Review

Determinants of Disuse-Induced Skeletal Muscle Atrophy: Exercise and Nutrition Countermeasures to Prevent Protein Loss

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Summary Muscle atrophy results from a variety of conditions such as disease states, neuromuscular injuries, disuse, and aging. Absence of gravitational loading during spaceflight or long-term bed rest predisposes humans to undergo substantial loss of muscle mass and, consequently, become unfit and/or unhealthy. Disuse- or inactivity-induced skeletal muscle protein loss takes place by differential modulation of proteolytic and synthetic systems. Transcriptional, translational, and posttranslational events are involved in the regulation of protein synthesis and degradation in myofibers, and these regulatory events are known to be responsive to contractile activity. However, regardless of the numerous studies which have been performed, the intracellular signals that mediate skeletal muscle wasting due to muscular disuse are not completely comprehended. Understanding the triggers of atrophy and the mechanisms that regulate protein loss in unloaded muscles may lead to the development of effective countermeasures such as exercise and dietary intervention. The objective of the present review is to provide a window into the molecular processes that underlie skeletal muscle remodeling and to examine what we know about exercise and nutrition countermeasures designed to minimize muscle atrophy.

Key Words Skeletal muscle disuse atrophy, microgravity, hindlimb suspension, protein synthesis and degradation, exercise and nutrition countermeasures

Because skeletal muscle is the most abundant tissue of the human body, we may hypothesize that decreases in its mass possibly will profoundly impact the whole-body metabolism and ultimately lead to the development of lifestyle-related diseases. In addition, muscle deconditioning (reduced strength, abnormal reflex patterns, increased fatigability) could limit the ability of astronauts to work in space and/or to rapidly egress the spacecraft in an emergency landing (1). Even though various functional and morphological alterations are noticeable in inactive muscles, decreased protein content has been regarded as the hallmark of skeletal muscle atrophy. Muscle protein can be either gained or lost by relative changes in protein synthesis and degradation rates, which are known to be modulated by contractile activity. Thus, understanding about the molecular regulators of protein turnover during different mechanical loading conditions and their response to specific treatments leads to the likelihood of developing effective countermeasures for preventing disuse-induced muscle wasting.

This article provides a review of muscular adaptations to disuse, with emphasis on protein kinetics. We describe first the general aspects of muscle wasting with regard to exposure to actual or ground-based, simulated microgravity and some of the most significant findings that provide evidence for a close connection between skeletal muscle plasticity and protein metabolism. Next, we present a review of the literature in which results of measurements of protein synthesis/degradation rates during unloading are available and discuss molecular signaling pathways involved in the regulation of protein turnover in inactive muscles. Following that, we focus on the impact of muscle reloading on protein kinetics and then discuss potentially effective countermeasure procedures. Finally, a few lines are devoted to outline our view of future directions for investigations in this field.

1) Overview

1.1) Microgravity exposure and skeletal muscle atrophy

Skeletal muscle atrophy is known to occur as a result of diseases (e.g., diabetes mellitus, cancer, renal failure), neural injuries, aging (sarcopenia), starvation, and lack of use (e.g., gravitational unloading, limb casting, long-term bed rest). In particular, disuse-induced atrophy became the focus of attention after the Skylab missions of the mid-1970s (2, 3), because morphological and physiological adaptations of skeletal muscles to microgravity were evident during long-term spaceflights and were recognized to endanger the well-being of astronauts (cosmonauts) as well as the successful completion of longer space missions (4). Therefore, studies on the cellular and molecular mechanisms that regulate such adaptations and on potentially effective counter-
measures against skeletal muscle wasting/weakening were started. However, because most space-related life science programs are expensive and time-consuming, requiring international cooperation and resources with trans-disciplinary expertise (5), several ground-based models of skeletal muscle disuse have been developed for both humans and animals.

1.2) Ground-based models

Basically, the most used ground-based models to simulate microgravity conditions in humans are bed rest (with head-down tilt or not), joint immobilization, and unilateral lower limb suspension (6). For animals, especially rats and mice, hindlimb suspension (7, 8), joint immobilization (9, 10), tenotomy (11), denervation (12), and spinal cord transection (13, 14) are well-known methods to induce muscle atrophy. Although all of these models effectively bring about skeletal muscle wasting, chiefly in slow-twitch muscles such as the soleus (9, 15), their impact on muscle fibers occurs with different degrees of severity and/or rapidity (16). Rodent hindlimb suspension by tail-casting/harnessing, one model that removes the weight-bearing function of the hindlimbs, has relatively easy procedures and mimics actual spacecraft with regard to its effect on postural/antigravitational muscles without the need of immobilizing joints or transecting nerves or tendons. Therefore, this model has been used in a countless number of studies and, for that reason, a large amount of data is available.

1.3) Skeletal muscle adaptations to inactivity

Regardless of the inciting event, skeletal muscle atrophy is characterized by a decrease in protein content, fiber diameter, force production, and fatigue resistance (17). Reduced mass and cross-sectional area of atrophied muscles, loss of thin filaments (18, 19), and transitions in the myofibrillar type (20–22) are also responsible for alterations in other functional properties such as tetanic tension and shortening velocity (23–25). Issues related to structural and functional adaptations of skeletal muscles to inactivity or spaceflight are well documented (1, 26–30) and therefore are not within the scope of the present review.

