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REVIEW

Muscle Wasting: Cellular and Molecular Mechanisms

Disuse-induced skeletal muscle atrophy in disease and nondisease states in humans: mechanisms, prevention, and recovery strategies

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Abstract

Decreased skeletal muscle contractile activity (disuse) or unloading leads to muscle mass loss, also known as muscle atrophy. The balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) is the primary determinant of skeletal muscle mass. A reduced mechanical load on skeletal muscle is one of the main external factors leading to muscle atrophy. However, endocrine and inflammatory factors can act synergistically in catabolic states, amplifying the atrophy process and accelerating its progression. In addition, older individuals display aging-induced anabolic resistance, which can predispose this population to more pronounced effects when exposed to periods of reduced physical activity or mechanical unloading. Different cellular mechanisms contribute to the regulation of muscle protein balance during skeletal muscle atrophy. This review summarizes the effects of muscle disuse on muscle protein balance and the molecular mechanisms involved in muscle atrophy in the absence or presence of disease. Finally, a discussion of the current literature describing efficient strategies to prevent or improve the recovery from muscle atrophy is also presented.

atrophy; disuse; muscle wasting; protein turnover; sarcopenia

Skeletal muscle serves a fundamental role in maintaining good health. Unfortunately, disuse-induced skeletal muscle atrophy can occur throughout the human lifespan. Complex cellular and molecular processes underpin disuse-induced muscle atrophy, and these processes differ substantially amid disease, nondisease, and aging states. A brief Ovid MEDLINE search (April 2022) for "disuse atrophy" yields 116 review articles, and the results are further limited to 39, 16, and 21 review articles when combined with "mechanisms," "prevention," and "recovery," respectively. What is noteworthy is that only two review articles (1, 2) were retrieved when all four search terms were combined. Still, both reviews focused on the role of nutritional interventions to counteract muscle atrophy, with a special reference to protein and amino acids. Herein, we discuss the alterations in protein metabolism and the underlying cellular and molecular mechanisms governing and strategies to mitigate and recover from disuse-induced atrophy in disease and nondisease states throughout the lifespan.

INTRODUCTION

Skeletal muscle is a highly dynamic tissue that responds to different stimuli, especially to changes in mechanical load. Consequently, increased use or disuse directly impacts skeletal muscle phenotype, affecting metabolism, protein expression, and morphological characteristics (3–5). Disuse describes a state of different periods of decreased or absence of physical activity (4). Most people would reduce physical activity because of pain or disabilities caused by chronic diseases, enforced lockdowns (e.g., stay-at-home calls due to the COVID-19 pandemic), other infections forcing bed rest, or decreased ambulatory activity (6–9). Furthermore, diseases affecting mental health (e.g., depression), hospitalizations, recovery from major injuries, bone fractures, or limb immobilizations are common causes of reduced overall physical activity leading to muscle atrophy (6–8). The decrement of mechanical load due to exposure to microgravity (10), reduced ambulation (5), or the incapacity to move a limb for days will induce an adaptive response in skeletal muscle leading to muscle atrophy (11). In addition, when facing trauma, infections, or diseases characterized by inflammatory responses, releasing a set of systemic mediators can amplify the effects of disuse in the skeletal muscle and accelerate muscle atrophy (8, 9, 12, 13).

Skeletal muscle atrophy plays a significant role in several aspects of human health and quality of life. Muscle atrophy is often associated with reduced quality of life, reduced

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mobility in general, and decreased individual independence (12, 14). Furthermore, the decrement in muscle mass due to muscle atrophy is linked to a higher prevalence of several chronic diseases (e.g., type 2 diabetes mellitus, cardiovascular diseases, and depression) and even higher mortality (4, 13, 15). Consequently, understanding and preventing muscle atrophy has a critical role in human health. Skeletal muscle atrophy occurs via a coordinated dismantling of the myofibrillar protein lattice and the loss of organelles and cytoplasmic proteins (16–18). Many chronic diseases that include muscle loss as a characteristic feature are associated with a significant increase in serum cytokines and cortisol, which are lower in "simple" or uncomplicated models of muscle disuse (i.e., not confounded by an underlying illness). As such, the molecular mechanisms regulating atrophy in diseased versus inactive (but otherwise healthy) skeletal muscle are, we argue, fundamentally distinct (3, 4, 9, 18).

This review aims to characterize and discuss the etiology of the main changes in muscle protein metabolism and mechanisms driving skeletal muscle atrophy. Our goal is to highlight some of the salient differences between the atrophy in inactivity versus inactivity associated with or enforced by disease and discuss strategies to prevent and recover from muscle atrophy.

DISUSE AND DISEASE INFLUENCE MUSCLE MASS AND PROTEIN TURNOVER

Muscular disuse is the primary process leading to muscle atrophy in healthy subjects (5, 19) and can range from abrupt and absolute to relative. Research models of disuse in humans include step reduction (11), limb immobilization (20), bed rest (21), and microgravity (10). Skeletal muscle mass is mainly dictated by the balance between two processes: muscle protein breakdown (MPB) and muscle protein synthesis (MPS). Both processes occur continuously and concomitantly (4, 22). In general, MPB and MPS maintain a state of balanced remodeling of proteins in the muscle tissue. MPB works with different systems with proteolytic activity to break down proteins and release peptides and amino acids into the intracellular pool of the muscle (23). However, in healthy individuals, MPB is generally offset by MPS, mainly in response to ingestion of protein meals, leading to hyperaminoacidemia and mechanical loading stress on skeletal muscle (4, 18, 23). During MPS, cellular protein synthetic machinery uses amino acids and energy (i.e., ATP) to synthesize new proteins that can be secreted to the extracellular space or incorporated into different cellular structures as contractile skeletal muscle proteins (23). Ingestion of a meal containing protein, a source of essential amino acids, stimulates a transitory increase in MPS and decrement in MPB, resulting in a positive state of muscle protein balance (24). Alternatively, resistance exercise (RE)-induced loading increases both MPB and MPS, but when combined with a diet of sufficient high-quality protein, the increment in MPS results in a net positive state leading to an increase in skeletal muscle protein mass in the long term (22, 25). Nevertheless, a chronic state of negative protein balance will lead to skeletal muscle atrophy (26–28).

Uncomplicated Disuse-Induced Changes in Muscle Protein Turnover

It has been recognized that decreased MPS is the primary driving process leading to loss of muscle protein over time in disuse muscle atrophy in humans (4, 15, 26, 27, 29) (Fig. 1). Declines of 50%–60% in both fasting (hypoaminoacidemia) and fed (hyperaminoacidemia) MPS during unloading support the theses that elevated MPB, and "bulk" proteolysis has little, if any, contribution to the decrement in muscle mass observed during simple muscle disuse (29). The decline in MPS during unloading has been investigated and confirmed in many human trials (20, 30–33). Early studies showed a 30% decrease in MPS in young men (19–57 yr) during fasting state who had their leg immobilized compared with their contralateral nonimmobilized limb (34, 35). Notably, other groups have shown reductions of up to 50%–60% in fasting and fed MPS (30, 33, 36, 37). The MPS response to feeding is one of the main determinants of muscle protein balance (23). However, disuse promotes an "anabolic resistance" to the feeding stimulus during feeding-induced hyperaminoacidemia (33) or intravenous infusion of essential amino acids (30). The reduction in fasting and fed MPS can quantitatively account for the observed muscle mass loss in experimental studies in humans (29). If MPB contributes substantively to muscle atrophy during disuse in healthy subjects, it remains to be shown. Nevertheless, the few studies measuring MPB during muscle unloading seem to show no significant increments in MPB (4, 29, 38).

