Title

The Molecular Basis for Load-Induced Skeletal Muscle Hypertrophy

Journal

Calcified Tissue International, 96(3)

ISSN

0171-967X

Authors

Marcotte, George R
West, Daniel WD
Baar, Keith

Publication Date

2015-03-01

DOI

10.1007/s00223-014-9925-9

Peer reviewed

HHS Public Access

Author manuscript

Calcif Tissue Int. Author manuscript; available in PMC 2016 March 28.

Published in final edited form as:

Calcif Tissue Int. 2015 March ; 96(3): 196–210. doi:10.1007/s00223-014-9925-9.

The Molecular Basis for Load-Induced Skeletal Muscle Hypertrophy

George R. Marcotte, **Daniel W.D. West**, and **Keith Baar**

Neurobiology, Physiology and Behavior, University of California Davis

Abstract

In a mature (weight neutral) animal, an increase in muscle mass only occurs when the muscle is loaded sufficiently to cause an increase in myofibrillar protein balance. A tight relationship between muscle hypertrophy, acute increases in protein balance, and the activity of the mechanistic target of rapamycin complex 1 (mTORC1) was demonstrated 15 years ago. Since then, our understanding of the signals that regulate load-induced hypertrophy has evolved considerably. For example, we now know that mechanical load activates mTORC1 in the same way as growth factors, by moving TSC2 (a primary inhibitor of mTORC1) away from its target (the mTORC activator) Rheb. However, the kinase that phosphorylates and moves TSC2 is different in the two processes. Similarly, we have learned that a distinct pathway exists whereby amino acids activate mTORC1 by moving it to Rheb. While mTORC1 remains at the forefront of load-induced hypertrophy, the importance of other pathways that regulate muscle mass are becoming clearer. Myostatin, is best known for its control of developmental muscle size. However, new mechanisms to explain how loading regulates this process are suggesting that it could play an important role in hypertrophic muscle growth as well. Lastly, new mechanisms are highlighted for how β2 receptor agonists could be involved in load-induced muscle growth and why these agents are being developed as non-exercise-based therapies for muscle atrophy. Overall, the results highlight how studying the mechanism of load-induced skeletal muscle mass is leading the development of pharmaceutical interventions to promote muscle growth in those unwilling or unable to perform resistance exercise.

Skeletal muscle mass: a context for understanding its regulation

The regulation of tissue size is dictated by the balance between the rates of protein synthesis and degradation [1]. More specifically, it is the balance of the synthesis and degradation of functional and structural proteins within the cell that determines tissue size. Consequently, in muscle a net positive or net negative myofibrillar protein balance results in hypertrophy or atrophy, respectively. In healthy adults, rates of myofibrillar protein synthesis fluctuate between periods of net positive (after protein feeding) and net negative (fasting) balance,

Address for correspondence: Dr. Keith Baar, Functional Molecular Biology Lab, University of California Davis, One Shields Ave, 174 Briggs Hall, Davis, CA, 95616, kbaar@ucdavis.edu.

Disclosure statement

Dr. Baar reports grants, personal fees and non-financial support from PepsiCo, grants, personal fees and non-financial support from USATF, personal fees and non-financial support from Novartis. In addition, Dr. Baar has a patent “Control of muscle size” licensed to Advanced Muscle Technologies. Mr. Marcotte and Dr. West have nothing to disclose.

such that the change in muscle mass over time is, broadly speaking, very small [2]. While both rates of myofibrillar protein synthesis and breakdown fluctuate during anabolic and catabolic conditions, using our current methods the rate of protein synthesis appears more dynamic than that of protein breakdown, ultimately suggesting skeletal muscle mass is primarily dictated by the regulation of muscle protein synthesis [3]. There is also a fallacy in the field that decreasing the rate of degradation would always increase muscle mass. In some cases this is true [4, 5]. However, It is now clear that some forms of protein degradation actually are necessary to drive protein synthesis and improve both muscle size and quality [6, 7].

Skeletal muscle is a plastic tissue that rapidly adapts to its mechanical environment [8]. Increased load across a muscle, such as from strength (resistance) exercise or heavy work, results in a compensatory increase in muscle size and strength. This increase in size occurs largely from the growth of existing cells (hypertrophy) rather than an increase in cell number (hyperplasia) [9]. Mechanical loading also results in strong adaptive responses in a host of other tissues including bone [10], tendon [11] and the extracellular matrix in muscle [12], protecting these tissues against future injury. Conversely, tissue atrophy occurs with the introduction of catabolic stimuli including dietary protein withdrawal [13], mechanical unloading [14, 15] or during disease/injury states [16]. The ability of skeletal muscle to hypertrophy in response to load will be the focus of this review. For more information on atrophy, the reader is directed to the following excellent reviews on the topic [17, 18].

Bigger and stronger skeletal muscles improve both the quality and length of our lives [19–21]. Currently, resistance training is the only *bona fide* intervention to increase muscle mass that can be implemented on a population-wide basis. Despite its wide-ranging benefits [22], resistance training is under-prescribed [23] and regularly practiced only by a small minority of individuals. Further, there are a host of situations (e.g., injury, illness, bed rest, limited accessibility to facilities, social constraints) that preclude many individuals from resistance training. Therefore, adjunct therapies to increase or preserve muscle mass would have a major impact on population health and well-being. Given this context, the goal of the present review is to highlight both established and emerging molecular mechanisms that regulate load-induced skeletal muscle hypertrophy and that might be exploited for developing new interventions designed to increase muscle mass.

Signals controlling load-induced skeletal muscle hypertrophy

Load

The most important signal for muscle growth in a mature (weight neutral) individual is the load across the muscle. This was best demonstrated in a series of experiments performed by Theodore Wong and Frank Booth in the late 1980's [24–26]. In these experiments, all of the hindlimb muscles of the right leg of a rat were electrically stimulated. Because of the greater mass in the posterior compartment, the ankle would plantar flex resulting in high load lengthening contractions on the anterior compartment muscles (the tibialis anterior (TA) and extensor digitorum longus) and low load shortening contractions in the muscles of the posterior compartment (gastrocnemius (GTN), plantaris, and soleus). Ten weeks of training without an external load resulted in hypertrophy of the TA without a change in muscle size

in the GTN [26]. The exact same stimulation protocol, only this time adding a weight to resist plantar flexion, resulted in significant growth of both the TA and GTN (Figure 1). Since the only difference between the groups was the external load, the most obvious conclusion is that load is the key to muscle hypertrophy. Further analysis of this data set can provide other insights, including the fact that power is not important for muscle hypertrophy. This conclusion comes from comparing TA muscle hypertrophy from the non-weighted and weighted group (Figure 1). In both cases the hypertrophy is the same. That makes sense if load is the stimulus for hypertrophy since the stretch of the TA by the GTN provides the load. However, the addition of load to the foot would slow the rate at which that load was developed, resulting in a decrease in the power applied to the muscle. Since the hypertrophy of the TA is the same with or without an external load [26], power cannot be the stimulus for muscle growth.

The fact that the load across the muscle is important for muscle hypertrophy in adult animals is widely accepted. However, recent studies showing that both high and low load resistance exercise could drive muscle protein synthesis and hypertrophy to the same degree [27, 28] and that blood flow restriction can drive hypertrophy at low loads [29, 30] raised some questions about the role of load in muscle hypertrophy. Briefly, Prof. Stuart Phillips and his group had subjects perform resistance exercise with either 90% of their 1 repetition maximum (1RM) or 30% of 1RM. For the groups performing 30% 1RM, the subjects were further divided into two groups: one that was matched for the work performed by the 90% 1RM group, and another that continued to lift 30% 1RM to failure. Interestingly, mixed muscle protein synthesis increased the same amount in the 90% and 30% to failure groups, whereas in the 30% work-matched group a smaller increase in protein synthesis was observed [27]. When they performed a follow up training study using either a high or low load to failure they found that the amount of muscle hypertrophy was the same in both groups [28]. So, how can this finding be juxtaposed with the loading findings in animals? The simplest explanation is that during human resistance exercise motor units are recruited based on the load: low loads need only small motor units (fewer muscle fibers), whereas high loads require more and bigger motor units (more fibers). However, as an individual progresses toward failure, more motor units are recruited until at failure all of the motor units within the muscle are activated in an attempt to lift the weight. Therefore, at failure all fibers within the working muscle are loaded. In animal studies, electrical stimulation overrides this issue by depolarizing and activating all of the fibers directly and therefore the effect of load is more obvious. Taken together, these data suggest that the load across the muscle is the primary stimulus for skeletal muscle hypertrophy and the only way to provide load across all fibers in humans is to perform exercise to failure.

Hormones

The first molecular regulators of skeletal muscle mass identified were hormones such as testosterone [31]. The fascinating history of the discovery of the hormone testosterone covers more than a thousand years [32]. However, for muscle physiologists the role of this hormone in the control of muscle size and strength starts just over a century ago. In 1889, a French physiologist named Charles Edouard Brown-Sequard claimed that daily injections of an extract isolated from the testes of dogs and guinea pigs had increased the strength of the

72-year-old physician/scientist [33]. Even though it is likely that this was nothing more than a placebo effect, his report started more than a century of work on what would be called hormones and their effect on skeletal muscle size and strength. With the isolation and production of synthetic testosterone in 1935 [32], scientists were able to inject testosterone propionate and show for the first time that this hormone could lead to “a definite hypertrophy” of all of the muscles in the body [31]. By 1942 [34], this had been shown in young men, starting more than 70 years of testosterone use to improve muscle size, strength and performance [33]. However, it is important to note that testosterone affects developmental growth far more than hypertrophic growth. For example, women, who have approximately one tenth the testosterone of men, have developmentally smaller muscles [35]. However, women show the same relative amount of muscle hypertrophy as men following 16 weeks of strength training [35]. Further, even though muscle mass is lost during androgen deprivation therapy, resistance exercise is still able to produce skeletal muscle hypertrophy in patients undergoing this treatment [36]. Lastly, training one arm in a hormonal milieu with 3-times more testosterone than the other arm has no effect on either myofibrillar protein synthesis rate, muscle hypertrophy, or strength changes that occur with training [37, 38]. Together, these data indicate that even though testosterone affects developmental processes that regulate muscle mass, physiological fluctuations (i.e., non-pharmacological-based changes) in testosterone have little effect on load-induced skeletal muscle hypertrophy.