Marked decreases in absolute and relative protein contents of skeletal muscles in early stages of disuse (15, 31, 32) is one of the most significant adaptations of this tissue to reduced tension, resulting in fiber shrinking and weakening. Disuse-induced skeletal muscle atrophy is accompanied by a whole-body negative nitrogen balance in humans during spaceflight (33–35) or bed rest (36–38). In addition, hindlimb suspension or immobilization for 7 d significantly decreased the total RNA content and the α-actin and cytochrome c mRNA expression in the muscles of rats (39). Moreover, other studies also revealed that the RNA-to-DNA ratio in atrophic muscles decreases considerably in rats (32, 40, 41) and humans (42), indicating reduced capacity for protein synthesis. Thus, it can be deduced that changes in protein turnover (i.e., concomitant upregulation of protein degradation and downregulation of protein synthesis) in myofibers is one of the key mechanisms that orchestrate adaptations of skeletal muscle to unweighting. However, the hormonal and molecular regulators that act in response to weightlessness or unloading, and ultimately bring about skeletal muscle wasting, are not fully understood.

2) Disuse-Induced Adjustments in Skeletal Muscle Protein Turnover

Various simulation models for microgravity investigations have been reported to promote protein breakdown and/or reduce the rate of protein synthesis in skeletal muscles (Table 1). It is noticeable the great number of rat studies which were performed using the hindlimb suspension model of disuse (thus, the results from this model will be focused on in our discussion). In addition, it is apparent that the predominantly slow-twitch oxidative soleus is one of the ankle plantarflexors muscles most affected by unloading (and therefore most studied), with protein synthesis rates reducing to levels less than 50% and protein degradation rates increasing to levels more than 150% of those of controls. Along with its unique fiber phenotype, this susceptibility of the soleus may be due to its function as an anti-gravitational muscle, which becomes tensed for the sole fact that the ankle is dorsiflexed, when the limb is weight-bearing (61). The importance of muscle shortening and stretching during unloading for modulation of protein turnover and muscle mass has been demonstrated elsewhere (45, 46). Furthermore, the data summarized in Table 1 illustrate many discrepancies concerning the levels at which unweighting influences protein turnover in muscles. Added to varied ages (i.e., growth rate influence), gender difference, and disparate disuse durations (from 2 to 28 d), these differences might be derived from diverse nutritional states of the animals or subjects and from variations in the unloading techniques and analyzing methodologies (e.g., assessment in vivo or in vitro, direct measurement or calculated estimation). Nevertheless, the cited studies point out the important role of protein turnover regulation in triggering lean body mass lessening under conditions of mechanical inactivity or unloaded muscle contractions.

Notwithstanding human studies which have found that inactivity brings about a negative nitrogen balance, as mentioned above, increases in protein degradation rates were not apparent in humans during unweighting challenges (Table 1). On the contrary, protein breakdown rates in humans have been shown to have a tendency to decrease in response to unweighting (37, 60), probably as a compensatory mechanism for decreased protein synthesis rates (protein deficit). Furthermore, neither spaceflight nor bed rest induces changes in the urinary 3-methylhistidine, a standard assessment of myofibrillar protein breakdown (38). Therefore, we may conclude that, in humans, disuse-induced skeletal muscle wasting is chiefly determined by downregulation of protein synthesis (36, 37, 62), whereas in animals proteolysis may be a greater contributor to protein loss (63). In relation to the time course response of the soleus muscle of rats, the estima-
Table 1. Disuse effects on skeletal muscle protein turnover (synthesis and degradation).

| Study  | Disuse model | Duration (d) | Muscle          | Synthesis (% control) | Degradation (% control) | Reference |
|--------|--------------|--------------|-----------------|-----------------------|-------------------------|-----------|
| Rat    | TN           | 3            | Gas             | 92*; 78*             | 186*; 158*               | Goldspink et al. 1983 (43) |
|        | DN           | 2            | EDL             | 12*; 5*              | 16*; 3*                  | Tischler et al. 1990 (49) |
|        | HS           | 6            | Sol             | 76; 6*               | 153; 8*                  | Jaspers et al. 1985 (44) |
|        |              | 3            | Sol             | 79; 44*              | 208; 6*                  | Loughna et al. 1986 (45) |
|        |              | 6            | Sol             | 83*                  | 96*                     | Jaspers et al. 1988 (46) |
|        |              | 7            | Pla             | 58; 53*              |                         | Thomason et al. 1989 (31) |
|        |              |              | Gas             | 65*; 46*             |                         |           |
|        | DN           | 3            | Sol             | 125*                 | 183*                    | Furuno et al. 1990 (47) |
|        |              | 10           | Gas             | 80*                  | 131*                    | Tischler et al. 1990 (48) |
|        |              | 3            | Sol             | 113*                 | 141*                    |           |
|        |              | 5            | Gas             | 47*; 33*             | ND                      | Linderman et al. 1994 (52) |
|        |              | 2, 3         | Sol             | ~42*                 | ~217*                    | Tischler 1994 (53) |
|        |              | 21           | TA              | 75*; 68*             | ND                      | Taillandier et al. 1996 (54) |
|        |              | 9            | Sol             | ND                   | 166*                    | Taillandier et al. 1996 (55) |
|        |              | 10           | Sol             | ~73*                 | ~177*                    | Zdanowicz and Teichberg 2003 (57) |
|        |              | 4            | Sol             | ~65*                 |                         | Fluckey et al. 2004 (58) |
| Human  | BR           |              | Leg             | 53*                  | 71*                     | Ferrando et al. 1996 (37) |
|        | ULLU         | 10           | LQuad           | 90*                  | ND                      | Garrain et al. 1998 (42) |
|        | BR           | 14           | WB              | 82*                  | 81*                     | Stein et al. 2003 (60) |