Disease-Induced Changes in Muscle Protein Turnover

Contrasting with periods of muscle disuse in the absence of disease, both a diminished MPS and an elevated MPB can contribute to muscle atrophy during infections and disease states characterized by an inflammatory burden (9, 11, 18, 28, 39) (Fig. 1). The muscle wasting that occurs in cancer cachexia, sepsis, burns, or critical illness, is almost certainly the result of muscle disuse in parallel to enhanced proteolysis brought on by one or a combination of undernutrition, increased systemic catabolic hormones, and inflammatory mediators (18, 40–42). For instance, it is known that high levels of proinflammatory cytokines [i.e., interleukin-1-β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)] can increase MPB and suppress MPS (39, 43, 44). Accordingly, elevated concentrations of systemic inflammatory markers during clinical illness are associated with lower muscle mass (39, 45). Furthermore, high levels of proinflammatory cytokines have also been shown to negatively impact other cellular systems contributing to protein turnover and muscle remodeling, such as ribosomal biogenesis (46). As a result, inflammatory disease states induce far more rapid and aggressive muscle atrophy, often highly correlated with morbidity and mortality (9, 47).

Aging-Induced Changes in Muscle Protein Turnover

Aging is an additional feature that can influence skeletal muscle protein balance and increase susceptibility to muscle atrophy (23, 41, 48) (Fig. 1). Typically, older compared with younger persons require ~1.5–2-times the amount of high-quality protein to maximally stimulate MPS (49–51). Such observation sparked research on aging-induced anabolic

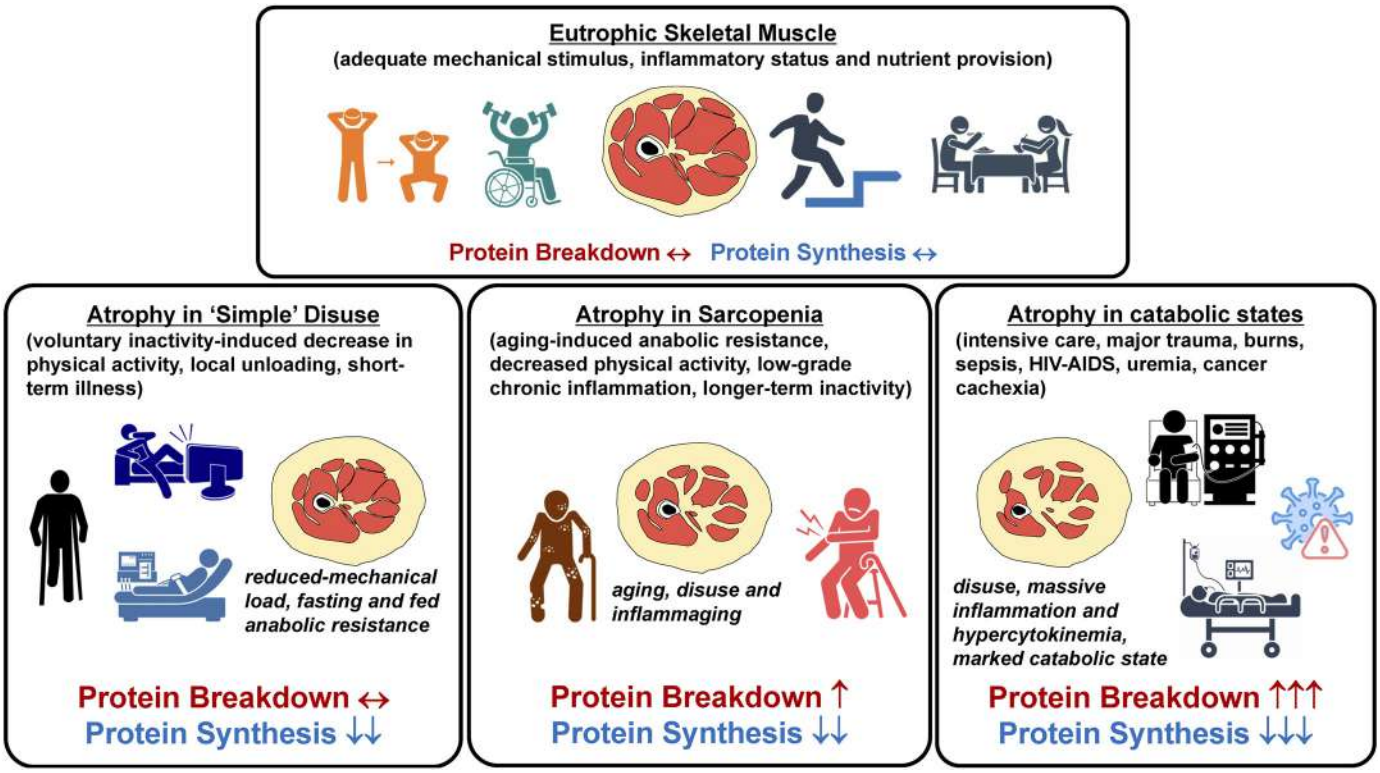

Figure 1. Muscle protein breakdown and synthesis in distinct atrophy scenarios.

resistance (23, 41, 49). The age-associated increase in inflammatory mediators (a condition also known as inflammaging) is one of the many mechanisms currently investigated in aging and skeletal muscle health that could be playing a role in age-related sarcopenia (52, 53). Sarcopenia affects mainly older adults and is also characterized by anabolic resistance in response to the normally robust stimulating effects of amino acids and RE on MPS (7, 54). However, muscle loss, or slow-atrophy, resulting from sarcopenia is likely multifactorial and results from poor dietary quality, decreased physical activity, chronic imbalances between anabolic and catabolic hormones, and increased concentration of proinflammatory cytokines and hormones (5, 7), all of which negatively influence muscle protein balance and contribute to the loss of muscle mass in sarcopenia (7).

MECHANISMS INVOLVED IN MUSCLE LOSS

Muscle loss assuredly occurs when the ability to generate new muscle – muscle protein synthesis (MPS) – and other cellular generative processes are impaired compared with the rate of muscle protein breakdown (MPB). An important question is which process, MPS or MPB, is impaired to the greater degree and, as a result, is the best candidate for treatment?

Proteolysis in Skeletal Muscle

The ubiquitin-proteasome system (UPS) degrades most cellular proteins (55). Degradation by this system begins with the ATP-dependent activation of ubiquitin by a single ubiquitin-activating enzyme (E1), which is subsequently transferred to one of several ubiquitin carrier proteins (E2) (56). Finally, E3 ubiquitin ligase enzymes catalyze the transfer of ubiquitin to the lysine residue of the target protein (56). Subsequent iterations of this cycle result in the polyubiquitination of target proteins, enabling their interaction with, and subsequent degradation by, the 26S proteasome. An estimated 500–1,000 E3 ligases exist in humans (57); however, research attention has primarily focused on MuRF1 (muscle RING-finger protein-1) and atrogin-1 because they are muscle-specific and consistently expressed in several preclinical models of muscle atrophy (58). Nonetheless, this myopic approach has hindered our understanding of muscle atrophy in contexts devoid of perturbations of these two E3 ligases.

