The influence of mechanical loading on skeletal muscle protein turnover

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Abstract

Skeletal muscle plays a fundamental role in human health and so understanding the biological processes that regulate skeletal muscle mass in health and disease is critical. We know that resistance exercise increases rates of muscle protein synthesis (MPS) in a mechanistic target of rapamycin complex 1 (mTORC1)-dependent manner. However, the exact molecule(s) that 'sense' mechanical loading and translate that signal to a biochemical event leading to upregulation of MPS remains elusive. Similarly, in response to periods of unloading there is a decrease in MPS and potentially a transient increase in muscle protein breakdown (MPB), but the relative contribution of MPS and MPB to muscle atrophy remains unknown. The aim of this review is to briefly outline the molecular mechanisms that regulate skeletal muscle protein mass in response to both mechanical loading and unloading (disuse). We discuss recent developments in the field of molecular exercise biology as well as present a working hypothesis as to the physiological basis for muscle disuse atrophy.

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Introduction

The human body is ~45% skeletal muscle by mass (Kim et al., 2002; Zhao et al., 2013). Skeletal muscle is a critical organ playing a key role in the generation of contractile force, post-prandial glucose disposal (Richter and Hargreaves, 2013) and is positively associated with improved metabolic health (Wolfe, 2006). It also is a highly plastic tissue capable of adapting to alterations in the load, frequency and duration of imposed contractile activity. It is known that contraction results in both acute and chronic changes in gene/protein expression and whole-body metabolism (Baar and Esser, 1999; Chen et al., 2002; Egan and Zierath, 2013). The nature of these adaptions often is highly specific to the type of stimulus. For example, performing repeated longer duration sessions of low intensity contractions (i.e., aerobic exercise) results in improved fatigue resistance in part due to a shift in mitochondrial density and oxidative capacity (Henriksson, 1977; Holloszy, 1967). Contrastingly, repeated bouts of exposure to fatiguing intense loaded contractions (i.e., resistance exercise), induces an increase in fiber cross sectional area (Campos et al., 2002; Davidsen et al., 2011; Goodman et al., 2011a; Mitchell et al., 2012). Resistance exercise (RE) training also induces increases in strength and minimally affects endurance (Sale, 1988). Training with RE has long been utilized as an effective strategy to increase strength and improve athletic performance as well as enhance health (American College of Sports Medicine position stand, 2009; Delorme and Watkins, 1948).

The size of human skeletal muscle mass is dictated by the coordinated relationship between diurnal changes in muscle protein synthesis (MPS) and muscle protein breakdown (MPB) (Phillips et al., 2009; Phillips et al., 1997). In healthy humans, it is the changes in MPS in response to protein feeding-induced and contraction-induced stimulation that is the major determinant of muscle mass and size (Phillips et al., 2009). For this reason, the focus of many studies in humans has been on factors that ultimately influence MPS. It is known that amino acid ingestion stimulates a rise in rates of MPS and that this effect is potentiated by RE. Put simply, RE sensitizes skeletal muscle to the anabolic effect of amino acid feeding an effect that lasts for up to 24 h (Burd et al., 2011). However, our understanding of how RE (loading) or disuse (unloading) influences the translation and subsequent incorporation of new muscle proteins into the contractile apparatus at the molecular level, remains unclear. We also are largely ignorant of the mechanisms responsible for the loss of muscle mass during periods of disuse. The aim of this review is therefore to provide a brief overview of existing knowledge in these areas with specific emphasis on current concepts in the field. We will not cover the intricacies of protein transcription, translation or elongation, nor will we discuss ribosomal biogenesis [for reviews see (Chaillou et al., 2014; Gingras et al., 1999b; Hershey, 1991; Mahoney et al., 2009; Sonenberg and Hinnebusch, 2007)]. Rather, we aim to provide a brief overview of how loading/unloading alters the putative regulators of protein turnover. For the interested reader we refer them to other excellent review articles on this topic (Goodman et al., 2011b; Jewell and Guan, 2013; Jewell et al., 2013; Mahoney et al., 2009; Murton et al., 2008; Wall et al., 2013a).