TN, tenotomy; DN, denervation; HS, hindlimb suspension; BR, bed rest; ULLU, unilateral lower limb unloading; Gas, gastrocnemius muscle; EDL, extensor digitorum longus muscle; Sol, soleus muscle; Pla, plantaris muscle; TA, tibialis anterior muscle; LQuad, lateral quadriceps femoris muscle; WB, whole body; ND, not determined; *difference not statistically significant; *fractional rate of protein synthesis (%/d); †absolute rate of protein synthesized or degraded (mg/d); ‡rate of protein breakdown calculated as the difference between synthesis and growth (%/d); §measurement in vitro (nmol of tyrosine incorporated or released/mg muscle/time); ¶protein synthesis or degradation rate in isolated myofibril; †measurement in vitro (nmol of phenylalanine incorporated/g muscle/h); # rate of whole body protein synthesis or breakdown (g protein/kg/d).

3) Regulatory Pathways

Skeletal muscle protein synthesis and degradation processes are regulated by intricate signaling pathways that turn activated or inactivated in response to stimuli such as nutritional molecules, hormones, specific drugs, and mechanical tension. To date, many details about these pathways have been discovered and an appreciable number of excellent reviews are available (66–74). Thus, we will abstain from describing full signaling cascades and will focus the present review on triggers of muscle atrophy and countermeasures.
expression patterns in skeletal muscle during disuse atrophy (75–79).

3.1) Regulation of protein synthesis

Specific transcriptional, translational, and posttranslational events within muscle fibers are understood to control the rates at which the protein synthetic machinery works with the purpose of building components necessary for the execution of specialized functions such as contraction. In this context, the mammalian target of rapamycin (mTOR) signaling pathway has been recognized to play a central role in the regulation of intracellular protein synthesis. mTOR mediates activation of protein synthesis by catalyzing phosphorylation of key molecules that directly or indirectly regulate the process of mRNA translation such as eukaryotic initiation factor 4E (eIF4E), and increases in the phosphorylation of 4E-binding proteins (4E-BP) associated with eukaryotic initiation factor 4E-binding proteins (4E-BP) and 70-kDa ribosomal protein S6 kinases (S6K). Besides, it has been well established that mTOR senses extracellular stimuli such as growth factors and nutrients (74). Therefore, skeletal muscle growth is thought to be chiefly modulated through activation of the downstream effectors of mTOR.

Modulation of the Ser2448 site in mTOR by phosphorylation/dephosphorylation has an important role in the control of protein synthesis in skeletal muscle. Plantaris muscle overloading by synergist muscle ablation increases mTOR Ser2448 phosphorylation and promotes hypertrophy of the plantaris muscle in rats; in contrast, gastrocnemius muscle unloading by hindlimb suspension decreases the phosphorylation levels of mTOR Ser2448 and promotes atrophy of the muscle, effects fully reversible by reloading (80). Furthermore, similar experimental treatments have been reported to produce analogous outcomes for the serine/threonine kinase Akt, a well known component of the insulin signaling pathway and an upstream of mTOR responsible for Ser2448 phosphorylation (81).

Correspondingly, soleus muscle atrophy induced by hindlimb suspension is associated with a significant decrease in the phosphorylation of eukaryotic elongation factor 2 (eEF2) Thr56 and S6K (82), whereas hypertrophy of the rat ankle dorsiflexor muscles (extensor digitorum longus and tibialis anterior) following high-resistance eccentric (lengthening) contractions is associated with long-lasting elevations in the rates of translation initiation and marked increases in S6K phosphorylation (83). Effects of eccentric contractions may be partially triggered by stretch-activated channels (84). In addition, compensatory hypertrophy of the rat plantaris muscle significantly correlates with increases in S6K activity, decreases in the amount of the translational repressor protein 4E-BP1 associated with eukaryotic initiation factor 4E (eIF4E), and increases in the amount of eIF4G bound to eIF4E, effects completely abolishable by daily injections of rapamycin (81). On the other hand, unloading-induced atrophy of the rat gastrocnemius muscle is associated with decreases in phosphorylated S6K and increases in the amount of 4E-BP1 bound to eIF4E, effects reversible by several days of recovery (81).