Despite its centrality in proteostasis, the UPS does not degrade intact myofibrillar proteins directly (59), implying that a preceding step is required for their initial release from the structured lattice. Caspases are activated by proapoptotic stimuli and cleave actomyosin complexes in vitro, which significantly accelerates ATP-dependent proteolysis (60). Furthermore, the Ca^{2+}-dependent calpains are also important for the initial liberation of myofibrillar proteins because they degrade proteins that maintain the structural integrity of the sarcomere, including nebulin, titin, α-actinin, and desmin (61).

Finally, the lysosomal-autophagy system is responsible for degrading damaged organelles (i.e., ribosomes, peroxisomes, and mitochondria), long-lived proteins, and protein aggregates (62). Macroautophagy (hereafter referred to as autophagy) begins with the formation and nucleation of an isolation membrane, followed by its elongation and closure

to form an autophagosome (62). The autophagosome fuses with the lysosome and cathepsin proteases degrade the engulfed protein cargo within the acidic lysosomal lumen. Selective breakdown of organelles, including the mitochondria (i.e., mitophagy), is accomplished by specific adaptor proteins that interact with the autophagy machinery and molecules on the damaged organelle (62).

Mechanisms of Muscle Loss in Disease States

Muscle wasting is a hallmark of many diseases, including sepsis, chronic kidney disease (CKD), diabetes, and cancer. Most, if not all, of these conditions are characterized by significant elevations of proinflammatory cytokines (i.e., TNF-α, IL-1β, and IL-6) (63) and glucocorticoids (i.e., cortisol) that accelerate proteolysis through the activation of select transcription factors including signal transducers and activators of transcription-3 (STAT3), small mothers against decapentaplegic 2/3 (Smad2/3), nuclear factor-κ-B (NF-κB), and Forkhead box transcription factors (FoxO) (63, 64). Plasma-derived from patients with septic shock, with IL-6 concentrations ~50-fold greater than in healthy controls, significantly increases NF-κB activity, MuRF1, and atrogin-1 expression, and ubiquitinated myosin in myotubes (63). In addition, patients with chronic kidney disease have suppressed skeletal muscle Akt activity that is ostensibly driven by activating the IL6-JAK (Janus kinase)-STAT3 signaling axis, leading to increased intramuscular myostatin and suppressor of cytokine signaling 1 and 3 (SOCS1 and SOCS3) levels (64, 65). SOCS1 and SOCS3 have been shown to downregulate the activity of the phosphoinositide 3-kinase (PI3K)-Akt pathway contributing to muscle wasting (65, 66). This series of events coincide with the nuclear translocation of FoxO transcription factors and the induction of MuRF1, atrogin-1, and several FoxO-sensitive autophagy genes (Fig. 2).

Glucocorticoids also increase catabolic drive via similar downstream effectors; however, they operate through distinct upstream mechanisms. Upon receptor binding, glucocorticoids activate the transcription of myostatin, FoxO, REDD1 (regulated in development and DNA damage responses 1), and Kruppel like factor 15 (KLF15) genes, among others (67). REDD1 and KLF15 reduce mTOR activity by increasing the activity of tuberous sclerosis complex 1/2 (TSC1/2) and branched-chain amino acid transaminase 2 (BCAT2)-mediated amino acid catabolism (67), respectively. In addition, KLF15 increases the expression of MuRF1 and atrogin-1 cooperatively with FoxO transcription factors (67) (Fig. 2). Finally, the accumulation of ubiquitinated myofibrillar proteins is accompanied by a corresponding increase in 20S proteasomal subunit activity in critically ill patients (68). The UPS is robustly activated and important for the loss of muscle protein in many disease states (68).

Diseased skeletal muscle is also characterized by elevated calpain and caspase gene expression and activity. Indeed, the expression of calpain-1, -2, and -10 genes is increased in mechanically ventilated patients (69), and activity is increased by ~70% in muscle extracts taken from patients with gastric cancer (70). The disease-state-induced activation of calpains is probably caused by impaired calcium handling since Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which is activated by calcium, is increased in, for example, skeletal muscle of patients with gastric cancer and correlates with the degree of wasting observed (71). In addition, there is evidence of increased apoptosis and caspase activity in mechanically ventilated diaphragm muscle (72), quadriceps samples from end-stage renal failure (73), and patients with chronic obstructive pulmonary disease (COPD) (74). Although certainly context-dependent, the activation of caspase enzymes may result from an imbalance in the cellular redox state, the release of proapoptotic factors from mitochondria, reduced PI3K-Akt signaling, or proinflammatory cytokine signaling, suggesting that similar antecedents modulate caspase and UPS activation likely in several diseases.

The role of autophagy in disease-induced muscle wasting is unclear. Tardif and colleagues (75) demonstrated that diaphragm and vastus lateralis muscle samples obtained from patients with esophageal cancer have an increased microtubule-associated protein light chain 3 (LC3)II/LC3I ratio indicative of autophagosome formation without apparent activation of other proteolytic systems. The same patients also have elevated cathepsin B activity compared with controls (75). The extent of induction is greater in patients with cachectic versus noncachectic cancer, suggesting that autophagy plays a general role in the wasting phenotype (76) Autophagy-related gene expression is also activated in skeletal muscle of patients with CKD (77) and COPD (78), which is likely driven by increased FoxO3 activity and/or reduced Akt-mTORC1 (mechanistic target of rapamycin complex 1) activity (77, 78). What is difficult to discern from these studies is whether autophagy flux is increased or whether autophagosome clearance is impaired, leading to an accumulation of the proteins mentioned above (76, 79). However, given the increase in energy requirements associated with disease (80), combined with reduced appetite (81, 82), it is more likely that autophagy is chronically upregulated to match the demand for nutrients imposed by the disease.

It is important to appreciate that diseases are distinct in their etiology and the severity of clinical features; therefore, the relative contribution of proteolytic and protein synthetic pathways to muscle atrophy will be context-dependent. However, it is clear that the disease-associated dysregulation of systemic hormones and cytokines, combined with inactivity and malnutrition, accelerates proteolysis (83–85) and, although not consistently (85), impairs protein synthesis (86, 87), favoring the net efflux of amino acids from skeletal muscle (85).