Setting the scene: the mechanistic (mammalian) target of rapamycin complex 1 (mTORC1)

An often-unifying feature of studies that aim to delineate the molecular regulation of MPS in response to exercise and nutrition is the assessment of changes in the activity of the mechanistic target of rapamycin complex 1 (mTORC1) (Apró

and Blomstrand, 2010; Areta et al., 2013; Churchward-Venne et al., 2014; Guertin and Sabatini, 2007; McGlory et al., 2014). The mTORC1 protein is an evolutionary conserved serine/threonine protein kinase that is critical for cell survival, cell proliferation, lipid synthesis and more relevant to this review, protein synthesis (Foster and Fingar, 2010; Lamming and Sabatini, 2013; Laplante and Sabatini, 2012). mTORC1 is composed of the catalytic subunit, mechanistic target of rapamycin (mTOR) and the associated partner proteins, regulatory-associated protein of mTOR (Raptor), Mammalian lethal with SEC13 protein 8 (mLST8), DEP domain-containing mTOR-interacting protein (Deptor) and Proline-rich Akt substrate of 40 kDa (PRAS40) (Foster and Fingar, 2010). Aberrant activity of mTORC1 is proposed to be implicit in the aetiology of ageing (Cuthbertson et al., 2005; Fry et al., 2011) and the pathophysiology of many diseased states, including cancer (Guertin and Sabatini, 2007). Hence, examination of how mTORC1 and its adapter proteins are regulated by both exercise, and nutrition, is relevant not only to exercise scientists, but also for those who aim to develop pharmaceutical countermeasures to combat disease.

There are numerous molecular input signals to mTORC1 that can regulate its activity. One such input is the guanosine triphosphatase (GTPase) Ras homolog enriched in brain (Rheb). When in a guanosine triphosphate (GTP)-bound state, Rheb is required for appropriate mTORC1 assembly; however, when in the guanasine diphosphate (GDP)-bound state mTORC1 assembly and activation is prevented. This specific process is controlled by the upstream GTPase activating protein (GAP) called tuberous sclerosis 2 (TSC2) (Aspuria and Tamanoi, 2004). When active, TSC2 activates Rheb driving its GTPase activity, increasing the guanosine diphosphate (GDP)/GTP-bound state of Rheb, thus inhibiting its interaction with, and activity of, mTORC1 (Inoki et al., 2003a). In turn, the influence of TSC2 on Rheb, is enhanced by adenosine monophosphate-activated protein kinase (AMPK) (Inoki et al., 2003b). As a cellular energy sensor, AMPK also has been shown to exert an inhibitory effect on mTORC1 via phosphorylation of Deptor (Gwinn et al., 2008). In contrast, protein kinase B (PKB) phosphorylates TSC2 to inhibit its ability to activate Rheb (Inoki et al., 2002) as well as directly targeting PRAS40, (Haar et al., 2007) exerting a dual stimulatory effect on mTORC1 activity. Once active, mTORC1 interacts with many downstream substrates including but not limited to the ribosomal protein of 70 kDa S6 kinase 1 (p70S6K1) and 4E-binding protein-1 (4E-BP1) (Dickinson et al., 2011; Gingras et al., 1999a; Pearson et al., 1995) (see Figure 1). Both p70S6K1 and 4E-BP1 play important roles in the MPS response to stimulation, in fact, phosphorylation of p70S6K1 has been shown be correlated with hypertrophy (Baar and Esser, 1999; Terzis et al., 2008). Indeed, mTORC1 phosphorylates p70S6K1 on Thr389 to upregulate translation initiation via the S6K1 Aly/Ref like target (SKAR) protein (Ma et al., 2008; Richardson et al., 2004) as well as translation elongation via eukaryotic elongation factor-2 kinase (eEF2K) (Wang et al., 2001). 4E-BP1 also is targeted by mTORC1 via phosphorylation on Thr37/46. Phosphorylation of 4E-BP1 reduces its affinity for eukaryotic initiation factor 4E (eIF4E), enabling eIF4E to interact with eukaryotic initiation factor 4G (eIF4G) to form the 43S preinitiation complex. These steps are key to the commencement of protein initiation (Gingras et al., 1999a). We acknowledge the existence other signaling proteins and protein kinases that either regulate (Hamilton et al., 2014), or are targets of mTORC1, however, the discussion of their role in mTORC1 signaling is not within the remit of the present review, so we refer interested readers to other informative articles (Dowling et al., 2010; Gkogkas et al., 2010; Laplante and Sabatini, 2013).

