Mechanisms regulating muscle mass during disuse atrophy and rehabilitation in humans

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Muscle mass loss accompanies periods of bedrest and limb immobilization in humans and requires rehabilitation exercise to effectively restore mass and function. Although recent evidence points to an early and transient rise in muscle protein breakdown contributing to this decline in muscle mass, the driving factor seems to be a reduction in muscle protein synthesis, not least in part due to the development of anabolic resistance to amino acid provision. Although the AKT signaling pathway has been identified in small animals as central to the regulation of muscle protein synthesis, several studies in humans have now demonstrated a disassociation between AKT signaling and muscle protein synthesis during feeding, exercise, and immobilization, suggesting that the mechanisms regulating protein synthesis in human skeletal muscle are more complex than initially thought (at least in non-inflammatory states). During rehabilitation, exercise-induced myogenesis may in part be responsible for the recovery of muscle mass. Rapid and sustained exercise-induced suppression of myostatin mRNA expression, that precedes any gain in muscle mass, points to this, along with other myogenic proteins, as being potential regulators of muscle regeneration during exercise rehabilitation in humans.

limb immobilization; hind-limb suspension; myogenesis; akt signaling; muscle protein synthesis

In this mini review, we summarize our current understanding of these processes in humans, with a significant portion of the review devoted to the complex nature of the regulatory mechanisms that are starting to emerge and an examination of how recent work in the regulation of muscle mass during disuse in humans has built on the work generated from animal-based approaches, providing unique insight. Last, we explore the myogenic processes responsible for the restoration of muscle mass during recovery following muscle disuse-induced atrophy in humans, which do not appear to be simply the converse of the mechanisms responsible for the initial loss of muscle mass (Fig. 1).

DISUSE-INDUCED LOSS OF MUSCLE MASS: RELATIVE CONTRIBUTIONS OF MUSCLE PROTEIN SYNTHESIS AND DEGRADATION

The maintenance of muscle mass is dependent on the balance between rates of muscle protein synthesis and muscle protein breakdown, where a chronic imbalance results in either the loss or gain of muscle mass. Work from animal studies utilizing in vivo tracer methodologies conclude that muscle protein synthesis is reduced (5) and muscle protein breakdown is increased (26) following 6 and 24 h of cast immobilization,
respectively. Furthermore, evidence that the proteasome inhibitor velcade resulted in an ~50% sparing of muscle weight following 3 days of cast immobilization in rodents, and with little change in the total amount and phosphorylation state of signaling proteins thought to modulate muscle protein synthesis, has been used to suggest that muscle protein breakdown is the dominant mechanism by which muscle protein content is reduced by disuse in animals (27). In contrast, it is unequivocal that muscle protein synthesis is suppressed in the immobilized, post-absorptive state in humans. For example, de Boer and colleagues detected a 50% decline in the rate of myofibrillar protein synthesis following 10 days of limb suspension, which the authors concluded was of sufficient magnitude on its own to account fully for the observed decline in muscle cross-sectional area, i.e., the contribution from muscle protein breakdown was insignificant (11). Reasons for these discrepancies are not clear but could reside in the notable metabolic differences present between humans and small animals. Most studies tend to utilize immature animals that are still in the growth phase, where rates of muscle protein synthesis and basal metabolic rate are known to be high and metabolic stability (capacity to maintain homeostasis) is low (13). Alternatively, it may in part be due to the differing degrees of stress that invariably exist between animal models of limb immobilization and those performed in informed, consenting adults. The influence these compounding issues may have is exemplified by the degree of muscle mass loss observed between species, where 3-day hindlimb cast immobilization of a rodent has been shown to result in an ~19% lower muscle mass compared with time-matched controls (27) vs. an ~5% decline in human quadriceps mass after 2-wk full-limb cast immobilization (25).

The observed decline in muscle protein synthesis following limb immobilization in humans combined with the lack of robust evidence for increased muscle protein breakdown during disuse atrophy has led some to speculate that the decline in muscle protein synthesis is solely responsible for the loss of muscle mass under these conditions (43). It is the authors’ opinion that the body of evidence published to date does not preclude a role for muscle protein breakdown during disuse atrophy in humans. Indeed, we have previously argued (41) that observations of increased amounts of ubiquitin-protein conjugates (17) and increased 3-methylhistidine (49) release in the first few days of muscle disuse point to an early and possibly transient contribution of muscle protein breakdown to the etiology of human disuse atrophy. However, although any contribution is likely to be comparatively small, the current lack of firm evidence utilizing tracer methodologies over the time course of muscle immobilization or disuse in humans makes the exact contribution of muscle protein breakdown to the etiology of disuse-induced muscle loss in humans speculative. Future studies should concomitantly assess both muscle protein synthesis and muscle protein breakdown to delineate the exact temporal contribution each makes to the etiology of disuse atrophy.