Mechanisms of Muscle Loss during Uncomplicated Disuse Atrophy

In contrast with disease states, uncomplicated (simple) muscle disuse (i.e., disuse in the absence of disease) does not appreciably alter systemic catabolic hormones (36, 88). The catabolic drive initiated by these factors is also absent, which may explain the lack of induction of MPB in healthy humans subjected only to muscle unloading (38). Indeed, the acute infusion of counter-regulatory hormones (epinephrine, glucagon, and cortisol) to mimic a stressed physiological state is sufficient to induce a 65% increase in leg muscle protein breakdown in healthy subjects (89). Furthermore, inactivity in otherwise healthy adults sensitizes skeletal muscle to the catabolic effects of hypercortisolemia, leading to significantly

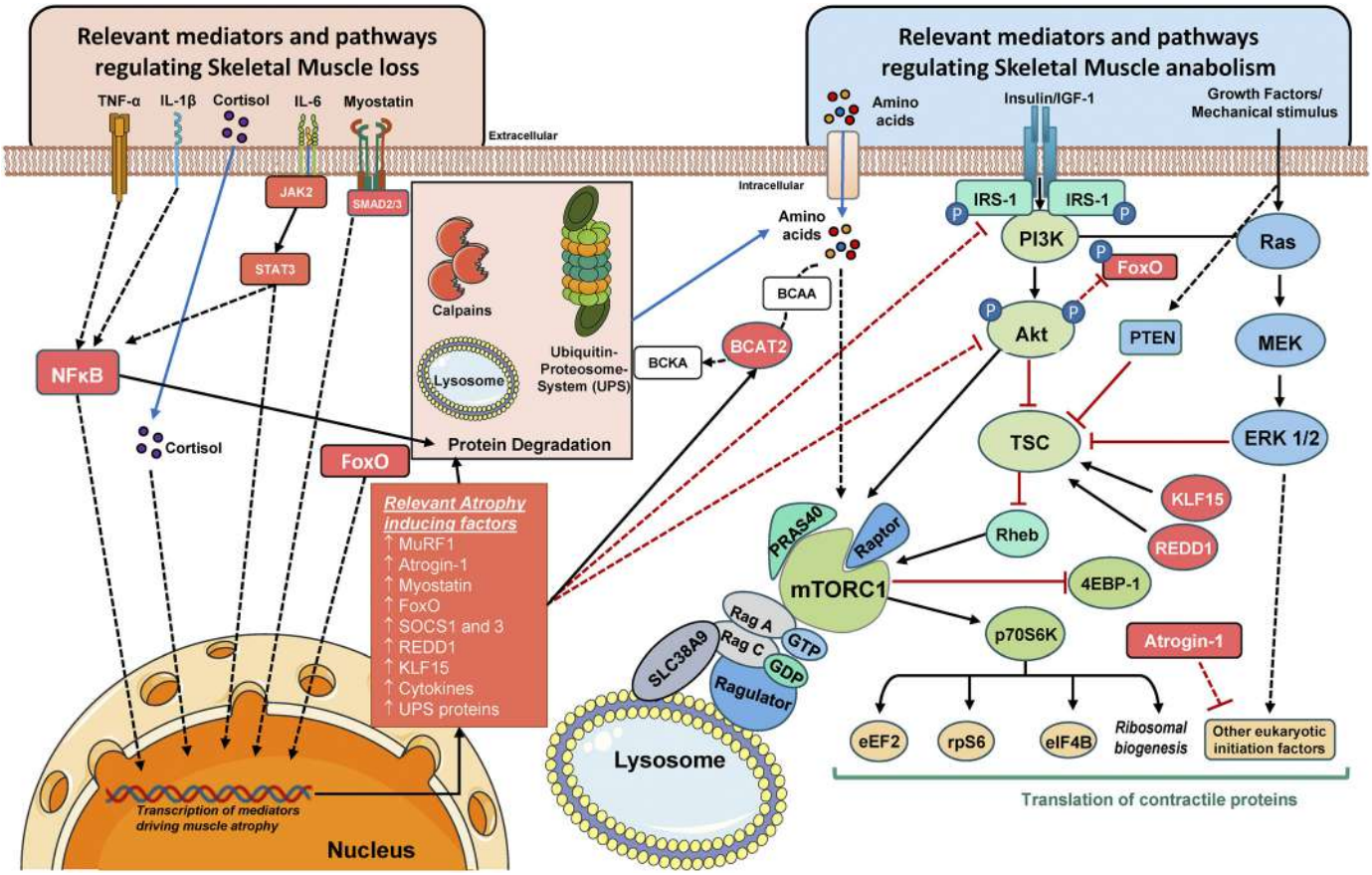

Figure 2. Summary of the main mediators and mechanisms regulating skeletal muscle loss (*left*) and anabolism (*right*). Mechanical stimulus, growth factors, and amino acids play a positive role in stimulating pathways contributing to maintaining anabolic tonus in skeletal muscle. During disuse, the lack of mechanical stimulus activated-pathways is sufficient to cause muscle atrophy. Still, muscle atrophy can be amplified by further inhibition of anabolic pathways and increment of the activity of catabolic pathways cause by various molecular mechanisms. 4EBP-1, eukaryotic translation initiation factor 4E-binding protein 1; Akt (PKB), protein kinase B; BCAA, branched-chain amino acids, BCKA, branched-chain keto-acids; eEF2, eukaryotic elongation factor 2; eIF4B, eukaryotic translation initiation factor 4B; ERK 1/2, extracellular signal-related kinase1/2; FoxO, Forkhead box transcription factors; IGF-1, insulin-like growth factor 1; IL, interleukin; JAK, Janus kinase; mTORC1, mechanistic target of rapamycin complex 1; MAPK, mitogen-activated protein kinase; myogenin; MEK, mitogen-activated protein kinase; MuRF-1, muscle RING-finger protein-1; NF-κB, nuclear factor-κ-B; p70S6K, p70 S6 kinase 1; PI3K, phosphoinositide 3-kinase; PRAS40, proline-rich AKT substrate 40 kDa; PTEN, phosphatase and tensin homolog; Raptor, regulatory-associated protein of mTOR; REDD1, regulated in development and DNA damage responses 1; Rheb, Ras homolog enriched in brain; rpS6, ribosomal Protein S6; SOCS3, Suppressor of Cytokine Signaling 3; SMAD, Small Mothers Against Decapentaplegic; STAT, signal transducers and activators of transcription; TNFα, tumor necrosis factor-α; TSC, tuberous sclerosis complex.

elevated MPB (90). These observations suggest that in the absence of systemic hormonal or cytokine-mediated dysregulation, muscle disuse atrophy is governed by distinct mechanisms from those described in patients with chronic disease.

Evidence of UPS activation during muscle disuse seems to depend on the timeline of the atrophy process. Our laboratory has observed an increase of ubiquitinated protein conjugates in vastus lateralis biopsy samples following 2 days of immobilization in humans (91). Although many studies have demonstrated either no change or even a decrease in the expression of MuRF1 or atrogin-1 following unloading periods lasting 14 days or longer, others have shown a transient induction of these atrogenes (92, 93) and their upstream transcriptional regulators, FoxO and NF-κB (94–97). However, in each case, the induction of gene expression or degree of ubiquitin conjugation is minimal compared with what is observed, for example, in critically ill patients (72). Thus, rather than orchestrating bulk protein breakdown per se, the early induction of atrogenes such as atrogin-1 may, we postulate, impair cellular protein synthetic capacity through, for example, the ubiquitination of translation initiation components (98, 99). This hypothesis is consistent with the rapid reduction of MPS observed within 2 days of unloading (97). In fact, seminal work from in preclinical model shows a significant decrease in contractile protein synthesis in the gastrocnemius muscle as soon as 6 h after hindlimb immobilization (100).