Resistance exercise and protein synthesis

Loading in the form of RE is a potent driver of human skeletal muscle strength and hypertrophy (Booth et al., 1998; Delorme and Watkins, 1948; Goldberg, 1968). More recent data have shown that isolated bouts of RE increase rates of MPS (Phillips et al., 1997) and this increase in MPS is largely driven by increased mRNA activity (protein synthesis per mRNA) most likely due to enhanced translation initiation (Baar and Esser, 1999; Chesley et al., 1992; Gingras et al., 1999b). Given that translation initiation is proposed to be orchestrated by mTORC1-p70S6K1 signaling (Gingras et al., 1999b; Ma et al., 2008), it is unsurprising that increased rates of MPS in response to RE are often accompanied by elevations in the phosphorylation status of mTOR and p70S6K1 (Areta et al., 2013; Baar and Esser, 1999; Churchward-Venne et al., 2012; Mitchell et al., 2012; Moore et al., 2009). In addition, as mentioned some have observed correlations between increases in the phosphorylation status of kinases/proteins and exercise-induced changes in MPS (Burd et al., 2010; Kumar et al., 2009) and even RE-induced hypertrophy (Baar and Esser, 1999; Mitchell et al., 2013; Terzis et al., 2008). Using the immunosuppressant rapamycin (an mTORC1 inhibitor) many studies have now demonstrated the importance of mTORC1 in the regulation of MPS. In one seminal study conducted by Bodine and colleagues (Bodine et al., 2001b) rapamycin administration reduced both mTORC1 signaling as well as muscle hypertrophy in response to synergist ablation in rodents and similar findings also have been demonstrated in mice with rapamycin resistant mutants of mTOR (Goodman et al., 2011a). But in contrast, to date, there are fewer studies in humans that have used rapamycin to show similar effects. In a landmark study by Drummond et al, (Drummond et al., 2009) the administration of 12 mg of rapamycin 2 h prior to RE significantly reduced MPS during the initial 1-2 h recovery period. The depression in MPS was accompanied by blunted $mTOR^{ser2448}$ and $p70S6K1^{thr389}$ phosphorylation at 1 h post-RE. Similar findings have been replicated in blood flow restricted models of RE (Gundermann et al., 2014) as well as in response to the consumption of amino acids, known to stimulate both MPS and mTORC1 (Dickinson et al., 2011), Taken together with data from animal models (Bodine et al., 2001b; Goodman et al., 2011a) these data (Dickinson et al., 2011; Drummond et al., 2009; Gundermann et al., 2014) show that mTORC1 signaling plays a key role in contraction-induced increases in human skeletal MPS. It is important to note though that in the study of (Drummond et al., 2009) rapamycin treatment did not inhibit p70S6K1 2 h post-RE, nor did it exert any impact on 4E-BP1 phosphorylation at any time point assessed during the recovery period, which is suggestive that there are redundant signaling pathways that may exert their influence on these proteins. In addition, whist rapamycin is successful in the amelioration of postprandial MPS (Dickinson et al., 2011), it does not reduce postabsoptive rates of MPS (Dickinson et al., 2013) so some constitutive mechanism must maintain MPS in this state that is not dependent on mTORC1. Thus, there are likely to be as yet unidentified pathways, which act in concert with mTORC1 to mediate MPS.