**THE MOLECULAR REGULATION OF DISUSE MUSCLE ATROPHY**

Previous work has highlighted translation initiation, where the ribosomal structure is formed and the associated mRNA transcript becomes bound, as a key point of regulation for muscle protein synthesis in a number of conditions where a decline in the rate of muscle protein synthesis is observed. More specifically, downstream mediators of the AKT signaling pathway have been identified as important regulators of this process (53–55). A detailed description of the AKT pathway is outside the scope of this review, and readers are directed to several comprehensive reviews on the topic (6, 14, 42), but, broadly speaking, the AKT signaling pathway J drives formation of the preinitiation complex by downstream inhibition of glycogen synthase kinase-3β; 2) promotes the synthesis of ribosomal components via the activity of p70S6K; 3) allows binding of the target mRNA transcript to the ribosomal complex via activation of mTOR, and thus inhibition of 4E-BP1; and 4) enhances protein synthesis in response to increased intramuscular amino acid availability by an as yet unknown mechanism that may involve mVps34 (20, 30). Therefore, in the day-to-day regulation of muscle mass, the AKT signaling pathway is considered to play a pivotal role in controlling muscle protein synthesis.

The central role of AKT signaling in the regulation of muscle mass is reinforced by experiments demonstrating the muscle-specific overexpression of AKT in transgenic mice results in profound muscle hypertrophy (4). Likewise, increased muscle protein synthesis in response to high-frequency electrical stimulation of rodent extensor digitorum longus muscle occurs in parallel with increased phosphorylation of downstream signaling proteins in the AKT pathway (2). Similar associations between rates of muscle protein synthesis and AKT signaling have been observed in animals following exercise (28) and disease (29). However, recent studies conducted in healthy human populations question the established notion that AKT signaling is driving muscle protein synthesis in the same context as has been described in animals. In 2008,
we described for the first time a disassociation between AKT signaling and protein turnover in human skeletal muscle under conditions of controlled amino acid delivery and insulin availability (18). We observed a stepwise increase in muscle AKT and p70S6K phosphorylation with increasing serum insulin concentrations in the postprandial state. However, although increasing amino acid provision (in the face of a fasting insulin concentration) increased leg protein synthesis compared with post-absorptive values, elevating serum insulin at the same amino acid infusion rate failed to modulate leg protein synthesis any further, despite markedly increasing muscle AKT and p70S6K phosphorylation. Subsequent reports by others suggest that this observation is not the result of an anomaly. By having subjects perform an acute bout of resistance exercise with one leg and endurance exercise in the contralateral limb, Wilkinson and colleagues (57) discovered the phosphorylation of signaling proteins in the AKT pathway to be increased by a similar magnitude in muscle biopsy samples obtained from the quadriceps of each leg; yet an increase in the rate of myofibrillar protein synthesis was restricted to the leg that performed resistance exercise. Likewise, following unaccustomed resistance exercise in young and elderly subjects, Mayhew and colleagues (31) observed an increase in muscle AKT phosphorylation in both groups, but the resistance exercise failed to augment the rate of muscle protein synthesis in the elderly and not in the young.

In combination, these observations, generated from studies conducted with protocols expected to result in an anabolic response, strongly suggest that the mere phosphorylation of the AKT signaling pathway is not sufficient for muscle protein synthesis to occur. This does not preclude AKT signaling from having an important function in the processes that govern human muscle protein synthesis but does suggest that enhanced AKT signaling does not commit muscle to elevated rates of protein synthesis. This view is in contrast to the central role of AKT signaling proposed by animal studies and suggests that the processes governing muscle protein synthesis in humans are more complex than currently perceived. Although data are sparse on the response of AKT signaling during interventions where a decline in muscle protein synthesis is expected, initial evidence would suggest AKT signaling has no obvious role in the observed decline in muscle protein synthesis following muscle disuse. It has been shown that neither the phosphorylation state nor the content of AKT, p70S6K, 4E-BP1, or eIF4E are altered by 10 or 21 days of limb suspension (11). Likewise, we have found the phosphorylation state of AKT$^{Ser473}$, p70S6K$^{Thr389}$, and 4E-BP1$^{Thr37/46}$ to be unaffected by 3 wk of limb immobilization (Constantin D, Miller J, Block M, Constantin-Teodosiu D, Hill R, Krasney P, Greenhaff PL., unpublished observations). Thus recent work in humans would cast doubt on the precise mechanisms that are responsible for the decline in muscle mass observed during disuse.