Hence, the abovementioned findings indicate that 1) disuse-induced skeletal muscle reduced protein synthesis is closely related with posttranslational modification (i.e., inactivation) of initiation and elongation factors downstream of Akt/mTOR and 2) a degree of muscle activity or mechanical loading is crucial for maintenance of skeletal muscle mass, through sustained activation of the translational machinery. Evidently, the contribution of decreased availability of specific mRNAs (i.e., transcriptional regulation) to the atrophic response of muscles to unloading should not be overlooked (85). Recently, we have found that soleus muscles unloaded for a few days had significantly lower amounts of S6K1 protein than controls, indicating transcriptional regulation of this kinase during disuse (unpublished data). Further research is needed to analyze whether the aforementioned findings are reproducible in human skeletal muscle and whether actual spaceflight induces the same molecular adaptations in this tissue.

3.2) Regulation of protein degradation

At least half of total muscle protein is myofibrillar protein (17), and this fraction is broken down more rapidly than other proteins during disuse-induced atrophy (51). The majority of studies regarding skeletal muscle protein degradation during disuse atrophy have focused on three primary proteolytic pathways: the cytosolic Ca2+-dependent proteolysis (calpains), lysosomal proteolysis (cathepsins), and ATP-dependent proteolysis (ubiquitin-proteasome degradation). Nevertheless, other degradative processes including intracellular and extracellular protease cascades (serine proteases and matrix metalloproteinases) and apoptosis (caspases) are all likely involved in muscle atrophy, though with unclear extents of involvement (86, 87). Recent investigations have indicated that the three primary proteolytic systems may work as partners during muscle proteolysis (17, 55). The ubiquitin-proteasome pathway appears responsible for degradation of the bulk of proteins, mainly myofibrillar proteins, in conditions of decreased muscle use (55, 88, 89). However, there is an extensive consensus that intact myofibrillar proteins cannot be degraded by the proteasome (90); consequently, the initial cleavage of myofibrillar proteins requires other proteases (87). So far, a vast number of atrophy-protein degradation-related studies have been done and, given that our objective is to review the impact of conditions comparable to actual space travel on unweighted muscles, here we shall not discuss the literature that describes results of experiments in which the factor innervation is not present (39).

3.2.1) Calcium-dependent proteolysis. The rate-limiting step in the degradation of myofibrillar proteins is their dissociation from the contractile filaments, before ubiquitin-dependent proteolysis takes place (90). Therefore, activation of calpains, cathepsins, and several other proteases (87) appears to represent an early rate-limiting step in myofibrillar protein degradation during unloading-induced skeletal muscle wasting. Calcium loading rates (91) and mRNA and protein levels of the
fast Ca\(^{2+}\) pump (92) and the calcium-binding protein calsequestrin (93) in the sarcoplasmic reticulum increase significantly in soleus muscles during hindlimb suspension, probably in response to oxidative stress-induced excess of intracellular calcium. Atrophying muscles have been found to have markedly increased calcium-dependent thiol protease (calpain) activity both in vivo and in vitro (48, 55, 94) along with enhanced m-calpain mRNA levels (55), suggesting transcriptional regulation of this enzyme. In disagreement with these data, a more recent investigation has shown that neither actual spaceflight nor simulated microgravity induces changes in the message levels of calpains in atrophic gastrocnemius muscles (89). Thus, it remains uncertain whether a muscle-specific regulation of these enzymes in response to unloading exists or not. Nevertheless, because transgenic mice with muscle-specific overexpression of calpastatin (an endogenous inhibitor of calpains) had significantly less (30%) muscle atrophy than non-transgenic animals during a 10-d unloading period (95), we may conclude that calpains are important triggers of skeletal muscle proteolysis degradation during gravitational unloading.

3.2.2) Lysosomal proteolysis. Notwithstanding lysosomal proteolysis having been suggested to play a minor role in atrophy caused by unweighting (48), marked increases in the activity and message of various isoforms of cathepsins in atrophic muscles have been reported (55, 89). Among these isoforms, cathepsin L appears to be the most responsive to unloaded contractions or weightlessness. However, inhibition of cathepsin B+L activity does not prevent disuse-induced myosin heavy chain fragmentation or muscle wasting (89). Cathepsins do not degrade cytosolic proteins; the major role of cathepsins is to degrade membrane proteins, including receptors, ligands, channels, and transporters (17). Thus, added to the fact that unloaded muscles are likely to undergo oxidative stress or altered calcium homeostasis (93, 96), this notion points toward a potential involvement of lysosomal activity with oxidative stress-induced myocyte apoptotic feedback (97). Oxidative stress occurs when antioxidant protein and scavenger protection are overwhelmed by oxidant production: increases in total hydroperoxides concurrent with decreases in nonenzymatic antioxidant scavenging capacity and activities of antioxidant enzymes such as catalase and glutathione peroxidase in unloaded muscles (96) may lead to increased apoptotic signaling, perhaps connected with increased lysosomal activity. Experimental evidence is needed to confirm or refute this hypothesis, and additional studies are required to clarify the genetic and molecular features of lysosomal-mediated protein cleavage in response to modified functional demands in muscles. Another issue that calls for further experimentation is the influence of muscle unloading on the interaction between lysosomal and ubiquitin-proteasomal mechanisms through differential ubiquitination of target proteins (17, 98).