Calpain and caspase systems are also inconsistently regulated in unloaded skeletal muscle. Indeed, there are data demonstrating reduced (101, 102), unchanged (96), or increased (97, 103) expression of calpains in response to muscle disuse. Calpains may be regulated through non-transcriptional mechanisms, including posttranslational modifications of substrates leading to enhanced cleavage activity. Consistent with this view, a recent series of preclinical studies demonstrated that desmin phosphorylation by glycogen synthase kinase (GSK)-3β and subsequent

ubiquitination by tripartite motif-containing-32 (TRIM32) are important for calpain-1-mediated desmin cleavage following denervation (61, 104). In humans, there is some evidence of enhanced GSK-3β activity following 3 days of unloading (96) and transcriptional upregulation of TRIM32 following bed rest (105). Future research should probe this interaction further; however, we contend that beyond an initial role in muscle remodeling, calpains do not play a significant role in unloading-induced atrophy in situations where calcium homeostasis is maintained. Likewise, beyond a minor increase following short-term immobilization (97), caspases are unlikely to play a significant role in muscle atrophy without proapoptotic signaling factors (i.e., elevated cytokines, oxidative stress, etc.). Although antioxidant defenses are seemingly reduced during unloading in humans (106), we could not detect any evidence of oxidative stress in young adults responding to immobilization (91).

Finally, no substantial evidence suggests that autophagy contributes significantly to muscle atrophy during disuse. Beclin1 is induced in response to short-term immobilization (97) and chronic bed rest (106), indicating that autophagosomes are being formed; however, the data are also consistent with an inhibition of autophagy. Indeed, there have been reports of the adaptor protein p62 increasing in response to disuse (107). Because p62 is degraded along with the cellular cargo during lysosomal organellar disassembly and proteolysis, its accumulation can be interpreted as induction of autophagy or a lack of autophagosome turnover (79). Consistent with the latter view, cathepsin L expression was unchanged following 24 wk of bed rest when beclin1 and p62 were increased (106), suggesting that protease activity remained unchanged. Similar interpretations can be made regarding mitophagy induction in response to inactivity; although Bnip3L is increased at the gene and protein level, it is also accompanied by an increase in p62 (107). It is difficult to conclude with confidence whether or not muscle disuse activates autophagy in the absence of data that quantifies the flux of autophagosomes rather than the abundance of its constituent proteins.

Reduced MPS as the Primary Driver of Skeletal Muscle Disuse Atrophy

Our discussion above clearly shows that an increase in MPB is not a primary determinant of muscle in simple disuse atrophy in humans. An overwhelming body of evidence has demonstrated reductions in postabsorptive (30, 31, 34, 36, 38, 108) and postprandial (30, 33, 109) MPS within days of disuse that together can easily explain the observed loss of muscle mass in the absence of any change in MPB (26, 31, 34). Furthermore, we and others have shown strong relationships between disuse-induced reductions in MPS and muscle atrophy by using integrated methods to determine daily protein synthetic rates that factor in fed and fasted states (97, 110). Despite the growing body of evidence implicating reduced MPS in disuse-induced muscle atrophy, the underlying mechanisms are unclear but could, we speculate, be related to reduced translational capacity and efficiency. Muscle RNA content (a reflection of translational capacity) is unchanged in humans (34) and rodents (111) in the early stages of response to muscle disuse, implicating translational efficiency as the initial primary defect. mTORC1 is considered a key regulator of translational efficiency because it regulates the rate-limiting step of protein synthesis — translation initiation; however, impaired mTORC1 signaling is not always observed following disuse (30, 33, 94, 97, 109). A lack of perturbation in mTORC1 signaling in response to muscle inactivity may be due to biopsy sampling time points; however, other pathways may also be operative. One example, consistent with the mitochondrial-dominated transcriptional signature of unloaded muscle (112), is the inter- and intramuscular accumulation of lipid species. Indeed, Manini and colleagues (113) demonstrated an increase in intermuscular adipose tissue following 4 wk of lower limb suspension associated with the loss of muscle volume observed (113). Direct lipid infusion has also been shown to blunt the mixed MPS response to insulin and amino acid administration without obvious mTORC1 dysregulation (114). These data (114) suggest a mTORC1-independent influence of lipids on translational signaling. A problem with this hypothesis, however, is that intramuscular triglyceride concentrations are unchanged following 5 days of limb immobilization (115) and 7 days of bed rest (116) — time periods in which anabolic resistance has already been established. These discrepancies may be resolved if we consider the possibility that individual lipid species, such as diacylglycerol (DAG) and/or ceramides, rather than bulk lipid accumulation per se, impair anabolic signaling. Lipin1, which converts phosphatidic acid to DAG, has been shown to increase after 24 h of hindlimb unloading in rodents (117) and in response to 24 and 48 h of lower limb immobilization in humans (112, 117). Likewise, ceramides are elevated by bed rest in humans (118). DAGs and ceramides are considered key regulators of lipid-induced insulin resistance (119), but recent data also suggest a role for these molecules in the development of anabolic resistance via mTORC-1 independent mechanisms.

In a rodent model of lipid-induced obesity, ceramides accumulate within the muscle cell and are associated with a reduced anabolic response to nutrition (120). Mechanistically, these effects were shown in C2C12 myotubes to be mediated specifically by ceramides and operate through the increased inhibitory phosphorylation of eukaryotic initiation factor 2 (eIF2)-α not mTORC1 (120). This signaling axis is interesting for several reasons. First, eIF2-α phosphorylation on serine 51 impairs global protein synthesis by preventing the formation of the ternary complex necessary for translation initiation (121). Second, although eIF2-α phosphorylation downregulates the synthesis of most cellular proteins, it increases the synthesis of activating transcription factor 4 (ATF4). ATF4 upregulates, among other molecules, Sestrin2, (122), which is considered the primary sensor of intracellular leucine (123). Conceivably, an elevation in Sestrin2 would increase the "leucine threshold" required to stimulate mTORC1 and thus could play a role in anabolic resistance. These hypotheses are speculative in nature and based on limited preclinical data but deserve future research attention given our lack of understanding of the underlying molecular processes mediating reductions in MPS in response to anabolic stimuli.

Transcriptomic profiling of skeletal muscle has consistently revealed downregulation of mitochondrial transcripts in response to muscle disuse (112). Mitochondrial and

cytoplasmic translational processes may be kinetically coordinated, which may be due to cytoplasm-located processes being the location of substantial ATP-consuming processes. Indeed, in preclinical models, the silencing of an important ribosomal subunit in the mitochondria leads to reduced translational efficiency via the transcription factor ATF4 (124). Our laboratory has demonstrated an increase in ATF4 gene expression and the expression of its downstream targets p53 and p21 after 3 days of immobilization in young women (22 ± 3 yr) (11). Preclinical data have shown that p53 and p21 are relevant in immobilization-induced muscle atrophy (125, 126). Noteworthy, expression of p21 in skeletal muscle fibers seems to be required for ATF4-mediated muscle fiber atrophy after immobilization in mice (125). Moreover, 48 h of unloading is associated with a coordinate downregulation of several genes encoding mitochondrial ribosomal subunits (112), many of which are reduced at the protein level after 21 days of bed rest (127). Thus, reductions in mitochondrial protein synthesis may precede and drive, or respond to, the reductions in myofibrillar protein synthesis in response to disuse, although future research is needed to address this hypothesis.