One observation that has received little attention to date but warrants further attention is the notion of anabolic resistance in humans. Although post-absorptive muscle protein synthesis is known to be reduced in limb-immobilized muscle, Glover and colleagues (16) demonstrated that suppressed muscle protein synthesis also extends to the postprandial state, even with high amino acid provision. The notion of anabolic resistance during immobilization could have significant implications on any therapeutic strategies that employed nutritional interventions.

Similar to previous observations discussed above, Glover and colleagues (16) observed no change in content of AKT, mTOR, or p70S6K between immobilized and non-immobilized legs before amino acid provision. Furthermore, the increase in muscle protein synthesis observed following both high (261 mg·kg$^{-1}$·h$^{-1}$) and low (43 mg·kg$^{-1}$·h$^{-1}$) dose amino acid provision was greatest in the non-immobilized legs but occurred in the face of similar changes in the phosphorylation of the AKT signaling pathway between immobilized and non-immobilized legs, again reinforcing suggestions that this pathway is not responsible for eliciting the decline in muscle protein synthesis.

Collectively, these observations highlight our lack of understanding of the mechanisms responsible for disuse-induced muscle loss in humans. Although currently employed animal models have helped suggest avenues to pursue, there remains a notable disparity between the observations collated between the two approaches. This begs the question if AKT signaling is not responsible for the observed decline in muscle protein synthesis during immobilization, what is? Naturally, this should be the basis of future work.

**REHABILITATION FOLLOWING MUSCLE DISUSE**

Although many reports in the scientific literature have focused on events during the period of muscle immobilization, the subsequent rehabilitation phase has by comparison been under investigated but should be considered of equal importance. Likewise, the investigation of the molecular events that underpin muscle rehabilitation following disuse in humans remain sparse, but initial information would suggest that myogenesis is an important response in the process. Muscle contraction of sufficient intensity and duration is known to result in activation of local satellite cells, aiding in subsequent muscle regeneration (10). Located between the plasma and basal membrane, satellite cells are normally quiescent, but once activated migrate to sites of injury or stress and subsequently proliferate and differentiate into multinucleated myofibres (for a review, see Ref. 48). A family of transcription factors, termed the myogenic regulatory factors (MRFs), include the members MyoD, Myf5, myogenin, and MRF4 and are responsible for inducing the transcription of skeletal muscle proteins and therefore coordinating the progression of satellite cell differentiation (3).

Myostatin, a member of the transforming growth factor-β protein family is known to be expressed in satellite cells and has been shown to negatively influence the expression of Myf4, Myf5, and MyoD in a tissue-type specific manner (1, 39). Furthermore, myostatin is widely known to inhibit muscle differentiation and growth in vivo, where animals (34, 38), including humans (47), lacking a functional copy of the myostatin gene, display extensive muscle hypertrophy. The effect of human limb immobilization on myostatin levels is ambiguous, with both elevated and unchanged mRNA levels reported in the literature (8, 11, 25). Increased protein levels of myostatin have been described in patients with chronic disuse due to osteoarthritis; however, the condition is characterized by persistent low-grade inflammation, making the relevance of the elevated myostatin levels to the etiology of disuse-induced muscle loss unclear (45). In contrast, during rehabilitation from immobilization-induced atrophy in humans, myostatin levels...
appear to be rapidly suppressed and sustained at a lower than basal level throughout rehabilitation. For example, we demonstrated that isokinetic exercise following 2 wk of immobilization (that produced ~5% loss of muscle mass) significantly suppressed myostatin mRNA levels within 24 h of cast removal and the first bout of rehabilitation exercise. Furthermore, myostatin mRNA expression remained below basal levels for the whole 6-wk period of investigation, during which muscle strength and mass returned to precast levels (25). This rapid and sustained downregulation of myostatin mRNA expression comcomitant with increases in muscle mass and in conjunction with its known effects on muscle mass regulation are suggestive of the protein having a central role in the regenerative process.