3.2.3) ATP-dependent ubiquitin-proteasome proteolysis. Together, the lysosomal and Ca\(^{2+}\)-dependent proteolytic pathways appear to account for not as much as 18% of total proteolysis in non-weight-bearing soleus muscles of rats (55). The ATP-dependent ubiquitin-proteasome system, therefore, accounts for the major part of myofibrillar protein degradation resulting from skeletal muscle disuse. According to several studies, the amount of ubiquitin-protein conjugates increases significantly in unloaded muscles during spaceflight (89), simulated microgravity (59, 89, 99), and immobilization (100). This increase in ubiquitinated proteins, which appears to be restricted to the myofibrillar fraction (101), is associated with marked increases in the mRNA expression of ubiquitin, 14-kDa ubiquitin-conjugating enzyme, and C2 and C9 subunits of the 20S proteasome (55, 89, 99). In addition, rat hindlimb joint immobilization (100, 102), hindlimb suspension (79, 102, 103), and spaceflight (79) have been shown to induce several fold increments in the mRNA content of the gastrocnemius muscle atrophy F-box (MAFbx/Atrogin-1 and muscle ring finger 1 (MuRF1), two recently discovered striated muscle-specific ubiquitin ligases (102, 104) that have been recognized very responsive to mechanical loading changes in skeletal muscle and rate-limiting of atrophy (i.e., knock-out mice for these genes are partially resistant to muscle atrophy). Message levels of MAFbx/Atrogin-1 and MuRF1 also increase significantly in soleus muscles after 2-wk hindlimb unloading (99). Recently, we have found that the message level of MAFbx/Atrogin-1 in soleus muscles increases about 3 fold following a short period of 36 h unloading (unpublished data). Furthermore, another ubiquitin-protein ligase, the neuronal precursor cell-expressed developmentally down-regulated-4 (Nedd4; which is implicated in targeting membrane proteins), has been reported to be upregulated similarly to MAFbx/Atrogin-1 in soleus muscles of rats after unweighting (77, 99). Overall, these results indicate that the components of the ubiquitin-proteasome pathway in skeletal muscle are very sensitive to disuse and are regulated transcriptionally. Thus, coordinated activation of the major proteolytic pathways, principally the ubiquitin-proteasome system, appears to be the most important determinant of muscle atrophy in animals.

3.3) Other potentially involved regulators

3.3.1) Insulin-signaling pathway. Decreased functional demand in skeletal muscles induces regulation of other intracellular signaling molecules and/or pathways that may be involved in the atrophic response. Substrate utilization for energy production in muscles of rats under conditions of actual (105) or simulated (106, 107) microgravity shifts away from lipid and towards glucose, with concomitant suppression of lipoprotein lipase activity (108). This change is supported by the findings that the abundance of insulin receptors (109) and the amount of the glucose transporter 4 (110, 111) in rat soleus muscles augment markedly in response to mechanical unloading. Conversely, atrophied muscles have a decreased total amount of the B-subunit of the insulin receptor, suppressed Akt and glycogen synthase kinase-3β (GSK-3β) activities, and
increased levels of phosphorylated insulin receptor substrate-1 (IRS-1) Ser107 and c-jun NH2-terminal kinase (JNK) Thr183/Tyr185, indicating impaired insulin signaling and suggesting JNK-dependent phosphorylation of IRS-1 during muscle disuse (111). Other studies have also suggested insulin resistance in humans during bed rest (112) and in rats during hindlimb unloading (113) or immobilization (114). Moreover, spaceflight and ground-based models are known to induce a subclinical diabetogenic state in humans (115). However, atrophied muscles uptake glucose independent of insulin; probably stimulated by elevated activities of Erk and p38 (111). Thus, impaired insulin (and perhaps IGF-1) signaling in inactive muscles does not impede glucose uptake but may decrease the sensitivity of myofibers to the effects of insulin on suppressing net protein degradation (113) and enhancing protein synthesis through Akt/mTOR activation.

3.3.2) Apoptosis. The reduced number of myonuclei in spaceflown rats was suggested to be a contributing factor to the reduction in fiber size associated with chronic unloading of the musculature (116). Compared with muscles of weight-bearing animals, muscles of hindlimb suspended rats have a significantly decreased number of myonuclei (117) and markedly increased number of fibers containing morphologically abnormal nuclei and myonuclei demonstrating double-stranded DNA fragmentation, indicating that the apoptosis process is ongoing in unloaded muscles (118). In fact, a very recent study has found that the number of apoptotic nuclei increases several fold in unloaded soleus muscles (99). Besides, spaceflight (119, 120), muscle unloading following hyprophytropy (121), and also acute resistance exercise (122) induce accumulation of p53 protein, a tumor suppressor gene product, in skeletal muscles. The p53-mediated signal transduction pathway functions as a cell-cycle checkpoint that is activated during muscle inactivity are not intended to be conclusive data is currently available for inactivity-induced atrophy, further information about the potential connection between NF-κB activation and proteasome-dependent proteolysis can be found in another very recent review (63). Incidentally, an intriguing question is arises that is about the apparently contradictory functions of activated p53 and NF-κB pathways during muscle disuse: one may turn on proapoptotic factors (Bax) and the other may turn on anti-apoptotic factors (Bcl-2), respectively. What is the functional role of these two paradoxical regulations?