Even though post-exercise testosterone does not affect load-induced skeletal muscle signaling, protein synthesis or hypertrophy, there is no doubt that it and a whole host of other hormones go up both in the circulation [37, 39] and within the working muscle [40, 41] following resistance exercise. These hormones, like IGF-1 [42] and growth hormone [43, 44] can all affect developmental skeletal muscle mass and strength. However, it is clear from both in vitro work [45], animal [42, 46–49], as well as human training studies [37, 38] that none of these hormones are required for load-induced skeletal muscle hypertrophy (explained in depth below). Therefore, even though hormones set our baseline muscle size and strength, they play little role in the adaptive response to resistance training.

Chalones

Chalones are tissue-specific secreted proteins that control organ size [50]. It is thought that each organ can secrete a distinct chalone that circulates through the body and inhibits the growth of that specific tissue. In many cases, these are N-substituted oligopeptides that can regulate genes involved in growth and differentiation [51]. However, skeletal muscle has a different kind of chalone protein known as myostatin [52]. Myostatin, or growth and differentiation factor 8, is a member of the transforming growth factor β (TGFβ) family of hormones. Myostatin was originally cloned by Alexandra McPherron and Se-Jin Lee as a highly conserved, muscle-specific, secreted protein [53]. True to its categorization as a chalone, when the protein was naturally absent, as in Belgian Blue and Piedmontese cattle or whippet dogs, the animals have an excessively muscled phenotype [53, 54]. Further, decreasing the receptor for myostatin, increasing the production of its pro-peptide (that inhibits myostatin-activin IIb receptor binding), or increasing the production of follistatin (a natural TGFβ family inhibitor) results in an increase in muscle mass [55]. It is important to

note that the increase in muscle mass due to inhibition of myostatin signaling early in development results in both hyperplasia and hypertrophy [52]. This is in contrast to what occurs following load-induced muscle growth, where muscle growth is primarily the result of skeletal muscle hypertrophy [9]. In fact, even though myostatin clearly plays a role in muscle mass, its role in load-induced skeletal muscle hypertrophy is still equivocal. For example, even though four days of training decreases myostatin mRNA in proportion to the load across the muscle [56], in human muscle myostatin mRNA goes down the most in "non-responders", those subjects who do not increase muscle mass after resistance exercise [57]. Further, if a decrease in circulating myostatin drives load-induced hypertrophy, why do only the loaded muscles grow [26] and not every muscle in the body? These data suggest that myostatin mRNA may not be the best predictor of muscle growth or that myostatin protein may not be a true chalone following resistance exercise. However, as we learn more about the complexity of myostatin signaling, a novel molecular model for load-induced inhibition of myostatin signaling is beginning to become clear (discussed below).

Feeding

The consumption of sufficient nutrients is needed for developmental muscle growth. However, in energy-restricted diets that in all other manners are of high-quality (i.e. have sufficient protein) there is no long-term negative effect on muscle size or function relative to body mass [58]. In contrast, in the absence of sufficient protein, muscle does not develop to the proper size [59]. Therefore, protein is a key component of muscle development. In rats however, there is no effect of caloric restriction [60] or either increasing or decreasing dietary protein on muscle hypertrophy following prolonged overload [61]. This is in stark contrast to the role of protein in human load-induced skeletal muscle hypertrophy [2]. In humans, providing protein after resistance exercise leads to more myofibrillar protein synthesis and has the potential to decrease protein degradation [62]. Further, the consumption of protein together with resistance training leads to greater muscle hypertrophy than consumption of isoenergetic carbohydrate [63], revealing that it is amino acids – not energy per se – that can augment protein accretion. After exercise, there is an increase in amino acid transport [64, 65] resulting in a concentration of the substrates for protein synthesis. Interestingly, this load-induced increase in amino acid sensitivity is maintained for at least 24 hours following the completion of exercise [66]. The role of protein feeding in human load-induced muscle hypertrophy is so important that the amount [67], type [68], and timing of protein intake [69] after resistance exercise has been extensively studied. These studies allow us to state that the consumption of ~0.25g/kg body weight of a rapidly digested and leucine-rich protein source every 4–5 hours throughout the day and soon after the completion of resistance exercise is needed to optimize load-induced skeletal muscle hypertrophy in humans.

The difference between the role of protein in rodent and human load-induced skeletal muscle hypertrophy is likely due to the extreme difference in the load itself and not a species difference. Many rodent studies use a synergist ablation model whereby a large muscle is removed and the resulting intact muscles receive a constant mechanical stimulus to grow [46]. This is in stark contrast to human resistance exercise where subjects exercise intermittently several times per week resulting in a time under tension that is much lower.

Together these data suggest that for an intermittent loading paradigm (human resistance training), the provision of sufficient amino acids at an optimal interval is essential for optimal load-induced skeletal muscle hypertrophy. However, if the load is more chronic, the need for dietary amino acids to potentiate the effect of loading is reduced.

Catecholamines

Resistance exercise is a whole body stress. During resistance exercise, catecholamine levels rise significantly [70, 71]. Catecholamines bind to and activate adrenergic receptors [72]. With respect to skeletal muscle hypertrophy, the beta-2 (β2) adrenergic receptors receive the most attention due to the ability of β2-agonists to induce skeletal muscle hypertrophy [73]. Therefore, the potential of catecholamines, acting through β2 receptors to induce muscle hypertrophy is an attractive model. However, there has always been the issue of how a global stress signal, such as a rise in catecholamines, could result in a response only in the muscles that have performed resistance exercise. An attractive model to address this concern has been put forward by Bruno et al. [74]. In this model, calcium, released during exercise, converges with the β2-receptor to activate a downstream signaling cascade that drives skeletal muscle hypertrophy (see more detail below). With this new insight, the potential role of whole body stress, in the form of a rise in catecholamines, warrants serious consideration.

Molecular signals underlying load-induced skeletal muscle hypertrophy

mTORC1 and the activation of translation initiation

On the foundation of a great deal of physiological research into skeletal muscle hypertrophy [8, 24–26, 46, 75], in 1999 the first molecular regulator of load-induced skeletal muscle hypertrophy was identified [76]. The protein identified was a serine/threonine protein kinase called the mechanistic/mammalian target of rapamycin (mTOR) [77]. Fifteen years later, we know that mTOR is an evolutionarily conserved protein kinase that is important in relaying information from nutrients, growth factors and mechanical loading to drive protein synthesis and cell growth [77]. However, mTOR has limited kinase activity on its own [78]. Instead, mTOR forms complexes with other proteins that dictate its location, activity and downstream targets. Currently, we know mTOR to exist within two complexes: complex 1 (mTORC1) and complex 2 (mTORC2). The primary components of complex 1 are the regulatory associated protein of mTOR (raptor), the mammalian lethal with SEC13 protein (mLST8/GβL), the proline rich akt substrate (PRAS40), and the DEP domain-containing mTOR interacting protein (DEPTOR). Complex 2 contains the rapamycin insensitive companion of mTOR (rictor), mLST8/GβL, protein observed with Rictor-1 (PROTOR), DEPTOR and MAP kinase interacting protein 1 (mSIN1). Complex 1 is rapamycin-sensitive and integral to controlling cell size, and will therefore be the focus of our discussion.

Over the last 5 years, the mechanisms underlying the activation of mTORC1 by amino acids, growth factors and mechanical loading have been largely elucidated (see below). However, more work is needed before we fully understand the activation of mTORC1 and the identity of its downstream targets. The importance of mTORC1 in the regulation of muscle mass is seen by the fact that inhibiting mTORC1 in humans with the macrolide drug

rapamycin has been shown to block acute amino acid- [79] and resistance exercise- [80] induced increases in muscle protein synthesis. Further, rapamycin blocks hypertrophy that normally occurs after chronic mechanical loading [81], an effect specific to decreased mTORC1 activity and not to off-target effects of the inhibitor [9]. Similarly, the constitutive genetic up regulation of mTORC1 activity results in muscle hypertrophy [82] and the increase in mTORC1 activity following a single bout of exercise correlates with the increase in muscle mass following training [76, 83]. Together these studies suggest that mTORC1 is a critical regulator of skeletal muscle cell size and that for us to understand load-induced skeletal muscle hypertrophy we must understand how mTORC1 is regulated.

Growth factor activation of mTORC1

The activity of mTORC1 is regulated by a number of pathways allowing for tight control of cell growth in concert with changes in cellular energy levels [84, 85], the availability of nutrients [86];Menon, 2014 #9261;Zhang, 2014 #9479}, tissue load [87], and stress [77]. Of the various signaling pathways that activate mTORC1, the insulin/growth factor signaling pathway remains one of the best characterized, owing to its role in diabetes. In this context, mTORC1 becomes activated when it associates with the small G-protein Rheb (Ras homolog enriched in brain; Figure 2). Following insulin/IGF1 binding to its receptor on the membrane, a series of events ensue that releases the inhibition of Rheb, resulting in increased mTORC1 activity [88]. Rheb induced activation of mTORC1 is dependent on its GTPase activity, as GTP bound Rheb increases mTORC1 activity while GDP bound Rheb does not [89]. In the activation of mTORC1 by growth factors, a negative regulator of Rheb, called the TSC2 complex, is sequestered away from Rheb following insulin binding. The TSC2 complex is composed of hamartin (TSC1), tuberin (TSC2), and TBC1D7 [90]. The TSC2 complex acts as a GTPase activating enzyme (GAP) that keeps Rheb in the GDP-bound state [91]. Following insulin treatment, TSC2 is phosphorylated by protein kinase B (PKB)/akt [92] resulting in its relocalization away from Rheb [88]. Akt not only inactivates the TSC2 complex, but also inhibits PRAS40 (proline-rich akt substrate of 40KDa); a negative regulator of mTORC1 that directly inhibits the ability of mTOR to phosphorylate other targets [93]. Akt becomes activated as a direct result of the insulin/growth factor-dependent movement of a series of proteins to the plasma membrane. When insulin binds to its receptor, the receptor autophosphorylates creating a docking site for the insulin receptor substrates 1/2 (IRS1/2) [94]. IRS1/2 then moves to the plasma membrane where it recruits the regulatory subunit of the phosphoinositide 3-kinase (PI3K) [94]. This brings PI3K to the membrane where it can phosphorylate its target, the membrane phospholipid phosphoinositol (4,5) bisphosphate (PIP2). The resulting phosphoinositol (3,4,5) trisphosphate (PIP3) then acts as a docking site in the plasma membrane for akt and its kinase the 3-phosphoinositide dependent protein kinase-1 (PDK1) [95]. When akt and PDK1 co-localize at the membrane, PDK1 phosphorylates akt at one site [96] and the membrane-associated mTORC2 [97] phosphorylates a second site; resulting in full activation of akt and the subsequent phosphorylation of TSC2. In the end, this long complex pathway strives simply to move the TSC2 complex away from its target (Rheb) so that mTORC1 can be turned on (Figure 2). This same final step is shared by mechanical loading (see below) and this is why there is very little additive effect of both loading and growth factors. However, amino acids signal in a parallel fashion (described in detail below) and as a result amino

acids can increase mTORC1 signaling when supplied together with either growth factors or loading.