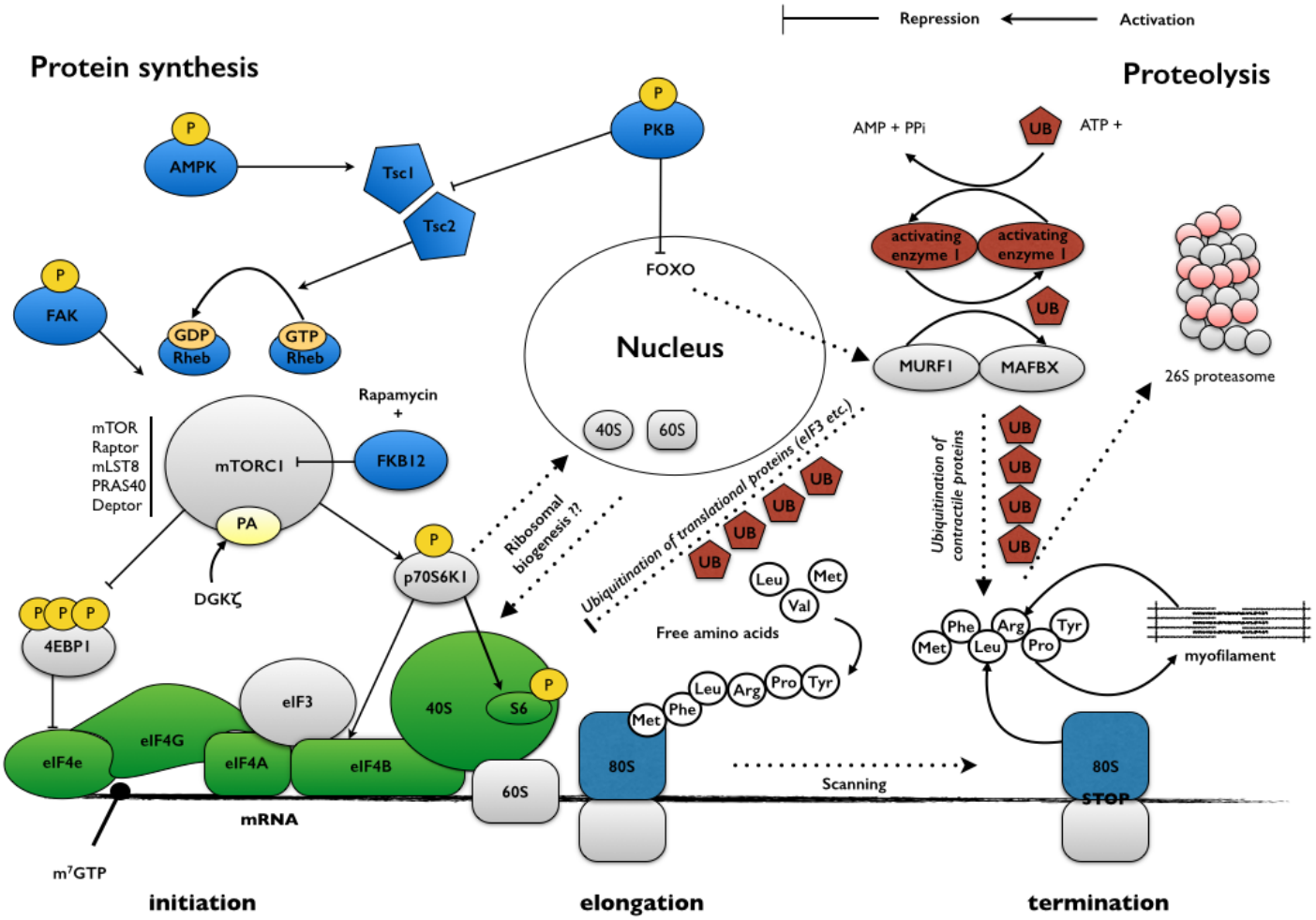

Figure 1. In response to mechanical loading, tuberous sclerosis 2 (TSC2) activation of Rheb guanosine triphosphatase (GTPase) is removed, leading to increased phosphorylation of mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 activation leads to phosphorylation of many downstream targets, resulting in accompanying increases in muscle protein synthesis (MPS). Phosphorylation of 4E-binding protein-1 (4E-BP-1)$^{thr37/46}$ by mTORC1 enables the formation of the 43S preinitiation complex that includes eukaryotic initiation factor 4E (eIF4E) and eukaryotic initiation factor 4G (eIF4G) interaction. The phosphorylation of 70 kDa S6 kinase 1 (p70S6K1)^{thr389} by mTORC1 upregulates the pioneer round of translation initiation and elongation and may promote ribosomal biogenesis. During muscle disuse, skeletal muscle loss is often associated with the increased mRNA expression of E3 ubiquitin ligases including muscle RING finger-1 (MuRF-1) and muscle atrophy-box protein (MAFbx/Atrogin-1), which target proteins for degradation by the 26S and 20S proteasome. Importantly, accompanying measures of MPB during muscle disuse are rarely evident. Therefore, it is possible that these E3 ubiquitin ligases are targeting proteins other than the myofilaments, such as proteins and protein kinases involved in translation.