Figure 1 summarizes our current knowledge about the main intracellular pathways that mediate signaling during muscle disuse.

4) Reloading Effects on Protein Kinetics

4.1) Myofiber damage and recovery of muscle mass

The period of recovery from spaceflight is critical for the readaptation of several metabolic and physiologic
functions of the body to 1 g, including recuperation of the contractile capacity of the muscles and normalization of in-flight altered musculoskeletal protein turnover. Once humans start adventuring forth to the moon, Mars and beyond, they have to be able to successfully and rapidly adapt to different levels of gravity (60). But this is not an easy undertaking, particularly because landing is associated with a metabolic stress response (34) and reloading of atrophied muscles upon re-exposure to terrestrial gravity results in mechanical stress-induced structural lesions of myofibers (129, 130), particularly sarcomere damage (131), which is responsible for the delayed-onset muscle soreness experienced by astronauts upon returning to Earth (19). Furthermore, reloaded muscles of animals have significantly increased hydroperoxide levels (132) and undergo inflammation and superoxide-mediated membrane damage (133–135). To our knowledge, however, few studies have focused on the effects of reload on muscular protein kinetics in either humans or animals. In humans, decreased whole body protein synthesis rates and nitrogen retention tend to be improved in the early-recovery phase from bed rest (60) and subsequent to microgravity exposure (38). In animals, reloading or recovery from unweighting produces selective changes in the proteome (136) and markedly boosts the disuse-induced decreased RNA-to-DNA ratio (40), protein synthesis rate (137), and myofibril protein content (15) in soleus muscles. In addition, most of the genes downregulated and several genes upregulated during unloading return to basal levels within a few hours of recovery (78). These effects result in relatively rapid recovery of the fiber cross-sectional area (135, 138) and muscle mass (15, 136–141).

4.2 Reactivation of the protein synthetic machinery

As mentioned previously, reloading the hindlimbs of rats after several days of suspension produces significant increases in the gastrocnemius muscle Akt (81), mTOR (80), and S6K (81) phosphorylation levels, as well as marked decreases in the amount of 4E-BP1 bound to eIF4E (81). In addition, others have demonstrated that the downstream pathway of Akt, including ribosomal protein S6, is activated early in recovery from disuse muscle (soleus) atrophy, suggesting that the recovery of atrophied muscles is facilitated by increased mRNA translation during the early stages of resumption of loading (140). Moreover, another study concluded that a 2-wk recovery period from nonweight bearing significantly increases the activation levels of S6K and ribosomal protein S6 in medial gastrocnemius muscles of sham-operated (ovariectomy) rats, but does not influence appreciably the levels of phosphorylated Akt or mTOR in the same animals (141). Concerning these contradictory outcomes, our opinion is that different regulation feedbacks detected for different muscles during unloading (82) and greatly variable time-course regulatory responses of protein translation factors during reloading (139) should be carefully considered when analyzing the effects of disuse and recovery, respectively.

4.3 Role of the proteolytic systems

Despite its importance, until few years ago nothing was known about regulation of protein degradation after reloading of unweighted muscles. Taillandier et al. (137) demonstrated for the first time that protein degradation rates in soleus muscles of rats reloaded for 18 h remain significantly higher than in muscles of weight-bearing animals. This elevated rate of protein breakdown during early recovery was attributable to the activation of non-lysosomal and Ca2+-independent proteolysis (137), mRNA levels of ubiquitin, m-calpain, and C8 and C9 subunits of the 20S proteasome were markedly elevated at 18 h of reload, whereas cathepsin D and 14-kDa ubiquitin-conjugating enzyme mRNA levels were decreased, indicating early transcriptional control of proteolysis. Practically all of these adaptations were finished following 7 d of reloading; however protein synthesis was still elevated at this time point (137), most probably engaged in myofibril rebuilding. Therefore, the authors concluded that enhanced protein synthesis and breakdown are both necessary during recovery from atrophy and that non-coordinate regulation of proteolytic systems is presumably required to target specific classes of substrates (atrophy-specific protein isoforms, damaged proteins) for replacement and/or elimination. Further research is needed to make clear what proteins are most susceptible to targeting for degradation during reloading.