Load-induced activation of mTORC1

The ability of rapamycin to inhibit the acute load-induced increase in muscle protein synthesis in humans [80] highlights the importance of mTORC1 in the response to mechanical loading. The exact mechanism underlying the transduction of the mechanical signal into increased mTORC1 activity remains relatively elusive. However, at least two distinct events appear to regulate mechanical loading mediated activation of mTORC1: an increase association of mTORC1 with Rheb [87]; and an increase the abundance of phosphatidic acid [98]. The mechanism underlying these two events will be described below.

Recent work from Troy Hornberger's lab demonstrates that, like insulin and growth factors, loading results in the translocation of TSC2 away from Rheb [87]. In fact, Jacobs and her colleagues were the first to show that the activation of mTORC1 by any stimulus was the result of the forced removal of TSC2 from Rheb [87]. In mouse muscle, at rest, TSC2 localizes with Rheb. Following resistance exercise, TSC2 becomes phosphorylated on RxRxxS*/T* residues and this phosphorylation event corresponds to the movement of TSC2 away from Rheb [87]. Therefore, just like insulin and growth factors the final step in the activation of mTORC1 is the phosphorylation of TSC2 (Figure 3). However, unlike insulin and growth factors the kinase responsible for the phosphorylation of TSC2 in response to resistance exercise does not appear to be akt. This conclusion comes from data collected in a number of different labs using very different methods. First, Hornberger showed that stretching isolated muscles in the presence of the PI3K inhibitor wortmannin could completely block the activation of akt without altering mTORC1 activation [45]. He further showed that mTORC1 activation in response to loading occurred normally in muscles that lacked akt1 [45]. Second, Espen Spangenberg used a mouse model that carried a knockin mutation in the insulin receptor, that prevents the insulin/IGF1-induced activation of akt, to show that in response to load these animal turned on mTORC1 and their muscles grew normally [42]. Third, in most human studies akt phosphorylation is not related to the load across the muscle, but rather the feeding state. In fact, in many human resistance exercise studies akt phosphorylation decreases, despite mTORC1 activation [99]. Fourth, we have shown that following resistance exercise the recruitment of PI3K to the membrane and the activation of akt decreases in muscles where mTORC1 activity rises up to 80-fold. From these data, it is clear that while insulin and growth factors activate mTORC1 through akt, load induces an akt-independent activation of mTORC1. Since the end result of both loading and growth factors is the movement of TSC2 away from Rheb, using different upstream kinases, this explains why loading and growth factors do not have an additive effect on muscle hypertrophy. While it remains unclear what mediates load-induced TSC2 phosphorylation a number of potential candidates are currently being assessed.

The second direct activator of mTORC1 associated with load-induced muscle hypertrophy is a glycerophospholipid known as phosphatidic acid (PA). Phosphatidic acid results in marked increases in mTORC1 activity when provided exogenously or through the transgenic

overexpression of the enzymes that synthesize PA [100]. Similarly, limiting PA production results in a decrease in mTORC1 activity (for review see [98]). PA is thought to mediate mTORC1 activation through direct binding to the FKBP12-rapamycin binding domain of mTOR [100]. Mechanical loading of muscle results in increased production of PA and this is required for mTORC1 activation. Recently, diacylglycerol kinase zeta (DGKζ), one of many enzymes responsible for the synthesis of PA, has been shown to play a role in load-induced mTORC1 activation [98]. You et al. [100] demonstrated that DGKζ activity increased biphasically with stretch and that adding more DGKζ to muscle cells *in vitro* could increase the activation of mTORC1 by serum. Further, muscles from DGKζ knockout mice did not show as great an increase in PA as wild type controls in response to stretch [100]. Lastly, electroporating DGKζ into muscle resulted in the activation of mTORC1 and skeletal muscle hypertrophy, whereas the kinase dead DGKζ did not [100]. These data suggest that DGKζ is involved in the activation of mTORC1 in skeletal muscle in response to stretch. However, whether DGKζ is required for load-induced activation of mTORC1 has yet to be determined. In fact, the absolute activation of mTORC1 in response to load in the DGKζ knockout muscles is the same as the wild type controls [100], suggesting that even though DGKζ can modulate mTORC1 activity, it is not required for the load-induced activation of mTORC1.

Amino acid activation of mTORC1

Amino acids are thought to activate mTORC1 using a mechanism that is distinct from loading or growth factors; allowing for an additive effect of combining these stimuli (as discussed above). The initial evidence that amino acid activation of mTORC1 was growth factor and loading-independent came from studies in TSC2 knockout cells. As described above, the removal of TSC2 by either loading or growth factors activates mTORC1. Therefore, in the absence of TSC2, basal mTORC1 activity is higher. Interestingly though, TSC2 knockout cells retain amino acid sensitivity [101]. Whether in the presence or absence of TSC2, amino acids are thought to regulate mTORC1 activity by the translocation of mTORC1 towards Rheb positive membranes (review by [102]). In amino acid deprived cells, mTORC1 is dispersed throughout the cytoplasm [103]. However, within 10 minutes of the addition of amino acids, mTORC1 relocalizes to Rheb-positive membranes [104]. The importance of this translocation event was demonstrated by fusing raptor to the last 15 amino acids of Rheb (Raptor-Rheb15). This fusion protein resulted in constitutively active mTORC1 that was insensitive to amino acid withdrawal [105]. In contrast, the fusion of raptor to Rheb via the CAAX Box, a site that normally acts to anchor Rheb to the membrane, was not able to activate mTORC1 during amino acid withdrawal. These data demonstrate that amino acid mediated activation of mTORC1 requires only the association of mTORC1 with Rheb and the localization of these proteins at the lysosome [105]. The importance of lysosomal localization of mTORC1 and Rheb can be attributed to additional regulators of mTORC1 at the lysosome surface of these cells. Included in the proteins that regulate mTORC1 at the lysosome in dividing cells are the Ragulator, the tumor suppressor folliculin and the Rag family of small G-proteins.

The Rags are a family of four small G-proteins denoted, RagA, B, C and D, that exist as heterodimers consisting of one RagA or B bound to one RagC or D [106]. The Rag

heterodimers localize to the lysosomal membrane via the Ragulator and interact with mTORC1 via direct binding to raptor [103, 107]. The interaction between RagA/B and raptor is dependent on the GTP loading of the Rag heterodimer, which in turn is regulated by a series of GAP and GEF proteins that are sensitive to nutrients. [103];Zhang, 2014 #9479}. Loss and gain of function studies have demonstrated that the knockdown of Rag or the overexpression of a dominant negative form of the Rags suppresses the effects of amino acids on mTORC1 activity [107]. Conversely, a constitutively active Rag heterodimer ($RagB^{GTP}$/$RagD^{GDP}$) relocalized mTORC1 to Rheb positive membranes, and thereby activated mTORC1, in a leucine-independent manner [103]. These data suggest that the Rags mediate mTORC1 movement to the lysosome, whereupon Rheb can then activate mTORC1 [103].

Nutrient regulation of Rag activity, while still in its infancy, is believed to involve two complexes of proteins; one called the Ragulator [105] and the other GATOR [108]. The Ragulator is tethered adjacent to Rheb at the lysosomal membrane and exhibits amino acid dependent guanine nucleotide exchange factor (GEF) activity towards RagA/B [109];Sancak, 2010 #7529}. The GEF activity of the Ragulator complex is regulated by the vacuolar H^+-adenosine triphosphatase ATPase (v-ATPase) [110]. The v-ATPase is a multi-protein complex that has been primarily characterized for its role in the acidification of the lysosomal lumen [111]. However, the inhibition of v-ATPase activity also impairs amino acid induced mTORC1 localization at the lysosome and S6K1 phosphorylation [112]. The underlying mechanism appears to be that low amino acid concentrations within the lysosomal lumen are sensed by the v-ATPase resulting in a conformational change that alters the interaction between the v-ATPase and the Ragulator resulting in the loss of GEF activity towards RagA/B [112] for further review see [102].

Recently, a number of proteins that may have GAP activity towards the Rag proteins have been discovered including GATOR1, folliculin and the leucyl tRNA synthase. In the absence of amino acids, RagA/B is held in the inactive GDP bound state by the heterotrimeric GAP protein complex GATOR1 [108]. When amino acids are high, the GATOR2 complex moves the GATOR1 complex away from the Rags thereby removing the GAP activity towards RagA/B [108]. The Ragulator complex then acts as a GEF towards RagA/B activating this half of the heterodimer [109]. Meanwhile, either the tumor suppressor folliculin or the leucyl tRNA synthase (LRS) acts as a GAP towards RagC/D [113, 114]. If the LRS is the GAP, this would provide the importance of leucine that we see in muscle following resistance exercise. However, there is some question as to whether the LRS can perform this role *in vivo* [108]. Furthermore, treatment of mouse embryonic fibroblasts with a mixture of amino acids devoid of leucine, blocks signaling downstream of mTORC1 without affecting mTORC1 association with the lysosome [115]. These data suggest that folliculin is the RagC/D GAP and that leucine affects mTORC1 signaling in a separate manner. Regardless, once RagA/B is GTP bound and RagC/D is GDP bound, the Rag heterodimer can bind to raptor and through its connection with the Ragulator recruits mTORC1 to the lysosome [105] where it can be activated by the association with GTP bound Rheb [91].

None of this detail of nutrient signaling is known to occur in muscle following exercise. However, we do know that intracellular amino acid levels (leucine in particular) increase acutely after strength exercise [64, 65], possibly due to an increase in amino acid transporters, including the primary leucine transporter in muscle LAT1 [116, 117]. The accumulation of amino acids following strength exercise activates VPS (vesicular trafficking protein) 34 [65], a kinase that promotes the movement of membrane vesicles, like the lysosome [118]. The activation of Vps34 by amino acids after resistance exercise likely facilitates the movement of lysosomal membranes to further promote the interaction of mTORC1 with the Rag-Ragulator complex.