Mechanisms of Muscle Loss during Aging (Sarcopenia)

In contrast to disease and disuse, aging is associated with a much slower but progressive atrophying of skeletal muscle (48, 128) (Fig. 3). Because of the time course of muscle loss in aging, it is far more difficult to establish which process, MPS or MPB, is predominantly affected, and it may be that disease, whether overt or covert, as well as periodic inactivity, could be playing, at times, important roles in muscle loss. Reductions in daily physical activity (130, 131) and changes in the systemic concentration of hormones and proinflammatory cytokines that accompany the aging process are likely to contribute to age-related muscle loss (132, 133). However, an intrinsic characteristic of aging skeletal muscle that distinguishes it from disuse and disease is a loss of proteostasis. Indeed, whereas the body arguably responds appropriately to the altered demands imposed by disease and disuse through differential modulation of MPS and MPB, aging is associated with the accumulation of damaged or dysfunctional proteins and organelles that are not cleared (134, 135). This loss of proteostasis is ostensibly fiber-type specific; whereas type 1 fibers increase protein synthetic and UPS machinery components, and chaperone complexes, type 2 A fibers demonstrate a decrease in these proteins (136). These observations are consistent with the preferential atrophy of type 2 fibers with age (137).

Although the antecedent cause of myofiber atrophy in type 2 fibers in aging is unclear, it may be driven by an inability to generate sufficient ATP to sustain protein quality control mechanisms. Mitochondrial respiratory function (138), as well as gene (139) and protein abundance (136), are significantly reduced in older skeletal muscle. Type 1 fibers can compensate for these reductions to some extent by upregulating components of the glycolytic machinery, but these adaptations do not occur in type 2 fibers (136). In addition, the remaining mitochondria appear to be dysfunctional as indicated by an uncoupling of oxidative phosphorylation, greater ROS production relative to younger adults, increased permeability transition susceptibility, and increased release of proapoptotic factors (i.e., endonuclease G) (134, 140). Genes associated with mitochondrial biogenesis and mitophagy are also reduced in older adults, resulting in the accumulation of dysfunctional mitochondria and an exacerbation of the previously mentioned processes (141). Presumably, physical inactivity contributes to impairments in mitochondrial respiratory function with increasing age (142); however, in advanced age (>75 yr), it is thought that an accumulation of persistently denervated fibers, consequent to numerous failed denervation-reinnervation cycles, leads to further mitochondrial impairment (142) that may induce myofiber atrophy and loss through induction of apoptosis (143).

The stimulation of MPS in response to anabolic stimuli is also attenuated in older adults compared with younger adults (51, 144–148), despite similar basal protein synthesis rates (149). In regard to protein metabolism, greater splanchnic extraction of amino acids (150) and impaired insulin-mediated vasodilation (151) would theoretically reduce amino acid delivery to skeletal muscle and contribute to an attenuated anabolic response. However, intracellular concentrations of amino acids are not lower in older adults (152), implying that the primary defect is in the usage of amino acids by the muscle cell. The mTORC1 signaling axis

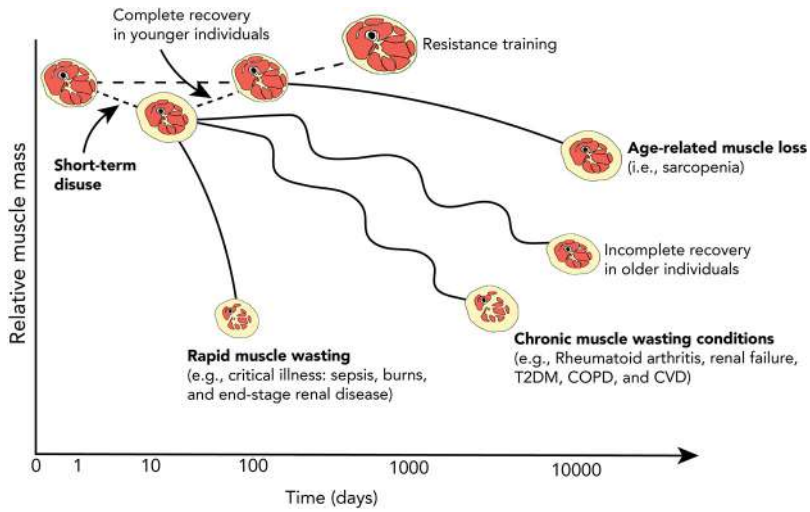

Figure 3. Graphic illustration of the timeline of skeletal muscle atrophy according to different underlying causes. Voluntary or involuntary decreased physical activity, anabolic resistance, and catabolic plus proinflammatory states are some proposed causes. CVD, cardiovascular diseases; COPD, chronic obstructive pulmonary disease; T2DM, type 2 diabetes. Nonlinear lines emphasize the role of periodic inactivity as accelerated periods of muscle loss in aging and disease states. Adapted from Little et al. (129).

primarily mediates the amino acid-induced activation of MPS. In the basal state, mTORC1 activity is elevated in older adults (60–87 yr) compared with younger adults (18–40 yr), and this dysregulation is thought to impair subsequent activation of the pathway in response to nutrition and exercise (149). These data imply that sustained activation of the mTORC1 signaling axis (i.e., an absence of normal fluctuations in signaling activity with fasting and feeding) leads to a global reduction in the sensitivity of the system to anabolic inputs. In preclinical models, dysregulated mTORC1 activity induces muscle atrophy (153, 154) and neuromuscular instability, suggesting a causative role in the development and progression of sarcopenia. Furthermore, in addition to impaired mTORC1 activation in response to protein ingestion, the insulin-mediated suppression of MPB is attenuated in older adults (155), likely as a result of impaired mitochondrial function and intramuscular lipid accumulation (156). Taken together, age-related muscle atrophy is a multifaceted process involving defects in cellular signaling leading to impaired activation of protein synthesis in response to anabolic stimuli.

STRATEGIES TO PREVENT OR ATTENUATE SKELETAL MUSCLE ATROPHY

Muscle contraction represents a potent anabolic stimulus that counters skeletal muscle atrophy. Briefly, mechanosensors, including costameres, focal adhesion kinases, and integrins, detect mechanical stimuli and activate intracellular signaling cascades that promote muscle anabolism, namely, mTORC1, mitogen-activated protein kinases, and Hippo signaling (157–160). The mechanisms linking mechanical stimuli to intracellular signaling remain to be precisely characterized; however, forceful muscle contractions, such as during resistance exercise training (RT), seem to attenuate atrophic losses. Oates et al. (161), for example, demonstrated that RT effectively prevents atrophy during 14 days of unilateral knee immobilization. Specifically, completing a low volume (1 set of 10 repetitions at 80% 1RM) of three lower body exercises every other day ablated the reductions in muscle fiber cross-sectional area (CSA), thigh thickness, and strength observed in a non-RT control group (161). Interestingly, RT attenuates muscle atrophy induced by substantially more harmful stimuli. Alkner et al. (162) subjected 17 healthy young (26–41 yr) adult males to 90 days of microgravity (6° head-down bed rest) with or without supine RT every third day. As expected, significant knee extensor atrophy (18%) and functional losses (31%–60%) were observed in subjects not performing resistance exercise (162). Impressively, there was no reduction in quadriceps volume in the RT group and functional declines were markedly reduced (162). Exercising before atrophy-inducing events or prehabilitation is an emerging area of investigation (163). Recently, four RT sessions before 5 days of bed rest did not diminish declines in quadriceps CSA and integrated MyoPS rates (164). Muscle contraction offsets atrophy, but further research is needed to develop effective prehabilitation strategies. Exercise, notably, is not feasible for many individuals contending with atrophy-inducing events; thus, alternative strategies should be explored.