5) Countermeasures

Prevention of protein loss and concurrent lean body mass wasting during space missions is fundamental for avoidance of weakening or deconditioning. Prevention of skeletal muscle atrophy during prolonged inactivity is also important for decreasing time needed for readaptation. The effects of a variety of potential countermeasures have been tested and, in many cases, proved ineffective or unsatisfactorily helpful for counteracting disuse-induced muscle wasting. One example is antioxidant supplementation, which successfully increases the antioxidant capacity of unloaded muscles but does not prevent atrophy (142). Another example is administration of allopurinol, a xanthine oxidase inhibitor with antioxidant properties, which mitigates muscle contractile dysfunction caused by hindlimb unloading but does not inhibit muscle atrophy in mice (143). Intraperitoneal injection of calcium-binding agents (EDTA, EGTA) with the aim of preventing increases in the intrafibrillar calcium content and so the activation of Ca2+-dependent proteases (144, 145) in rats is an interesting approach, one, however with not so expressive effects. Another interesting alternative is heating, which proved useful to some extent during immobilization (146) or before hindlimb unweighting (147). Nonetheless, injection of a β-adrenergic agonist with anabolic effects, clenbuterol, has been suggested to be of benefit in attenuating muscle atrophy and dysfunction in hindlimb-suspended animals (148–150), at least in part through a muscle-specific inhibition of the ubiquitin-proteasome pathway (59). A positive feature of the lat-
Several hormones are known to have an influence on anabolic and catabolic pathways; and the mechanisms by which either thyroid deficiency or spaceflight impacts skeletal muscle growth in neonatal rats appear to have a common pathway involving the control of plasma and muscle IGF-1 concentrations (151). However, neither inhibition of glucocorticoids (53) nor overexpression of IGF-1 (152) attenuates unloading-induced muscle atrophy. In a more recent study, daily subcutaneous injections of the complex IGF-1/binding protein-3 during hindlimb suspension showed beneficial effects such as proteolysis inhibition and preservation of muscle protein content and mass (57). So far, however, the most promising countermeasures to offset muscle protein loss during inactivity are exercise (or periodic loading) and dietary supplementation. Further information can be found in an excellent review written some years ago (153).

5.1 Exercise intervention

Inactivity is a risk factor for metabolic diseases, and even non-vigorous exercise provides marked protection against disorders involving poor lipid metabolism (108). Thus, performing regular exercise during spaceflight or hospitalization is not just a way to preserve muscle mass, but a way to keep healthy. Motion is an important element for preventing muscle loss: however tensionless or unloaded contractions have no substantial effect on protein turnover and therefore do not differ significantly from absolute inactivity. Muscle tension is fundamental. Tension of unloaded muscles by stretching has been proven beneficial for preventing atrophy (24) and increasing rates of protein synthesis and decreasing rates of protein degradation in animals (45, 46, 154). Rat hindlimb plantar support, which maintains plantarflexor muscles tensed, is also effective to prevent some of the muscular alterations brought about by unloading (155). In addition, short-duration, periodic weight support (156–158) or centrifugation (158) and intermittent high-load exercise (159) performed during hindlimb unloading have shown positive effects on the mass of the antigravitational soleus muscle, with lesser effects on the gastrocnemius (159, 160). However, a combination of resistance exercise and growth hormone treatment increases myofibrillar protein synthesis and attenuates atrophy of unweighted fast-twitch skeletal muscles (52). Furthermore, exercise and growth hormone treatments have shown a strong interactive effect in maintaining the mass of unloaded muscles (161) and ameliorating the apoptosis associated with inactivity (118). Exercise is also required to potentiate the anabolic effect of insulin in unloaded muscles, which appears to be mediated independently of a rapamycin-sensitive pathway (58) and to involve a mitogen activated protein (MAP)-kinase signaling pathway (162).

A novel form of resistance exercise training using flywheel technology has been tested for its efficacy as a countermeasure to offset the loss of musculoskeletal mass during hindlimb suspension (56). A 3-d per week exercise training regimen using the flywheel apparatus significantly increased protein synthesis rates and attenuated disuse-induced loss of soleus muscle mass in adult rats (56). Therefore, maintenance of protein synthesis rates could be one mechanism whereby resistance training attenuates skeletal muscle atrophy following unweighting. However, the anabolic response to a single bout of contraction in aged animals is less than that seen in adult animals (163). In addition, an electric stimulation-based isometric resistance exercise performed during the initial stages of unloading failed to counteract skeletal muscle (gastrocnemius) atrophy; in that study, isometric resistance training was not sufficient to activate essential components of the synthetic machinery, but could fully blunt increases in mRNAs of genes such as MAFbx/Atrogin-1 and MuRF1 (103).

Furthermore, a flywheel-based resistance exercise regimen performed during hindlimb suspension has been reported to induce significant decreases in MAFbx/Atrogin-1 and MuRF1 mRNAs in atrophied muscles of rats, with no effect on Nedd4 or components of the 20S proteasome (99). Because unloading-induced activation of NF-κB can be reversed by 10 min of fatiguing exercise (124), we may infer that NF-κB is one of the underlying mechanisms of the effects of exercise.

Several models of resistance training devices using the flywheel ergometry principle have been developed for human use on the International Space Station or on space shuttles traveling on long-duration missions to other planets (164–167). Resistance exercise composed of maximal concentric and eccentric actions and performed every third day during middle- (166) and long-term (168) bed rest could prevent atrophy of knee extensors (quadriceps muscle) and attenuate atrophy of plantar flexors (triceps surae muscle group). Furthermore, unilateral knee extension resistance exercise performed during unilateral lower limb unloading was capable not only of offsetting atrophy but promoting marked hypertrophy of chronically unloaded muscles (169). In another study, a 2-mo bed rest with 10 h per day wearing an antigravity, elastic device that provided a modest but continuous resistance at the leg muscles was shown to prevent decreases in muscle fiber size and myonuclear domain (170). In addition, leg resistance exercise carried out every other day throughout a 2-wk bed rest challenge could counteract the decrease in muscle protein synthesis observed during strict inactivity (171), probably through blunting total RNA and mRNA deficits and improving IGF-1 signaling (172). Additional research is needed to ascertain the specific molecular determinants of human skeletal muscle plasticity during loading changes.