Even though it is clear that the Rag proteins are necessary for mTORC1 activation by amino acids, the role of Rag GTP loading in the activation of mTORC1 has recently been questioned [119]. Prof. Joseph Avruch's group, have demonstrated that the amount of GTP bound RagA/B was not changed by amino acid withdrawal and that GTP loading did not alter mTORC1 association [119]. These authors showed that GTP loading of the Rag heterodimer was rapid, insensitive to amino acid withdrawal, and that the Rags have very low intrinsic GTPase activity. Further, using a specific inactivating mutation in the Rag, that prevents GTP loading of RagA/B, did not interfere with amino acid mediated mTORC1 binding to the lysosome or activation [119]. Even though the data from Oshiro et al. argues that the GTP bound state of Rag is inconsequential for the activation of mTORC1, they show that the Rag heterodimer is still required for mTORC1 activation by amino acids. The differences shown in this paper suggest the requirement of an as yet unidentified protein that is required for the activation of mTORC1 by the Rag heterodimer. Whether this protein is absent in HeLa cells (the previous studies were performed in HEK293 cells) or there is some other explanation, it is clear that further work is needed to fully understand how amino acids activate mTORC1 and even more work needs to determine which of these processes are at work in muscle following resistance exercise.

Smad2/3 transcriptional activity and growth

As discussed above, the myostatin/TGFβ signaling pathway can limit developmental muscle size and strength. The TGFβ family of ligands that activate this pathway, such as myostatin, TGFβ, or Activin, activate receptors that are coupled to a class of effector molecules known as the small mother of decapentaplegic (Smad) proteins [120]. Smad proteins are transcription factors that can directly regulate hundreds of gene targets, many of which are involved in muscle growth [121] and wasting [53]. Myostatin, like insulin, binds to a receptor tyrosine kinase in the membrane of the muscle cell [122] to exert biological action (Figure 4), predominantly through receptor-activated Smad2 and/or Smad3 (Smad2/3). The phosphorylation of either Smad2 or 3 by the Activin IIB and ALK4/5/7 co-receptors, as a result of myostatin binding, changes the conformation of the Smad allowing it to bind to the common mediator Smad4 [123]. Upon Smad2 or 3 binding to Smad4, the complex translocates to the nucleus where it can directly modulate gene expression [121] or modulate chromatin structure to more broadly decrease the expression of genes associated with muscle growth [123].

Beyond the classical TGFβ signaling pathway that takes Smad2/3 into the nucleus to alter transcription (Figure 4), the phosphorylation of Smad2/3 also appears to directly affect akt [124]. Winbanks and her colleagues have shown that the myostatin inhibitor follistatin can increase PIP3 levels in the membrane resulting in an increase in akt phosphorylation and the activation of mTORC1 [124]. Thus, high myostatin levels leads to phosphorylation of Smad2/3, inhibition of akt phosphorylation, and low mTORC1 activity. This inhibition of mTORC1 by myostatin is a consistent finding across models [124–126] and as a result the mTORC1 inhibitor rapamycin is able to block approximately half of the increase in muscle mass seen with myostatin inhibition [124, 127]. However, the other half of the muscle growth cannot be explained by the activation of mTORC1 and points to the importance of the transcriptional regulation of Smad2/3.

As mentioned above, the transcriptional role of Smad2/3 signaling requires Smad2/3 translocation into the nucleus with Smad4. The importance of this step has been highlighted by two recent reports showing that a separate group of Smad proteins, the receptor activated Smad1/5/8 family, can modulate Smad2/3 activity. In the first report, Sartori et al. [128] used transgenic mouse models to demonstrate that association with Smad4 was a limiting step in the regulation of muscle mass by myostatin. They achieved this by showing that inhibition of Smad1/5/8 increased denervation mediated muscle wasting. The increase in wasting corresponded with increased amounts of Smad2/3 binding to Smad4 and translocation into the nucleus, whereas increasing activated Smad1/5/8 prevented the negative regulation of muscle mass by myostatin. Almost at the same time, Winbanks and her colleagues used adeno-associated viral vectors (AAVs) to activate Smad1/5/8, and showed that this resulted in significant muscle growth in adult mice [129]. AAV-mediated overexpression of bone morphogenetic protein (BMP)7 or introduction of a constitutively active ALK3 (both of which activate Smad1/5/8) was sufficient to promote muscle growth. Additionally, AAV-mediated overexpression of the endogenous Smad1/5/8 inhibitor, Smad6, blocked the ability of follistatin to drive muscle growth [129]. Together, these studies demonstrate that the ability of myostatin to regulate muscle growth is dependent on competition for Smad4 binding. When myostatin signaling is high, Smad4 binds to Smad2/3 and this shuttles Smad2/3 into the nucleus where it can decrease the expression of genes needed for muscle growth and/or increase the expression of genes that drive muscle wasting. When BMP signaling is high, Smad4 shifts binding toward Smad1/5/8 and this either results in the targeting of a different set of genes or reverses the expression effects of myostatin.

While these pathways clearly play an important role in developmental muscle growth, their role in load-induced muscle growth remains equivocal. As described above, myostatin mRNA levels do not predict load-induced skeletal muscle hypertrophy [57]. Furthermore, we have not seen changes in Smad2/3 [130] or Smad1/5/8 (Aguirre et al. in preparation) phosphorylation following resistance exercise. In spite of the absence of differences in Smad phosphorylation, the expression of a Smad2/3 inhibited mRNA increases in proportion to the degree of muscle hypertrophy following training [130]. This suggests that following resistance exercise there is a decrease in Smad2/3 transcriptional activity. One possible explanation for this observation is that the Smad2/3 transcriptional inhibitor Notch is activated following resistance exercise [130]. In this model, resistance exercise causes the cleavage of the intracellular domain of Notch at the plasma membrane (Figure 4). Active

Notch then moves to the nucleus where it acts as an activator of transcription and also decreases the transcriptional repression of Smad2/3 [131–133]. In this way, Smad activity can be regulated locally within the exercised muscle, resulting in a muscle-specific signal for load-induced skeletal muscle hypertrophy.

G-protein coupled receptors as signalers of muscle activity

As discussed above, the stress of resistance exercise can be signaled in part through the activation of β2-adrenergic receptors. The β2 receptor is one member of a large class of G-protein coupled receptors (GPCR). There are over 800 distinct GPCRs encoded in the human genome, making this the largest family of signaling receptors in our bodies [134]. On top of the structural diversity of this family of signaling proteins lies their functional selectivity, meaning that the output of the receptor is not simply determined by the binding of an agonist, but also the context of the cellular environment [135]. As discussed above, functional selectivity may explain the ability of epinephrine to turn on growth-related proteins only in the exercised muscles [74]. In this example, whole body stress increases catecholamine levels resulting in increased binding to the β2-adrenergic receptor. In the working muscle, there is a concomitant increase in calcium flux as a direct result of contraction. When both of these signals are present in the same cell, the cyclic-AMP response element binding protein (CREB) regulated transcriptional co-activator (CRTC) is activated [74] and the activation of this protein can drive skeletal muscle hypertrophy. However, if epinephrine can only drive growth in an activated (calcium rich) environment, how is it that other β2 agonists, such as clenbuterol, formoterol and salmeterol can drive growth in all muscles regardless of activity [73]? This could potentially be explained by the ability of these drugs to simultaneously alter calcium homeostasis, increasing the passive release of calcium from the sarcoplasmic reticulum within the muscle without the need for exercise [136, 137]. In fact, much of the growth response to β2 agonists can be blocked by altering calcium-activated signals within muscle [138]. Together, these data suggest that GPCR activation, in the presence of a calcium-rich environment, can signal through CRTC to increase muscle mass.

The β2-adrenergic receptor is not the only GPCR that can induce skeletal muscle growth. Distinct GPCRs that are activated by Wnt [139], ghrelin [140], and lysophosphatidic acid [141] can all increase muscle mass or prevent muscle atrophy. Interestingly, the ability of GPCRs to alter muscle mass is dependent on mTORC1 since inactivation of mTORC1 by rapamycin prevents G-protein coupled muscle hypertrophy [139, 142]. Furthermore, the molecular explanation for muscle hypertrophy for many of the GPCR agonists seems to be through the upregulation of IGF-1 and the activation of the PI-3K/akt/mTORC1 canonical growth factor pathway [74, 139, 141]. As discussed above, this pathway plays a limited role in load-induced skeletal muscle hypertrophy, suggesting that GPCRs may be more important for developmental growth and likely play a limited role in the adaptation to resistance exercise.

Conclusions

In adults, increases in skeletal muscle mass are dependent on the load across the muscle. In the past 15 years, we have learned a great deal about the acute response to an increase in load across a muscle. However, this understanding is far from complete. For example, even though the acute activation of mTORC1 is needed to increase muscle protein synthesis after resistance exercise [80], in humans, long-term muscle growth is inversely related to mTORC1 transcriptional activity [143]. Therefore, it is clear that we have a long way to go before we completely understand how a mechanical signal (load on a muscle) is converted to a chemical signal (mTORC1 activation or Smad2/3 inhibition) that can change muscle phenotype (bigger/stronger muscles). As a result, the creation of a drug that can mimic the effects of resistance exercise on skeletal muscle mass and strength is many years in the future.

Acknowledgments

This work was supported by a project grant from the National Institute on Aging of the National Institutes of Health under award number R01AG045375. DWDW was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Postdoctoral Fellowship. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW. Control of the size of the human muscle mass. Annual review of physiology. 2004; 66:799–828.
2. Phillips, S.; Baar, K.; Lewis, N. Nutrition for Weight and Resistance Training. In: Lanham-New, S.; Stear, S.; Shirreffs, S.; Collins, A., editors. Nutrition Society Textbook on Sport and Exercise Nutrition. Wiley-Blackwell; 2011. p. 120-133.
3. Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A, Rennie MJ. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol Endocrinol Metab. 2008; 295:E595–604. [PubMed: 18577697]
4. Ferrando AA, Sheffield-Moore M, Wolf SE, Herndon DN, Wolfe RR. Testosterone administration in severe burns ameliorates muscle catabolism. Critical care medicine. 2001; 29:1936–1942. [PubMed: 11588456]
5. Tuvdendorj D, Chinkes DL, Zhang XJ, Suman OE, Aarsland A, Ferrando A, Kulp GA, Jeschke MG, Wolfe RR, Herndon DN. Long-term oxandrolone treatment increases muscle protein net deposition via improving amino acid utilization in pediatric patients 6 months after burn injury. Surgery. 2011; 149:645–653. [PubMed: 21333314]
6. Baehr LM, Tunzi M, Bodine SC. Muscle hypertrophy is associated with increases in proteasome activity that is independent of MuRF1 and MAFbx expression. Front Physiol. 2014; 5:69. [PubMed: 24600408]
7. Hwee DT, Baehr LM, Philp A, Baar K, Bodine SC. Maintenance of muscle mass and load-induced growth in Muscle RING Finger 1 null mice with age. Aging Cell. 2014; 13:92–101. [PubMed: 23941502]
8. Goldberg AL. Protein synthesis during work-induced growth of skeletal muscle. J Cell Biol. 1968; 36:653–658. [PubMed: 5645554]
9. Goodman CA, Frey JW, Mabrey DM, Jacobs BL, Lincoln HC, You JS, Hornberger TA. The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth. The Journal of physiology. 2011; 589:5485–5501. [PubMed: 21946849]