Whilst mTORC1 has been firmly established as a central governor of MPS in response to loaded contractions, rather little is understood as to what signals regulate mTORC1. It is widely accepted that mTORC1 activation following contraction occurs in a 3-phosphoinositide-dependent protein kinase (PI3K)-PKB-independent manner (Philp et al., 2011), potentially via a mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) mechanism (Miyazaki et al., 2011). In addition, exercise-induced increases in circulating 'anabolic' hormones (insulin-like growth factor [IGF-1] and testosterone) also play no, or at least a very minor role (Spangenburg et al., 2008; West et al., 2010). Even the autocrine production of growth factors is unlikely to be playing a role in RE induced signaling processes in muscle (Hamilton et al., 2010). In fact, it is now believed that the transmission of mechanical tension to the biochemical signals that regulate MPS is mediated by mechanisms that are intrinsic to the muscle, specifically via mTORC1. Yet the exact molecule(s) that sense mechanical tension remain elusive. The costamere complex, a sarcolemmal protein complex that connects the extracellular matrix to the contractile machinery, is a principle site of investigation (Ervasti, 2003; Hughes et al., 2015; Pardo et al., 1983). Indeed, it has been suggested that focal adhesion kinase (FAK), an integrin-associated within the costameres of skeletal muscle (Burridge and Chrzanowska-Wodnicka, 1996), exhibits mechanical sensitivity and this effect has been observed in human and animal models of contraction (Klossner et al., 2013; Li et al., 2013). For example, in one study chronic overload of avian *anterior latissimus dorsi* was associated with an increase in FAK content and activity as well as the content of paxillin (Fluck et al., 1999). Furthermore, two weeks of leg immobilization in humans reduced FAK$^{Tyr576/577}$ phosphorylation in humans (Glover et al., 2008) corroborating other reports (de Boer et al., 2007; Li et al., 2013) in which muscle cross sectional area also was reduced at the same time. Whilst informative, the functional relevance of FAK towards human, *in vivo* changes in rates of MPS remains unknown. Some data demonstrate that FAK is required for IGF-1-mediated increases in protein synthesis in myotubes via a TSC2-mTORC1-p70S6K1 signaling cascade (Crossland et al., 2013). Yet, in humans, RE-induced increases in systemic IGF-1 are not correlated with *in vivo* rates of MPS (West et al., 2009) or hypertrophy (West et al., 2010; West and Phillips, 2012). Clearly, more work is now needed to elucidate how changes in FAK content and activity influence muscle protein mass.

Another molecule that has been proposed to regulate mTORC1 activity is phosphatidic acid (PA) (O'Neil et al., 2009). Increases in PA content following mechanical loading appear to influence mTORC1 activity by binding directly to the FKBP12 rapamycin-binding domain of mTOR (You et al., 2014). Originally, it was believed that this occurred in a phospholipase D-dependent mechanism (Hornberger et al., 2006) but more recent data demonstrates that an enzyme responsible for the production of PA, diacylglycerol kinase-ζ (DGKζ), also is increased following passive stretch (You et al., 2014). The authors were able to show that stretch-induced increases in PA and mTORC1 activity were significantly compromised in DGKζ knockout mice while the over expression of DGKζ resulted in muscle hypertrophy. However, whether DGKζ plays a role in load-induced muscle hypertrophy in a physiological context in humans remains to be determined. Similarly, promising data have recently been published alluding to the role of the Yes-associated protein (YAP) in regulating cell size. In one of these studies, mechanical loading resulted in an increase in YAP whilst overexpression of YAP induced skeletal muscle hypertrophy in an mTORC1-independent mechanism (Goodman et al., 2015). In the same year, another group demonstrated that overexpression of YAP promoted muscle hypertrophy again in an mTORC-1 independent mechanism (Watt et al., 2015). However the latter study the authors were also able to show that this increase in skeletal muscle size was associated with increased rates of MPS, potentially via enhanced ribosomal biogenesis. These novel data now provide critical information that could be used to investigate the role of YAP in regulating MPS in response to exercise in a physiological context in humans (Watt et al., 2015).