5.2 Dietary supplementation

Beyond its motile function, skeletal muscle in mammals serves as a protein reservoir that is mobilized in stressful states as a source of amino acids for energy metabolism (173). Therefore, muscle remodeling associated with disuse atrophy may imply decreased synthetic activity and increased recruitment of the pro-
Effects were in part accredited to enhanced positive increased non-oxidative leucine disappearance (i.e., vented increases in whole-body leucine oxidation, and kg body weight/d) improved nitrogen balance, pre-

these conflicting results.

could prevent loss of lean leg mass and stimulate net tion, essential amino acid and carbohydrate supple-

the overall musculature during weightlessness in rats protein diet is unlikely to have any beneficial effect on

thesis nor prevented the reduction in the fast-twitch tib-

despite of inducing increased protein synthesis rates, a high-protein (30%) diet had no beneficial effect in preventing soleus muscle atrophy in unloaded rats (177). In addition, the same diet neither sustained protein synthesis nor prevented the reduction in the fast-twitch tibialis anterior muscle growth, indicating that a high-protein diet is unlikely to have any beneficial effect on the overall musculature during weightlessness in rats (54). More research is required to clarify the reasons for these conflicting results.

In humans, increased dietary amount of protein (1 g/kg body weight/d) improved nitrogen balance, prevented increases in whole-body leucine oxidation, and increased non-oxidative leucine disappearance (i.e., increased protein synthesis) during bed rest, and these effects were in part accredited to enhanced positive action of insulin on protein metabolism (62). In addition, essential amino acid and carbohydrate supple-

during prolonged inactivity (28 d of bed rest) could prevent loss of lean leg mass and stimulate net muscle protein synthesis (178, 179). Moreover, dietary intake supplementation with 30 mmol/d each of the three branched-chain amino acids (BCAAs) during bed rest has been reported to improve nitrogen retention by increasing the accretion of amino acids in the tissue free amino acid pool, however with no significant influence on protein kinetics (60). Increased concentrations of skeletal muscle free BCAAs observed during unloading (42) and plasma BCAAs seen during exposure to micro-

gravity (180) suggest a protein catabolic state where protein degradation exceeds protein synthesis. Therefore, activation of the muscle synthetic machinery through intake of appropriate amounts of protein and carbohydrate, supplemented with essential amino acids such as leucine, one of the BCAAs with the strongest protein anabolic effect (181, 182), may be helpful for preventing disuse-induced skeletal muscle atrophy. Because the concentration of plasma BCAAs decreases significantly in the early-recovery phase from spaceflight (180), indicating deactivation of proteolytic pro-

cesses and reactivation of the protein synthetic machin-
ery, BCAA supplementation may be of particular help just before and immediately after landing.

BCAAs, especially leucine, function as nutritional signaling molecules in skeletal muscle. Translational control of protein synthesis (i.e., phosphorylation of 4E-BP1 and S6K1) in rat skeletal muscle by high doses of leucine administered by oral gavage ceases within 2 h (183, 184) and this transient effect may be sustained for longer periods when other essential amino acids are abundantly available (185). In addition, because the first two steps in BCAA catabolism are common to the three BCAAs (186) and are upregulated by increased plasma leucine concentrations (187), resulting in abnormally low levels of circulating iso-leucine and valine (183, 187), supplementation with leucine alone with the aim of preventing muscle protein loss during inactivity is not recommended. Continuous infusion of 10% Travasol, which contains the 9 essential plus 6 nonessential amino acids, has been reported to result in increased rates of mixed muscle protein synthesis in humans (188). Furthermore, infusion of essential amino acids suppresses muscle protein breakdown in hindlimb-immobilized rats (185). BCAA may be partic-

ularly beneficial for activating the protein synthetic machinery in skeletal muscle when supplemented before and after exercise (189, 190).

6) Perspectives

Perhaps, during space missions, there is nothing more discomforting for astronauts (cosmonauts) than seeing their physical capacity dwindling. Even short-duration spaceflight can result in significant muscle atrophy (191) and changes in myosin heavy chain iso-

form expression (192) in humans. Prevention of myo-

fibrillar protein wasting, which occurs as a consequence of muscle disuse, is crucial for the successful accomplishment of space-related missions and for a faster recovery after returning from space. This involves countermeasure actions such as periodic loading (e.g., flywheel-based resistance exercise) and dietary supple-

mentation intended to keep protein metabolic processes near basal levels. Supplementation with BCAAs, which may elicit several positive effects other than those men-
tioned above (193–196), and addition of anabolic agents (e.g., clenbuterol, growth hormone) and pro-
tease inhibitors such as Bowman-Birk (87) in the diet may be helpful. However, loaded contractions performed on a regular basis and improved heat and/or CO2 dissipation after exercise (35) should not be neglected.

Future investigations related to disuse-induced mus-

cle atrophy are supposed to focus on the molecular mechanisms that regulate protein kinetics in humans. In addition, further research is needed to make clear the precise pathways and transcription factors that regulate intracellular adaptations to loading changes. Finally, more experimentation is needed on the effects of different exercise protocols and diet regimens, as well as specific supplements, during unloading.

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