10. Skerry TM. The response of bone to mechanical loading and disuse: fundamental principles and influences on osteoblast/osteocyte homeostasis. Archives of biochemistry and biophysics. 2008; 473:117–123. [PubMed: 18334226]
11. Kjaer M, Langberg H, Heinemeier K, Bayer ML, Hansen M, Holm L, Doessing S, Kongsgaard M, Krogsgaard MR, Magnusson SP. From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scandinavian journal of medicine & science in sports. 2009; 19:500–510. [PubMed: 19706001]
12. Mackey AL, Brandstetter S, Schjerling P, Bojsen-Moller J, Qvortrup K, Pedersen MM, Doessing S, Kjaer M, Magnusson SP, Langberg H. Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2011; 25:1943–1959. [PubMed: 21368102]
13. Wycherley TP, Moran LJ, Clifton PM, Noakes M, Brinkworth GD. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. The American journal of clinical nutrition. 2012; 96:1281–1298. [PubMed: 23097268]
14. Breen L, Phillips SM. Interactions between exercise and nutrition to prevent muscle waste during ageing. Br J Clin Pharmacol. 2013; 75:708–715. [PubMed: 22957963]
15. de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV. Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. The Journal of Physiology. 2007; 583:1079–1091. [PubMed: 17656438]
16. Paddon-Jones D, Sheffield-Moore M, Cree MG, Hewlings SJ, Aarsland A, Wolfe RR, Ferrando AA. Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. J Clin Endocrinol Metab. 2006; 91:4836–4841. [PubMed: 16984982]
17. Bodine SC. Disuse-induced muscle wasting. The international journal of biochemistry & cell biology. 2013; 45:2200–2208. [PubMed: 23800384]
18. Jackman RW, Cornwell EW, Wu CL, Kandarian SC. Nuclear factor-kappaB signalling and transcriptional regulation in skeletal muscle atrophy. Experimental physiology. 2013; 98:19–24. [PubMed: 22848079]
19. Blair SN. Physical inactivity: the biggest public health problem of the 21st century. British journal of sports medicine. 2009; 43:1–2. [PubMed: 19136507]
20. Ruiz JR, Sui X, Lobelo F, Morrow JR Jr, Jackson AW, Sjostrom M, Blair SN. Association between muscular strength and mortality in men: prospective cohort study. BMJ. 2008; 337:a439. [PubMed: 18595904]
21. Wolfe RR. The underappreciated role of muscle in health and disease. The American journal of clinical nutrition. 2006; 84:475–482. [PubMed: 16960159]
22. Hurley BF, Hanson ED, Sheaff AK. Strength training as a countermeasure to aging muscle and chronic disease. Sports medicine. 2011; 41:289–306. [PubMed: 21425888]
23. Villareal DT, Smith GI, Sinacore DR, Shah K, Mittendorfer B. Regular multicomponent exercise increases physical fitness and muscle protein anabolism in frail, obese, older adults. Obesity. 2011; 19:312–318. [PubMed: 20489691]
24. Wong TS, Booth FW. Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. J Appl Physiol. 1990; 69:1718–1724. [PubMed: 1703146]
25. Wong TS, Booth FW. Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. J Appl Physiol. 1990; 69:1709–1717. [PubMed: 1703145]
26. Wong TS, Booth FW. Skeletal muscle enlargement with weight-lifting exercise by rats. J Appl Physiol. 1988; 65:950–954. [PubMed: 2459101]
27. Burd NA, West DW, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK, Phillips SM. Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. PLoS ONE. 2010; 5:e12033. [PubMed: 20711498]
28. Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK, Phillips SM. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. J Appl Physiol (1985). 2012; 113:71–77. [PubMed: 22518835]

29. Abe T, Kearns CF, Sato Y. Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. J Appl Physiol (1985). 2006; 100:1460–1466. [PubMed: 16339340]
30. Fujita S, Abe T, Drummond MJ, Cadenas JG, Dreyer HC, Sato Y, Volpi E, Rasmussen BB. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. J Appl Physiol (1985). 2007; 103:903–910. [PubMed: 17569770]
31. Papanicolaou GN, Falk EA. General Muscular Hypertrophy Induced by Androgenic Hormone. Science. 1938; 87:238–239. [PubMed: 17770410]
32. Nieschlag E, Nieschlag S. Testosterone deficiency: a historical perspective. Asian J Androl. 2014; 16:161–168. [PubMed: 24435052]
33. Hoberman, JM.; Yesalis, CE. Scientific American. 1995. The History of Synthetic Testosterone; p. 77-81.
34. Samuels LT, Henschel AF, Keys A. Influence of Methyl Testosterone on Muscular Work and Creatine Metabolism in Normal Young Men. The Journal of Clinical Endocrinology & Metabolism. 1942; 2:649–654.
35. Cureton KJ, Collins MA, Hill DW, McElhannon FM Jr. Muscle hypertrophy in men and women. Medicine and science in sports and exercise. 1988; 20:338–344. [PubMed: 3173042]
36. Hanson ED, Sheaff AK, Sood S, Ma L, Francis JD, Goldberg AP, Hurley BF. Strength training induces muscle hypertrophy and functional gains in black prostate cancer patients despite androgen deprivation therapy. The journals of gerontology Series A, Biological sciences and medical sciences. 2013; 68:490–498.
37. West DW, Burd NA, Tang JE, Moore DR, Staples AW, Holwerda AM, Baker SK, Phillips SM. Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. J Appl Physiol. 2010; 108:60–67. [PubMed: 19910330]
38. West DW, Kujbida GW, Moore DR, Atherton P, Burd NA, Padzik JP, De Lisio M, Tang JE, Parise G, Rennie MJ, Baker SK, Phillips SM. Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. J Physiol. 2009; 587:5239–5247. [PubMed: 19736298]
39. Ahtiainen JP, Pakarinen A, Kraemer WJ, Hakkinen K. Acute hormonal and neuromuscular responses and recovery to forced vs maximum repetitions multiple resistance exercises. Int J Sports Med. 2003; 24:410–418. [PubMed: 12905088]
40. Adams GR, Haddad F. The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. J Appl Physiol. 1996; 81:2509–2516. [PubMed: 9018499]
41. McKoy G, Ashley W, Mander J, Yang SY, Williams N, Russell B, Goldspink G. Expression of insulin growth factor-1 splice variants and structural genes in rabbit skeletal muscle induced by stretch and stimulation. J Physiol. 1999; 516(Pt 2):583–592. [PubMed: 10087355]
42. Spangenburg EE, Le Roith D, Ward CW, Bodine SC. A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. J Physiol. 2008; 586:283–291. [PubMed: 17974583]
43. Cuneo RC, Salomon F, Wiles CM, Hesp R, Sonksen PH. Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol (1985). 1991; 70:688–694. [PubMed: 2022560]
44. Cuneo RC, Salomon F, Wiles CM, Sonksen PH. Skeletal muscle performance in adults with growth hormone deficiency. Hormone research. 1990; 33(Suppl 4):55–60. [PubMed: 2245969]
45. Hornberger TA, Stuppard R, Conley KE, Fedele MJ, Fiorotto ML, Chin ER, Esser KA. Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase B- and growth factor-independent mechanism. Biochem J. 2004; 380:795–804. [PubMed: 15030312]
46. Goldberg AL. Work-induced growth of skeletal muscle in normal and hypophysectomized rats. Am J Physiol. 1967; 213:1193–1198. [PubMed: 6054866]
47. Goldberg AL. Role of insulin in work-induced growth of skeletal muscle. Endocrinology. 1968; 83:1071–1073. [PubMed: 5685568]

48. Goldberg AL. Protein turnover in skeletal muscle. I. Protein catabolism during work-induced hypertrophy and growth induced with growth hormone. J Biol Chem. 1969; 244:3217–3222. [PubMed: 5792657]

49. Goldberg AL, Goodman HM. Relationship between growth hormone and muscular work in determining muscle size. J Physiol. 1969; 200:655–666. [PubMed: 5765853]

50. Bullough WS. The control of mitotic activity in adult mammalian tissues. Biological reviews of the Cambridge Philosophical Society. 1962; 37:307–342. [PubMed: 13874622]

51. Elgjo K, Reichelt KL. Chalones: from aqueous extracts to oligopeptides. Cell Cycle. 2004; 3:1208–1211. [PubMed: 15326370]

52. Lee SJ. Extracellular Regulation of Myostatin: A Molecular Rheostat for Muscle Mass. Immunol Endocr Metab Agents Med Chem. 2010; 10:183–194. [PubMed: 21423813]

53. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci U S A. 1997; 94:12457–12461. [PubMed: 9356471]

54. Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, Parker HG, Ostrander EA. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. PLoS genetics. 2007; 3:e79. [PubMed: 17530926]

55. Lee SJ, McPherron AC. Regulation of myostatin activity and muscle growth. Proc Natl Acad Sci U S A. 2001; 98:9306–9311. [PubMed: 11459935]

56. Heinemeier KM, Olesen JL, Schjerling P, Haddad F, Langberg H, Baldwin KM, Kjaer M. Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. J Appl Physiol (1985). 2007; 102:573–581. [PubMed: 17038487]

57. Kim JS, Petrella JK, Cross JM, Bamman MM. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. J Appl Physiol (1985). 2007; 103:1488–1495. [PubMed: 17673556]

58. Kagawa Y. Impact of Westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. Prev Med. 1978; 7:205–217. [PubMed: 674107]

59. Montgomery RD. Muscle morphology in infantile protein malnutrition. J Clin Pathol. 1962; 15:511–521. [PubMed: 16810988]

60. Almurshed K, Grunewald K. The effects of dietary energy restriction on overloaded skeletal muscle in rats. The British journal of nutrition. 2000; 84:697–704. [PubMed: 11177183]

61. Almurshed KS, Grunewald KK. Dietary protein does not affect overloaded skeletal muscle in rats. The Journal of nutrition. 2000; 130:1743–1748. [PubMed: 10867045]

62. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol. 1999; 276:E628–634. [PubMed: 10198297]

63. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. The American journal of clinical nutrition. 2007; 86:373–381. [PubMed: 17684208]

64. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol. 1995; 268:E514–520. [PubMed: 7900797]