Nutritional interventions are another method to reduce skeletal muscle atrophy. Protein nutrition has been the primary focus of atrophy-reducing nutritional strategies as amino acid provision supports increases in protein synthesis, and leucine directly activates mTORC1 (5, 27). Protein supplementation has been shown to reduce lean mass losses during disuse (20, 37, 165), but not uniformly (166). Hypo- and hypercaloric diets can also accelerate muscle loss, so energy-balanced diets should be considered (5). Biolo et al. (167), for example, found that a hypocaloric diet (80% of energy expenditure), compared with an energy-balanced diet, exacerbated protein catabolism and lean mass losses during 14 days of bed rest. Beyond optimal protein and energy consumption, specific nutritional compounds may also combat muscle atrophy. For example, omega-3 polyunsaturated fatty acids (n-3 PUFAs) (11) and creatine (168) have been shown to reduce quadricep CSA and lean mass losses, respectively, during limb immobilization. Muscle atrophy-related mitochondrial dysfunction may also be mitigated by n-3 PUFAs (169), and nutritional compounds containing polyphenols may effectively counter inflammation and oxidative stress damage (170), though evidence of oxidative stress-induced atrophy is lacking. Ursolic acid and tomatidine, found in foods such as apples and unripe tomatoes, respectively, are candidate molecules to inhibit skeletal muscle atrophy and targets for future nutraceutical development (171). Immense progress has been made, but further work is required to optimize nutritional interventions that mitigate skeletal muscle atrophy.

Skeletal muscle atrophy is a facet of several pathologies, and many concomitant attributes should be considered when mitigating muscle loss. Chronic systemic inflammation, for example, accompanies various cancers, and exercise at intensities lower than typical levels may be a possible treatment to increase muscle mass and physical function in patients with cancer (172). Oldervoll et al. (173) randomized 231 patients with cancer to complete 8 wk of circuit exercise or standard care. Notably, exercising patients improved their shuttle walk and handgrip strength performance, though disease progression prevented 43 individuals from completing the intervention (173). Furthermore, intensive care unit (ICU)-acquired weakness can be exacerbated by immobilization. However, mechanically ventilated ICU patients randomized to an exercise versus standard care group experienced a markedly improved return to functional independence (59% vs. 35% of patients, respectively) and more ventilator-free days (174). Patients with spinal cord injury also experience substantial muscle atrophy, and Thomaz et al. (175) suggested in a recent meta-analysis that neuromuscular electrical stimulation (ES) is an effective treatment to increase muscle volume in patients with spinal cord injury. However, these conclusions should be cautioned as the analysis only included two studies (n = 26 patients) with differing follow-up periods and ES protocols (175).

Devries et al. (176) showed that unilateral low-load resistance exercise during 14 days of step reduction (SR; <1,500 steps) yielded significantly greater MyoPS rates and skeletal muscle mass. Similarly, a subanalysis of this cohort showed significantly greater muscle fiber CSA, satellite cell content, and capillarization in the exercise compared with the

nonexercised leg (177). Essential amino acid supplementation has also been shown to mitigate disuse-induced atrophy in young (37) and older (68 ± 5 yr) (178) adults; however, a recent trial in older adults (> 65 yr) at risk for sarcopenia showed no beneficial effect of leucine-enriched protein, nor leucine-enriched protein plus n-3 PUFAs, supplementation on lean mass, and integrated MyoPS compared with an isoenergetic placebo (179). Whether protein nutrition can independently ablate skeletal muscle atrophy remains unclear; adequate protein nutrition can certainly potentiate anabolic stimuli, such as resistance exercise (180). Muscle atrophy presents in numerous pathologies not highlighted herein, and several pharmaceutical treatments are being developed to mitigate muscle wasting (181). These drugs aim to downregulate catabolic (e.g., follistatin, which inhibits myostatin-SMAD) or upregulate anabolic [e.g., β_2-adrenoreceptor agonists, insulin-like growth factor (IGF)-1 analogs, which promote myoblast proliferation] pathways (182); however, clinical trials remain underway, and few pharmaceuticals are currently available. Together, exercise effectively mitigates disease-induced atrophy, which nutritional interventions may enhance. Continued refinement and development of strategies, particularly pharmaceuticals, are necessary to counter disease-induced muscle atrophy effectively.

Several nondisease states promote skeletal muscle atrophy. Musculoskeletal injuries or illness can result in acute periods of disuse and subsequent atrophy that can be studied with limb immobilization and acute bed rest models. In addition to the abovementioned interventions, ES may be an effective strategy to counter these acute periods of disuse. Dirks et al. (183) demonstrated that twice daily ES ameliorated declines in quadriceps CSA following 5 days of unilateral leg immobilization in young males (23 ± 1 yr). ES may also complement existing strategies to reduce atrophy during spaceflight — a unique atrophy-inducing stimulus often mimicked with head-down bed rest (184). Overall, atrophy-inducing events, such as illness, injury, and aging, are unavoidable throughout the lifetime. Muscle contraction via RT or ES and nutrition can mitigate skeletal muscle atrophy during catabolic periods, but further work is needed to refine existing interventions and establish pharmaceutical treatments. Nonetheless, strategies highlighted herein and optimal recovery strategies can effectively reduce skeletal muscle atrophy.

RECOVERY FROM MUSCLE ATROPHY

In preplanned situations that will involve disuse (i.e., elective surgery), where time permits that sufficient skeletal muscle tissue may be amassed before an upcoming disuse event, strategies to bolster muscular strength and muscle mass—progressive RT—should be implemented wherever feasible. However, unavoidable emergencies arise whereby disuse-induced muscle atrophy occurs unexpectedly and, often, rapidly. Irrespective of the instigating event (i.e., disease, aging, disuse, or malnutrition), much of the current research focuses on preventing muscle mass loss during the disuse event, and although prevention is important, what individuals do to recover from a severe catabolic crisis may be just as, if not more, important. Many of the physiological reformations that occur within, and surrounding, skeletal muscle in response to disuse — capillary rarefaction (185–187), satellite cell dysfunction (185, 188), ectopic fat deposition (189), and altered myocellular signaling (93) — are similar to those that underpin anabolic resistance with aging, and therefore impair skeletal muscle adaptation. The incomplete recovery of skeletal muscle mass, such that what is lost is not fully regained, exacerbates the canonical age-related decline of muscle mass and lowers an individual's protection against subsequent spells of muscle disuse.

After a disuse event and mobility permitting, physical activity interventions effectively restore skeletal muscle mass and function. Reambulation soon after a disuse event may be sufficient to restore lost muscle. A recent study by Bowden-Davies and colleagues (190) demonstrated in middle-aged men and women (36 yr) that, following 14 days of step reduction (<1,500 steps/day), just 2 wk of reambulation (>10,000 steps/day) was sufficient to restore lost lower-limb lean body mass. However, in a cohort of older middle-aged men (50 yr), muscle mass lost during 2 wk of unilateral lower limb immobilization was only partially recovered following ambulatory recovery — irrespective of nutritional intervention (20 g/day dairy protein) (166). Together, the aforementioned studies indicate that ambulation may, in some cases (i.e., younger individuals), be adequate to restore disuse-induced muscle loss, but as individuals age, more purposeful interventions are required.