In addition to the phosphorylation status of anabolic signaling molecules, emerging data suggest that their subcellular localization in response to RE also may play an important regulatory function. The trafficking of mTOR to the lysosome where its co-activator Rheb resides has already been shown to mediate mTORC1 assembly in response to leucine provision in cell models (Sancak et al., 2010). This finding is particularly important given the critical role mTORC1 plays in protein synthesis. However, recently it was demonstrated that in response to eccentric contractions phosphorylated TSC2 disassociates from the lysosome to the cytoplasm (Jacobs et al., 2013a; Jacobs et al., 2013b). The significance of this work, together with the knowledge that amino acids simulate mTORC1 movement to the lysosome, is that it provides a mechanistic model by which RE and amino acids stimulate MPS. In this model, RE (through an unknown mechanism) removes TSC2 inhibition of Rheb that in the presence of amino acids interacts with mTOR to enhance translation initiation. It is important to reiterate that these data were generated in rodent and cell lines and additional work in humans is now required to experimentally corroborate this hypothesis in humans. These data provide an interesting working model to explain the potentiation of MPS in response to amino acid after RE.

Impact of muscle unloading on skeletal muscle mass

Although loaded contractions are known to stimulate a rise in MPS, and ultimately skeletal muscle growth, periods of skeletal muscle unloading/disuse have been shown to result in muscle atrophy (Wall et al., 2013b). Indeed, there are many reports characterizing the negative impact of periods of muscle unloading on muscle size, strength and mass (Hvid et al., 2013; Thorlund et al., 2011). For example, prolonged periods of muscle disuse (> 10 days) have been estimated to result in muscle loss at a rate of ~0.5% per day (Wall et al., 2013a) with the decrement in strength occurring at an even more precipitous rate (Thom et al., 2001). Even short periods of muscle unloading lasting as little as 5 days result in measurable declines in muscle mass (Wall et al., 2014; Wall et al., 2013b). Despite the advances made in our understanding of the mechanisms that drive skeletal muscle hypertrophy, the exact physiological and molecular mechanisms underpinning skeletal muscle disuse atrophy remain disputed (Phillips and McGlory, 2014a; Phillips and McGlory, 2014b; Reid et al., 2014a; Reid et al., 2014b). It has been proposed by some that muscle disuse-mediated atrophy is predominantly driven by accelerated rates of proteolysis that is driven by the ubiquitin-proteasome pathway (Reid et al., 2014a). Indeed, there are numerous reports of increases in the mRNA expression of the E3 ubiquitin ligases such as muscle RING finger-1 (MURF-1) and muscle atrophy-box protein) (MAFbx/Atrogin-1) in response to muscle unloading in a variety of models, that are associated with a reduction in skeletal muscle mass (Bodine et al., 2001a; Brocca et al., 2012; de Boer et al., 2007; Jones et al., 2004). Moreover, unloading has been proposed to stimulate an increase in the activity of the 26S and 20S proteasome complexes in both human and animal models, a process that is key to the breakdown of muscle proteins (Ikemoto et al., 2001; Jones et al., 2004; Levine et al., 2008). Thus, in contrast to mechanical loading, based on the available evidence, muscle unloading induces muscle atrophy via an increase in rates of proteolysis. However, it is important to note that in most instances, due to the technical limitations, actual rates of muscle protein breakdown (MPB) are rarely assessed. Instead, muscles are weighed pre- and post-disuse, molecular markers are measured and a conclusion is drawn as to a mechanism. However, we submit that such evidence fails to mechanistically prove that MPB is elevated sufficiently enough to render it the predominant mechanism underpinning muscle disuse atrophy. Another important consideration is that the majority of research in this area has been conducted in rodent models of disuse that may not accurately reflect the physiological impact of muscle disuse in humans (Cunningham, 2002; Phillips et al., 2009).