65. MacKenzie MG, Hamilton DL, Murray JT, Taylor PM, Baar K. mVps34 is activated following high-resistance contractions. J Physiol. 2009; 587:253–260. [PubMed: 19015198]

66. Burd NA, West DW, Moore DR, Atherton PJ, Staples AW, Prior T, Tang JE, Rennie MJ, Baker SK, Phillips SM. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. The Journal of nutrition. 2011; 141:568–573. [PubMed: 21289204]

67. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. The American journal of clinical nutrition. 2009; 89:161–168. [PubMed: 19056590]

68. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol (1985). 2009; 107:987–992. [PubMed: 19589961]
69. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, Hawley JA, Coffey VG. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. The Journal of Physiology. 2013; 591:2319–2331. [PubMed: 23459753]
70. Fatouros I, Chatzinikolaou A, Paltoglou G, Petridou A, Avloniti A, Jamurtas A, Goussetis E, Mitrakou A, Mougios V, Lazaropoulou C, Margeli A, Papassotiriou I, Mastorakos G. Acute resistance exercise results in catecholaminergic rather than hypothalamic-pituitary-adrenal axis stimulation during exercise in young men. Stress. 2010; 13:461–468. [PubMed: 20666650]
71. Kraemer WJ, Noble BJ, Clark MJ, Culver BW. Physiologic responses to heavy-resistance exercise with very short rest periods. International journal of sports medicine. 1987; 8:247–252. [PubMed: 3667019]
72. Roatta S, Farina D. Sympathetic actions on the skeletal muscle. Exercise and sport sciences reviews. 2010; 38:31–35. [PubMed: 20016297]
73. Ryall JG, Sillence MN, Lynch GS. Systemic administration of beta2-adrenoceptor agonists, formoterol and salmeterol, elicit skeletal muscle hypertrophy in rats at micromolar doses. British journal of pharmacology. 2006; 147:587–595. [PubMed: 16432501]
74. Bruno NE, Kelly KA, Hawkins R, Bramah-Lawani M, Amelio AL, Nwachukwu JC, Nettles KW, Conkright MD. Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. The EMBO journal. 2014; 33:1027–1043. [PubMed: 24674967]
75. Goldberg AL, Etlinger JD, Goldspink DF, Jablecki C. Mechanism of work-induced hypertrophy of skeletal muscle. Med Sci Sports. 1975; 7:185–198. [PubMed: 128681]
76. Baar K, Esser K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. Am J Physiol. 1999; 276:C120–127. [PubMed: 9886927]
77. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012; 149:274–293. [PubMed: 22500797]
78. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell. 2002; 110:177–189. [PubMed: 12150926]
79. Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, Timmerman KL, Dhanani S, Volpi E, Rasmussen BB. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. The Journal of nutrition. 2011; 141:856–862. [PubMed: 21430254]
80. Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, Volpi E, Rasmussen BB. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. J Physiol. 2009; 587:1535–1546. [PubMed: 19188252]
81. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 2001; 3:1014–1019. [PubMed: 11715023]
82. Goodman CA, Miu MH, Frey JW, Mabrey DM, Lincoln HC, Ge Y, Chen J, Hornberger TA. A phosphatidylinositol 3-kinase/protein kinase B-independent activation of mammalian target of rapamycin signaling is sufficient to induce skeletal muscle hypertrophy. Molecular biology of the cell. 2010; 21:3258–3268. [PubMed: 20668162]
83. Terzis G, Georgiadis G, Stratakos G, Vogiatzis I, Kavouras S, Manta P, Mascher H, Blomstrand E. Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. Eur J Appl Physiol. 2008; 102:145–152. [PubMed: 17874120]
84. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, Yang Q, Bennett C, Harada Y, Stankunas K, Wang CY, He X, MacDougald OA, You M, Williams BO, Guan KL. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell. 2006; 126:955–968. [PubMed: 16959574]

85. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003; 115:577–590. [PubMed: 14651849]

86. Crozier SJ, Kimball SR, Emmert SW, Anthony JC, Jefferson LS. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. J Nutr. 2005; 135:376–382. [PubMed: 15735066]

87. Jacobs BL, You JS, Frey JW, Goodman CA, Gundermann DM, Hornberger TA. Eccentric contractions increase the phosphorylation of tuberous sclerosis complex-2 (TSC2) and alter the targeting of TSC2 and the mechanistic target of rapamycin to the lysosome. The Journal of Physiology. 2013; 591:4611–4620. [PubMed: 23732640]

88. Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, Cantley LC, Manning BD. Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell. 2014; 156:771–785. [PubMed: 24529379]

89. Sato T, Nakashima A, Guo L, Tamanoi F. Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. The Journal of biological chemistry. 2009; 284:12783–12791. [PubMed: 19299511]

90. Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, Finan PM, Kwiatkowski DJ, Murphy LO, Manning BD. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Molecular cell. 2012; 47:535–546. [PubMed: 22795129]

91. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol. 2003; 13:1259–1268. [PubMed: 12906785]

92. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol. 2002; 4:648–657. [PubMed: 12172553]

93. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nature cell biology. 2007; 9:316–323. [PubMed: 17277771]

94. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. The Biochemical journal. 1998; 333(Pt 3):471–490. [PubMed: 9677303]

95. Alessi DR, Cohen P. Mechanism of activation and function of protein kinase B. Curr Opin Genet Dev. 1998; 8:55–62. [PubMed: 9529606]

96. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol. 1997; 7:261–269. [PubMed: 9094314]

97. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. Science. 2005; 307:1098–1101. [PubMed: 15718470]

98. Hornberger TA, Chu WK, Mak YW, Hsiung JW, Huang SA, Chien S. 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. 2006; 103:4741–4746. [PubMed: 16537399]

99. Deldicque L, Atherton P, Patel R, Theisen D, Nielens H, Rennie MJ, Francaux M. Decrease in Akt/PKB signalling in human skeletal muscle by resistance exercise. Eur J Appl Physiol. 2008; 104:57–65. [PubMed: 18535836]

100. You JS, Lincoln HC, Kim CR, Frey JW, Goodman CA, Zhong XP, Hornberger TA. The role of diacylglycerol kinase zeta and phosphatidic acid in the mechanical activation of mammalian target of rapamycin (mTOR) signaling and skeletal muscle hypertrophy. The Journal of biological chemistry. 2014; 289:1551–1563. [PubMed: 24302719]

101. Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG. The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. The Journal of biological chemistry. 2005; 280:18717–18727. [PubMed: 15772076]

102. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. Trends in cell biology. 2014

103. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science. 2008; 320:1496–1501. [PubMed: 18497260]

104. Long X, Ortiz-Vega S, Lin Y, Avruch J. Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. The Journal of biological chemistry. 2005; 280:23433–23436. [PubMed: 15878852]

105. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010; 141:290–303. [PubMed: 20381137]

106. Sekiguchi T, Hirose E, Nakashima N, Ii M, Nishimoto T. Novel G proteins, Rag C and Rag D, interact with GTP-binding proteins, Rag A and Rag B. The Journal of biological chemistry. 2001; 276:7246–7257. [PubMed: 11073942]

107. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. Nat Cell Biol. 2008; 10:935–945. [PubMed: 18604198]

108. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM. A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science. 2013; 340:1100–1106. [PubMed: 23723238]

109. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. Cell. 2012; 150:1196–1208. [PubMed: 22980980]

110. Zhang CS, Jiang B, Li M, Zhu M, Peng Y, Zhang YL, Wu YQ, Li TY, Liang Y, Lu Z, Lian G, Liu Q, Guo H, Yin Z, Ye Z, Han J, Wu JW, Yin H, Lin SY, Lin SC. The Lysosomal v-ATPase-Ragulator Complex Is a Common Activator for AMPK and mTORC1, Acting as a Switch between Catabolism and Anabolism. Cell metabolism. 2014; 20:526–540. [PubMed: 25002183]

111. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nature reviews Molecular cell biology. 2007; 8:917–929. [PubMed: 17912264]

112. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science. 2011; 334:678–683. [PubMed: 22053050]

113. Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, Kim HK, Ha SH, Ryu SH, Kim S. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. Cell. 2012; 149:410–424. [PubMed: 22424946]

114. Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, Spooner E, Sabatini DM. The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. Molecular cell. 2013; 52:495–505. [PubMed: 24095279]

115. Averous J, Lambert-Langlais S, Carraro V, Gourbeyre O, Parry L, B'Chir W, Muranishi Y, Jousse C, Bruhat A, Maurin AC, Proud CG, Fafournoux P. Requirement for lysosomal localization of mTOR for its activation differs between leucine and other amino acids. Cellular signalling. 2014; 26:1918–1927. [PubMed: 24793303]

116. Drummond MJ, Fry CS, Glynn EL, Timmerman KL, Dickinson JM, Walker DK, Gundermann DM, Volpi E, Rasmussen BB. Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise. J Appl Physiol (1985). 2011; 111:135–142. [PubMed: 21527663]

117. Churchward-Venne TA, Burd NA, Mitchell CJ, West DW, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. The Journal of physiology. 2012; 590:2751–2765. [PubMed: 22451437]

118. Ktistakis NT, Manifava M, Schoenfelder P, Rotondo S. How phosphoinositide 3-phosphate controls growth downstream of amino acids and autophagy downstream of amino acid withdrawal. Biochemical Society transactions. 2012; 40:37–43. [PubMed: 22260663]

119. Oshiro N, Rapley J, Avruch J. Amino acids activate mammalian target of rapamycin (mTOR) complex 1 without changing Rag GTPase guanyl nucleotide charging. The Journal of biological chemistry. 2014; 289:2658–2674. [PubMed: 24337580]

120. Schmierer B, Hill CS. Kinetic analysis of Smad nucleocytoplasmic shuttling reveals a mechanism for transforming growth factor beta-dependent nuclear accumulation of Smads. Molecular and cellular biology. 2005; 25:9845–9858. [PubMed: 16260601]

121. Marshall A, Salerno MS, Thomas M, Davies T, Berry C, Dyer K, Bracegirdle J, Watson T, Dziadek M, Kambadur R, Bower R, Sharma M. Mighty is a novel promyogenic factor in skeletal myogenesis. Exp Cell Res. 2008; 314:1013–1029. [PubMed: 18255059]

122. Massague J, Seoane J, Wotton D. Smad transcription factors. Genes & development. 2005; 19:2783–2810. [PubMed: 16322555]

123. Gaarenstroom T, Hill CS. TGF-beta signaling to chromatin: how Smads regulate transcription during self-renewal and differentiation. Seminars in cell & developmental biology. 2014; 32:107–118. [PubMed: 24503509]