Resistance training is widely accepted as the most potent nonpharmacological intervention to augment skeletal muscle mass and function, but the response varies between individuals (191) and across the age spectrum (192). Nevertheless, resistance training remains a crucial strategy to counter disuse-induced muscle atrophy and strength losses (193, 194). After 2 wk of lower limb immobilization, younger individuals (21–27 yr) lost significantly greater muscle mass than older individuals (61–74 yr); however, younger individuals possess the capacity to recover lost muscle mass and strength in response to 4 wk of progressive resistance exercise retraining (195). Although older individuals recoup disuse-induced functional impairments (e.g., maximal voluntary contraction), muscle mass and architectural properties did not fully recover in response to 4 wk of retraining. Conversely, after 2 wk of bed rest, quadricep volume decreased to a greater extent in older than in younger individuals, and younger individuals experienced greater disruption to metabolic homeostasis (196).

In contrast, Hvid and colleagues (197) demonstrated that both younger (24.3 ± 0.9 yr) and older (67.2 ± 1.0 yr) individuals displayed similar decrements in mechanical muscle function in response to a much shorter period of muscle disuse (e.g., 4 days). Relative reductions in muscle mass and function aside, when compared with younger individuals, older individuals display a diminished capacity to recover from disuse-induced muscle atrophy. Older individuals are unable or take longer, to fully regain lost muscle mass and muscle function despite targeted recovery efforts — including reambulation, strength testing sessions, and resistance exercise programs (166, 193, 195–198).

Although in some cases, ambulation and some rehabilitation therapies are sufficient to restore lost muscle following a disuse event, in other populations, such as older individuals and those having undergone prolonged disuse or catabolic

event, a multifaceted approach is necessary. Studies in animal models seeking to identify nutritional strategies to prevent or, at least, curtail disuse-induced muscle atrophy are promising (199, 200), but human investigations using various nutritional approaches, including supplementation with protein (166, 201), essential amino acids (EAA) (20, 37, 202), leucine (21, 165, 203, 204), branched-chain amino acids (BCAA) (205), and β-hydroxy β-methyl-butyrate (HMB) (206) are inconsistent. Importantly, manipulating nutritional intake during disuse is not always possible. Nevertheless, since exercise and nutrition are synergistic in promoting skeletal muscle growth and enhancing muscular adaptation (207), it is likely that combining both loading activity- and nutrition-based approaches will confer the greatest benefits to restore muscle mass and function following disuse-induced atrophy (208). By employing an appropriate nutritional strategy following a disuse event, the rate at which an individual returns to their predisuse event condition may be accelerated.

Energy balance largely dictates changes in total body mass, but the protein content of one's diet is a critical determinant of net protein balance and, consequently, skeletal muscle mass. When coupled with physical activity, specifically resistance exercise, protein feeding provides a potent stimulus for skeletal muscle growth (24, 25). However, following a disuse event, studies demonstrating the effectiveness of protein intake to facilitate muscle mass and function recovery are scarce. Mitchell and colleagues (166) showed that supplemental consumption of dairy protein (20 g/day) did not assist in the recovery of lost muscle with 2 wk of passive reambulation (i.e., no structured rehabilitation) or 2 wk of resistance training in middle-aged males, despite augmenting myofibrillar fractional synthetic rate. Conversely, although whey protein supplementation (2 × 30 g/day) did not protect against muscle loss during step reduction and energy restriction, it did facilitate an enhanced rate of integrated MPS and a rapid regain of whole body and leg lean mass during return to habitual activity in older men and women (201). The consumption of whey protein also facilitated the recovery of knee extensor strength following 7 days of bed rest and 5 days of progressive rehabilitation (consisting of 45 min/day stretching and balance/strength-focused exercises) (209). The observed discrepancies may be due to the duration of postdisuse recovery (1 vs. 2 wk), total daily protein intake (1.3 vs. 1.6 g/kg/day), and mode of recovery (reambulation vs. resistance exercise), or protein source (whey vs. milk). Regardless, research focused on combining exercise and protein-nutrition interventions to accelerate muscle mass recovery following disuse warrants further attention.

An optimal protein-based intervention postdisuse muscle restoration has not been fully determined; however, this might be accomplished by manipulating the amount and quality (i.e., EAA content) of dietary protein or through various other established and nascent nutritional interventions (2). Creatine supplementation is one such nutrition strategy that holds promise. Creatine is one of the most widely researched nutritional supplements, touted as promoting muscular strength, power, and lean body mass accretion in young (210–212) and older individuals (213–215). Hespel and colleagues (216) showed that 2 wk of immobilization decreased quadriceps muscle CSA, dynamic power (W_{max}), and isometric torque (F_{max}) by 10, 25, and 22%, respectively, but during rehabilitation, creatine monohydrate supplementation (from 20–25 g/day) facilitated a greater recovery of quadriceps muscle CSA and W_{max} when compared with a maltodextrin placebo. The authors suggested that this rapid recovery may be mediated by a creatine-induced change in myogenic regulatory factor MRF4 and myogenin expression (216).

Another emerging nutritional intervention that has displayed encouraging results in attempts to offset disuse-induced muscle loss is the use of n-3 PUFAs, which contain biologically active constituents in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Supplementation with n-3 PUFAs in young females maintained a greater myofibrillar fractional synthetic rate throughout 2 wk of immobilization and during 2 wk of ambulatory recovery compared with placebo control (11). Furthermore, during ambulatory recovery, n-3 PUFAs supplementation facilitated a full recovery of lost quadricep muscle volume to preimmobilization levels, whereas the control group had not fully restored the disuse-induced muscle loss (11). Nutritional strategies to enhance recovery of muscle mass following disuse require significant attention. Given that disuse-induced muscle atrophy occurs ~5 times faster than muscle growth (110), any postdisuse nutrition-focused recovery strategies must be protracted to yield substantial benefits for muscle mass recovery to develop sufficient muscle tissue for protection against subsequent bouts of disuse.

Summary

Disuse-induced muscle atrophy is a response to the lack of mechanical loading. In the absence of disease, a decrement in MPS creates a negative protein balance leading to skeletal muscle atrophy. Disuse decreases fasting MPS and leads to resistance to the anabolic effects of essential amino acids during the fed state. In ill subjects, catabolic hormones and inflammatory mediators enhance the activity of intracellular proteolytic systems increasing MPB and further suppressing MPS. Moreover, several disease states are characterized by decreased nutrient ingestion and very low or absence of ambulatory activity. Because of its multifactorial nature, muscle atrophy occurring during disease states tends to be more pronounced and linked to higher mortality. Older subjects demand special attention when facing periods of reduced activity or disuse since the age-related anabolic resistance can amplify the effects of periods of disuse, increasing predisposition to sarcopenia. Furthermore, older subjects are less likely to recover from disuse-induced muscle atrophy when compared with young subjects. Among all strategies to prevent or recover from disuse-induced muscle atrophy, physical activity, mainly resistance exercise, and adequate nutrition seem the most efficient and reliable.

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AUTHOR CONTRIBUTIONS

E.A.N., T.S., J.M., B.S.C., and S.M.P. conceived and designed research; E.A.N., T.S., J.M., B.S.C., and S.M.P. prepared figures; E.A.N., T.S., J.M., B.S.C., and S.M.P. drafted manuscript; E.A.N., T.S., J.M., B.S.C., and S.M.P. edited and revised manuscript; E.A.N., T.S., J.M., B.S.C., and S.M.P. approved final version of manuscript.

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