The mechanisms by which rehabilitative exercise induces satellite cell proliferation and differentiation is currently unclear, but muscle contraction has been shown on several occasions to reduce skeletal muscle myostatin mRNA and/or protein levels (21, 22, 56). It is therefore interesting to speculate that contraction-induced decreases in myostatin expression, via action on the myogenic regulatory factors, results in satellite cell activation. This does not preclude myostatin from having a direct influence on muscle hypertrophy. Recent work suggests that myostatin inhibition results in increased AKT mRNA and protein levels and overall activity (37, 52).

Interestingly, under normal circumstances, greatest satellite cell activation, and presumably ultimately muscle mass gains, appear to occur following eccentric vs. concentric muscle contractions (46). Unaccustomed eccentric exercise is known to result in disruption to the sarcomere structure (32) and the production of several inflammatory cytokines, most notably interleukin-6 (IL-6) (7). Furthermore, increased mRNA levels of IkB-β, an indirect activator of the NF-κB inflammatory signaling cascade, has been observed in muscle samples obtained during the early rehabilitative exercise phase following 2-wk limb immobilization (25). Although not observed at later stages, potentially because the subject would have become accustomed to the exercise regime, these findings suggest that inflammation may play a positive role in the initial response to rehabilitation and, moreover, may be responsible for initiating the myogenic events observed.

The role of inflammation in the regenerative process may, at first glance, appear counterintuitive. In many circumstances, such as sepsis, cancer cachexia, and chronic obstructive pulmonary disease, inflammation has been associated with a catastrophic loss of skeletal muscle mass (40). Moreover, the administration of either of the inflammatory cytokines, IL-6 or tumor necrosis factor-alpha (TNFα), has been associated with increased muscle catabolism, principally as a result of increased ubiquitin-proteasome-mediated proteolysis (15, 24). However, the immune response appears central to the repair process in muscle. Cheng and colleagues demonstrated the inhibition of interferon-γ to reduce cell proliferation and decreased the formation of regenerating fibers (9). In humans performing eccentric exercise of sufficient intensity to elicit a rise in muscle IL-6 mRNA, McKay and colleagues demonstrated rapid and transient localization of the IL-6 receptor (IL-6Rα) and increased IL-6 protein expression in satellite cells (33). The presence of these changes comcomitant to increased satellite cell proliferation highlight IL-6 signaling as potentially important in the response of satellite cells to eccentric exercise.

The wide availability of anti-inflammatory medication has helped to further investigate the role of inflammation in the anabolic response to eccentric exercise. A good recent example of this is the work published by Mikkelsen and colleagues (35). Subjects were studied during and for several hours after completing 200 maximal eccentric contractions in each leg and received a localized infusion of a nonsteroidal anti-inflammatory drugs (NSAID) to one leg via a microdialysis catheter. When examined 8 days later, increases in satellite cell numbers were detected only in the control leg that had not received the NSAIDs (35). NSAIDs are known to reduce prostaglandin release by inhibiting the cyclooygenase enzyme (44), and it has been previously proposed that prostaglandins are responsible for stimulating satellite cell proliferation (36). In accordance with these observations, NSAIDs have also been shown to blunt resistance exercise-induced increase in postexercise muscle protein synthesis in healthy, male volunteers (51). These observations have significant implications on the use of NSAIDs where muscle mass gains are sought from exercise. This is particularly pertinent to elite-level sportsmen where regular NSAID use is prevalent (50) but also has ramifications for rehabilitation regimes where discomfort or inflammation may typically be treated by NSAIDs.

As noted earlier, given the classical association of inflammation with muscle catabolism, it has been suggested that the regular provision of NSAID may represent a potential strategy to prevent muscle wasting per se (19). This in essence sums up our current level of understanding of the processes responsible for regulating muscle mass in humans. Inflammation appears to have dual functions in muscle and can act as both an anabolic and catabolic trigger, but as of yet we do not understand why. From recent work, it has become apparent that the mechanisms responsible for the regulation of muscle mass in humans are more complex than originally thought and, moreover, that subtle differences exist between data generated from current animal and human models of muscle disuse. It is now acknowledged that although animals share genes, organ systems, and systemic physiology with humans, humans differ significantly in terms of morphometry, physiology, and lifespan. Therefore, it is only logical that to gain detailed insight into human disuse atrophy and rehabilitation we must ultimately study humans and use animal- and cell-based approaches to support and provide scientific justification for investigation in humans.

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