124. Winbanks CE, Weeks KL, Thomson RE, Sepulveda PV, Beyer C, Qian H, Chen JL, Allen JM, Lancaster GI, Febbraio MA, Harrison CA, McMullen JR, Chamberlain JS, Gregorevic P. Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. The Journal of cell biology. 2012; 197:997–1008. [PubMed: 22711699]

125. Amirouche A, Durieux AC, Banzet S, Koulmann N, Bonnefoy R, Mouret C, Bigard X, Peinnequin A, Freyssenet D. Down-regulation of Akt/mammalian target of rapamycin signaling pathway in response to myostatin overexpression in skeletal muscle. Endocrinology. 2009; 150:286–294. [PubMed: 18801898]

126. Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ. Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. Am J Physiol Cell Physiol. 2009; 296:C1258–1270. [PubMed: 19357233]

127. Welle S, Burgess K, Mehta S. Stimulation of skeletal muscle myofibrillar protein synthesis, p70 S6 kinase phosphorylation, and ribosomal protein S6 phosphorylation by inhibition of myostatin in mature mice. Am J Physiol Endocrinol Metab. 2009; 296:E567–572. [PubMed: 19141683]

128. Sartori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, Stantzou A, Mouisel E, Toniolo L, Ferry A, Stricker S, Goldberg AL, Dupont S, Piccolo S, Amthor H, Sandri M. BMP signaling controls muscle mass. Nature genetics. 2013; 45:1309–1318. [PubMed: 24076600]

129. Winbanks CE, Chen JL, Qian H, Liu Y, Bernardo BC, Beyer C, Watt KI, Thomson RE, Connor T, Turner BJ, McMullen JR, Larsson L, McGee SL, Harrison CA, Gregorevic P. The bone morphogenetic protein axis is a positive regulator of skeletal muscle mass. The Journal of cell biology. 2013; 203:345–357. [PubMed: 24145169]

130. Mackenzie MG, Hamilton DL, Pepin M, Patton A, Baar K. Inhibition of Myostatin Signaling through Notch Activation Following Acute Resistance Exercise. PLoS ONE. 2013 In Press.

131. Carlson ME, Hsu M, Conboy IM. Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature. 2008; 454:528–532. [PubMed: 18552838]

132. Chu J, Jeffries S, Norton JE, Capobianco AJ, Bresnick EH. Repression of activator protein-1-mediated transcriptional activation by the Notch-1 intracellular domain. The Journal of biological chemistry. 2002; 277:7587–7597. [PubMed: 11739397]

133. Ross DA, Kadesch T. The notch intracellular domain can function as a coactivator for LEF-1. Molecular and cellular biology. 2001; 21:7537–7544. [PubMed: 11604490]

134. Benovic JL, von Zastrow M. Editorial overview: Cell regulation: The ins and outs of G protein-coupled receptors. Current opinion in cell biology. 2014; 27:v–vi. [PubMed: 24529749]

135. Zhou L, Bohn LM. Functional selectivity of GPCR signaling in animals. Current opinion in cell biology. 2014; 27:102–108. [PubMed: 24680435]

136. Sirvent P, Douillard A, Galbes O, Ramonatxo C, Py G, Candau R, Lacampagne A. Effects of chronic administration of clenbuterol on contractile properties and calcium homeostasis in rat extensor digitorum longus muscle. PLoS ONE. 2014; 9:e100281. [PubMed: 24971566]

137. Bakker AJ, Head SI, Wareham AC, Stephenson DG. Effect of clenbuterol on sarcoplasmic reticulum function in single skinned mammalian skeletal muscle fibers. The American journal of physiology. 1998; 274:C1718–1726. [PubMed: 9611138]

138. Douillard A, Galbes O, Begue G, Rossano B, Levin J, Vernus B, Bonnieu A, Candau R, Py G. Calpastatin overexpression in the skeletal muscle of mice prevents clenbuterol-induced muscle hypertrophy and phenotypic shift. Clinical and experimental pharmacology & physiology. 2012; 39:364–372. [PubMed: 22300302]

139. von Maltzahn J, Bentzinger CF, Rudnicki MA. Wnt7a-Fzd7 signalling directly activates the Akt/mTOR anabolic growth pathway in skeletal muscle. Nature cell biology. 2012; 14:186–191. [PubMed: 22179044]

140. Porporato PE, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi VF, Prodam F, Ronchi G, Fagoonee S, Fornaro M, Chianale F, Baldanzi G, Surico N, Sinigaglia F, Perroteau I, Smith RG, Sun Y, Geuna S, Graziani A. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. The Journal of clinical investigation. 2013; 123:611–622. [PubMed: 23281394]

141. Minetti GC, Feige JN, Rosenstiel A, Bombard F, Meier V, Werner A, Bassilana F, Sailer AW, Kahle P, Lambert C, Glass DJ, Fornaro M. Galphai2 signaling promotes skeletal muscle hypertrophy, myoblast differentiation, and muscle regeneration. Science signaling. 2011; 4:ra80. [PubMed: 22126963]

142. Kline WO, Panaro FJ, Yang H, Bodine SC. Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. J Appl Physiol. 2007; 102:740–747. [PubMed: 17068216]

143. Phillips BE, Williams JP, Gustafsson T, Bouchard C, Rankinen T, Knudsen S, Smith K, Timmons JA, Atherton PJ. Molecular networks of human muscle adaptation to exercise and age. PLoS genetics. 2013; 9:e1003389. [PubMed: 23555298]

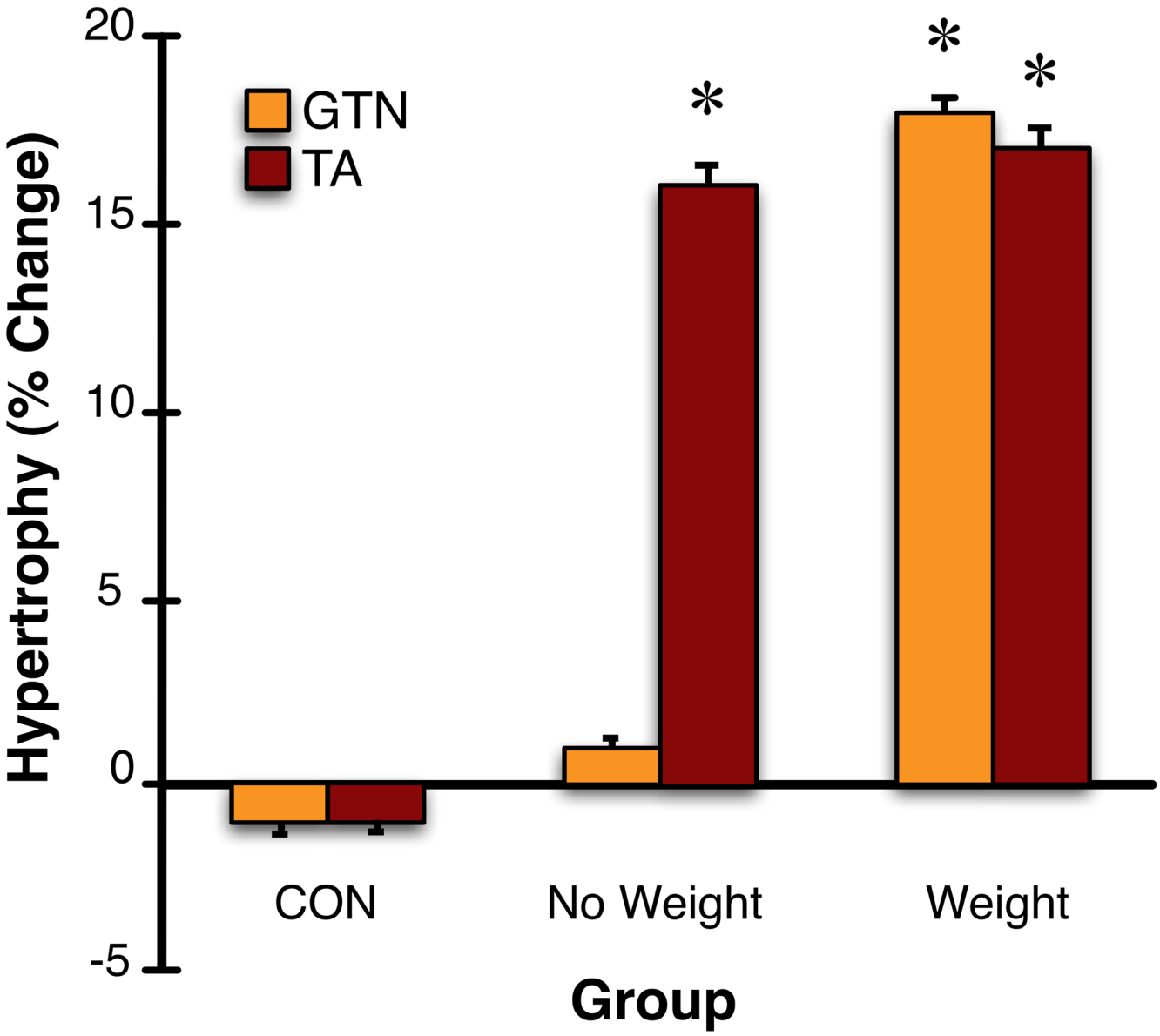

Figure 1.
The effect of load on muscle hypertrophy. The increase in muscle mass following 10-weeks resistance exercise in CON, animals that received no stimulation; No Weight, animals that exercised without an external weight to prevent dorsiflexion; and Weight, animals where dorsiflexion was resisted by an external load just less than maximal isometric load. Adapted from [26].

