinositol 3-kinase/Akt in regulating fibroblast survival in response to contraction of type I collagen matrices via a beta 1 integrin viability signaling pathway. J. Biol. Chem. **279**: 33024–33034. doi:10.1074/jbc.M313265200. PMID:15166238.

Yeung, E.W., Whitehead, N.P., Suchyna, T.M., Gottlieb, P.A., Sachs, F., and Allen, D.G. 2005. Effects of stretch-activated channel blockers on (Ca2+)i and muscle damage in the mdx mouse. J. Physiol. **562**: 367–380. doi:10.1113/jphysiol.2004.075275. PMID:15528244.