Calculations based on human models of muscle disuse using stable isotopic tracers demonstrate that the decline in muscle size with muscle disuse can be significantly, but not entirely, accounted for by a decrement in postabsorptive and postprandial rates of MPS (Phillips et al., 2009; Phillips and McGlory, 2014a). These calculations, while dismissed by some, show that increases in MPB, as opposed to reductions in MPS, if present would result in a much greater reduction in muscle mass than is observed. If declines in post absorptive and postprandial rates of MPS fail to fully account for the loss of muscle mass with disuse, it is likely that changes in rates of MPB do occur, albeit transiently. But, rather than adopting an either or approach, it has been suggested that there are integrated changes in both rates of MPS and MPB in response to disuse (Reid et al., 2014a). Indeed, there is emergent data to suggest that eIF3f, a key protein involved in mTORC1/p70S6K1 mediated translation initiation, is polyubiquitnated by MAFbx/Atrogin-1 under atrophic conditions in both cell and mouse skeletal muscle (Csibi et al., 2010; Csibi et al., 2008; Lagirand-Cantaloube et al., 2008; Sanchez et al., 2013). The targeted degradation of this, and potentially other protein/protein kinases involved in protein synthetic pathways, by the ubiquitin ligases under models of disuse would serve to limit the capacity of the protein synthetic machinery to mount a robust response to anabolic stimulation i.e., exercise and feeding (Figure 1.). Such a hypothesis has yet to be experimentally tested in human models using direct readouts of MPB, but it does seem logical given data to show that MPS is blunted in response to the consumption of amino acids following periods of reduced muscle loading (Breen et al., 2013; Drummond et al., 2012; Glover et al., 2008; Wall et al., 2013b). As such, pharmacological disruption of the ubiquitination of these proteins may serve to rescue the losses of muscle mass during periods of disuse. Although, pharmacological measures may not always be necessary, especially in uncomplicated models of

disuse, as we know that local muscle electrical stimulation also prevents unloading-induced declines in muscle size (Dirks et al., 2014; Gibson et al., 1988). Indeed, even as little as 10-15 minutes per day of can offset the decline in muscle size associated with disuse (Gibson et al., 1988). However, there are occasions where the rescue of losses in muscle mass with electrical stimulation fails to salvage the accompanying losses in muscle strength (Dirks et al., 2014). Finally, pharmacological interventions need to be carefully considered as recent evidence suggests that global downregulation of proteolysis may lead to myopathic conditions (Castets et al., 2013).

It is also critical to point out that a hypothesis that rests on disuse-induced declines in MPS as the predominant mechanism for atrophy it is not because MPB cannot be, or has not been, measured with disuse. In fact, in the studies (admittedly few in number) in which MPB has been measured with muscle disuse in humans it has been shown to be unchanged (Symons et al., 2009). Using a proxy marker of myofibrillar proteolysis (3-methylhisitidine: 3-MH) and microdialysis one report has shown an increase in interstitial 3-MH concentration (Tesch et al., 2008). However, as a static marker interstitial 3-MH concentration could have increased due to increased appearance or reduced clearance and subsequently definitive conclusions based on these data as to the role of MPB are simply not possible. However, we (Phillips et al., 2009; Phillips and McGlory, 2014a) and others (Wall and van Loon, 2013) have acknowledged that early, and potentially transient, changes in MPB are possible.

Conclusions

It appears clear that skeletal muscle hypertrophy is primarily driven by rises in rates of MPS in response to RE and amino acid ingestion, the relative contribution of both MPS and MPB to muscle disuse atrophy have, however, yet to be established. Dr. Donald Ingber stated *"It is becoming increasingly clear… mechanical and structural cues… have a central role in tissue physiology, as well as in a wide variety of diseases… it remains unclear how the whole cell processes this molecular scale information and orchestrates a physiologically relevant response. Thus, the time is now ripe… to put all the pieces together again and to understand how cells react to mechanical stimuli in their normal tissue context* (Ingber, 2006)". It has been nearly 10 years since the publication of that article and despite significant technological and scientific advancement, we are still unaware as to how muscle loading alters skeletal muscle morphology at the cellular level. Specifically, the key signal(s) that promotes muscle atrophy and muscle hypertrophy following bouts of mechanical loading/unloading in humans remain unknown. Future research using an integrative approach (measures of MPS and MPB) in combination with the application advanced molecular techniques may yield critical information in this regard, and thus, it may be possible to finally 'put all the pieces together'. However, it is our opinion that to achieve this aim it will require the collaborative efforts of many researchers in many fields using a variety of models.

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Conflict of interest

The authors declare no conflicts of interest.

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