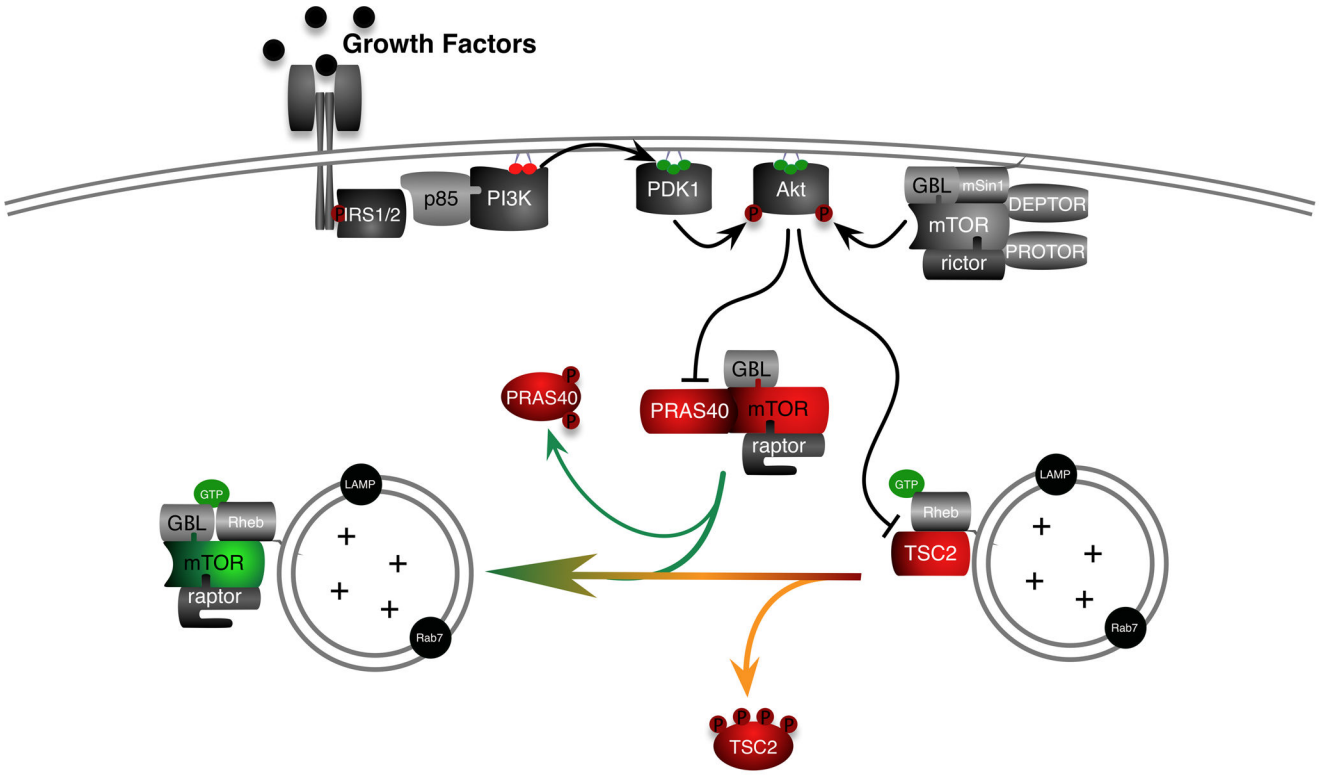

Figure 2.

The activation of the mechanistic target of rapamycin complex 1 (mTORC1) by growth factors. Growth factors bind to receptor tyrosine kinases that recruit the insulin receptor substrates (IRS1/2) and this bind PI3K to the membrane. When at the membrane, PI3K converts phosphoinositol (4,5) bisphosphate (the two red circles at the membrane) into phosphoinositol (3,4,5) trisphosphate (the three green circles at the membrane), which is a docking site for akt and its upstream kinase the 3-phosphoinositide dependent protein kinase-1 (PDK1). When akt and PDK1 co-localize at the membrane, PDK1 phosphorylates akt at one site and the membrane-associated mTORC2 phosphorylates a second site; resulting in full activation of akt. Active akt turns on mTORC1 by phosphorylating and removing PRAS40 and TSC2 from mTOR and Rheb, respectively.

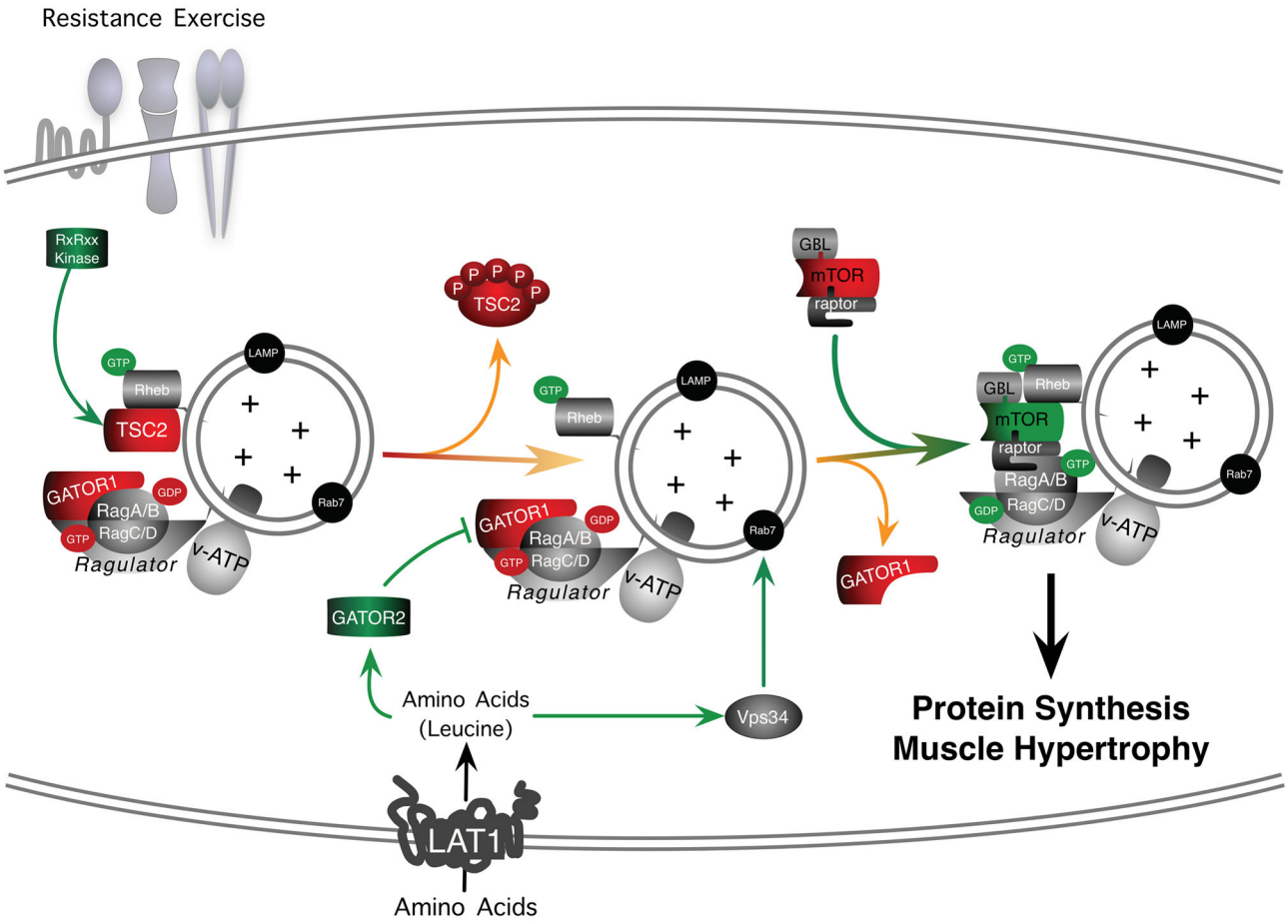

Figure 3.

The activation of the mechanistic target of rapamycin complex 1 (mTORC1) following resistance exercise and feeding. Lifting a heavy weight to failure stimulates a mechanoreceptor that in turn activates an RxRxxS*/T* kinase that phosphorylates and moves the tuberosclerosis complex (TSC2) away from the lysosome allowing Rheb (Ras homologue enriched in brain) to remain in the guanosine triphosphate (GTP) bound state. Simultaneously, amino acid uptake and intracellular amino acid levels increase. The extra amino acids stimulate the leucyl tRNA synthase (LRS) to act as a GTPase activating protein (GAP) towards RagC/D and GATOR (GAP Activity Towards Rags)2 blocks GATOR1 (the GAP of RagA/B) and the Ragulator GTP loads RagA/B and activates the complex. The active Rag complex then binds to raptor and positions mTOR beside its activator GTP bound Rheb. The resulting elevation of mTORC1 activity drives myofibrillar protein synthesis and eventually leads to an increase in muscle mass and strength. LAT1, L-type amino acid transporter; Rab7, Ras-related protein 7; LAMP2, lysosome-associated membrane protein 2; P, phosphorylation; DEPTOR, DEP domain-containing mTOR-interacting protein; GβL, G-protein beta subunit-like protein; PRAS40, proline-rich Akt substrate of 40 kilodaltons; and RAPTOR, the regulatory-associated protein of mTOR.

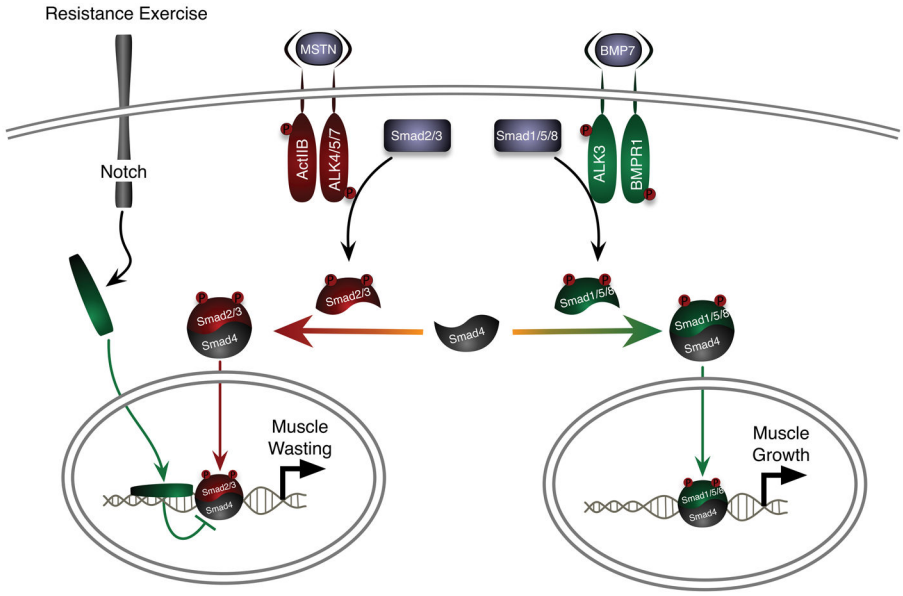

Figure 4.

The role of Smads in the control of muscle mass. Myostatin and similar members of the TGFβ superfamily can phosphorylate Smad2/3 allowing it to bind to the common mediator Smad4 and move to the nucleus. In the nucleus, Smad2/3 drives transcriptional events that result in the transcription of genes that limit muscle size. Smad2/3 signaling can be competitively inhibited at the level of Smad4 binding by Smad1/5/8. BMP7 and other members of the TGFβ superfamily activate Smad1/5/8 through ALK3 and the BMP receptor. Resistance exercise can also limit Smad2/3 signaling by cleaving and activating Notch. The intracellular domain of Notch then moves to the nucleus and blocks Smad2/3 transcription.