Alterations of protein turnover underlying disuse atrophy in human skeletal muscle

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Phillips SM, Glover EI, Rennie MJ. Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. J Appl Physiol 107: 645–654, 2009. First published July 16, 2009; doi:10.1152/japplphysiol.00452.2009.—Unloading-induced atrophy is a relatively uncomplicated form of muscle loss, dependent almost solely on the loss of mechanical input, whereas in disease states associated with inflammation (cancer cachexia, AIDS, burns, sepsis, and uremia), there is a pro-catabolic hormonal and cytokine environment. It is therefore predictable that muscle loss mainly due to disuse alone would be governed by mechanisms somewhat differently from those in inflammatory states. We suggest that in vivo measurements made in human subjects using arterial-venous balance, tracer dilution, and tracer incorporation are dynamic and thus robust by comparison with static measurements of mRNA abundance and protein expression and/or phosphorylation in human muscle. In addition, measurements made with cultured cells or in animal models, all of which have often been used to infer alterations of protein turnover, appear to be different from results obtained in immobilized human muscle in vivo. In vivo measurements of human muscle protein turnover in disuse show that the primary variable that changes facilitating the loss of muscle mass is protein synthesis, which is reduced in both the postabsorptive and postprandial states; muscle proteolysis itself appears not to be elevated. The depressed postprandial protein synthetic response (a phenomenon we term “anabolic resistance”) may even be accompanied by a diminished suppression of proteolysis. We therefore propose that most of the loss of muscle mass during disuse atrophy can be accounted for by a depression in the rate of protein synthesis. Thus the normal diurnal fasted-to-fed cycle of protein balance is disrupted and, by default, proteolysis becomes dominant but is not enhanced.

protein synthesis; proteolysis; proteasome; immobilization

All body proteins are in a constant state of turnover, with simultaneous synthesis and degradation. In adult human beings, in whom growth has ceased, the processes of synthesis and breakdown are equal and opposite on a daily basis. Alterations in protein balance occur throughout the day depending on environmental influences, such as the amount and composition of food and physical activity, which mainly affect protein synthesis. Protein synthesis is, by far, the most dynamic variable in the protein balance equation and changes several-fold throughout the day (Fig. 1A). Imbalances between protein synthesis and degradation result in increases or reductions in protein content, by a wide variety of possible changes in each arm of protein turnover, separately or together. Whenever protein breakdown chronically exceeds synthesis, the muscle protein mass declines, but it has not been a trivial matter to discover what actually happens to the two processes. It is now generally agreed that in humans, protein synthesis is down-regulated as a result of uncomplicated (i.e., nonpathological) disuse (38, 46–48, 90), but the relative importance of this compared with protein breakdown and the actual changes in direction and extent of breakdown in human muscle remain somewhat unclear. Nevertheless, it seems that in an increasing number of studies in situations in which there is a long-term loss of muscle mass, alterations in protein synthesis are facilitative and responsible for the majority of the observed changes in protein mass. Contrastingly, protein breakdown is often adaptive to the fall in protein synthesis and actually falls or remains unchanged but does not rise; this is a major conclusion of our review.

Naturally the problem of clinical muscle wasting is one that affects human beings rather than animals, but paradoxically, most of the literature dealing with disuse atrophy concerns animal models, which we suggest is inappropriate for reasons that we lay out in detail. In particular, we highlight suggestions for more definitive work on the mechanisms of human muscle atrophy using robust methods in vivo. In this review, we focus on results concerning human muscle in which, where possible, the dynamic processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) have actually been measured in vivo. These approaches are in contrast to inferences from static measures of mRNA or protein expression or from mea-
Mechanically comparable and induce similar outcomes in terms of rest, casting, limb suspension, and immobilization, are mechanisms that induce disuse atrophy in human muscle, such as chronic bed rest reviews (3, 34). In our view, commonly used techniques to measure physiological processes in muscle cells in vitro. For descriptions of the ground-based (i.e., nonspaceflight) models of disuse, we refer the reader to previous comprehensive reviews of the ground-based (i.e., nonspaceflight) models of disuse, see Adams et al. (3). For the leg muscles and particularly the quadriceps head-down bed rest induces the greatest rate of loss of muscle cross-sectional area (CSA) (5, 6, 12, 18, 29, 39, 90, 128); casting induces slightly less atrophy (46, 47, 56, 58, 66), whereas unilateral leg suspension (22, 28, 129) and knee immobilization induce atrophy with rates of muscle loss somewhat less than bed rest and casting (33, 48, 141). Nonetheless, based on available evidence that has accumulated to investigate why there is atrophy in all of these models, they share a common mechanism, which is a reduction in resting protein synthesis and no measurable change in proteolysis (12, 38, 48, 90).

Rates of muscle loss in all models of disuse are marginally faster within the first 30 days with a mean loss in muscle CSA of −0.6%/day (28, 29, 39, 46–48, 126, 141). After the first 30 days, rates of loss slow and reach a plateau in both the quadriceps femoris and the soleus (3). Muscle fiber CSA loss is apparently greater than that of muscle CSA with mean rates of −1.0%/day (7, 56, 126, 127, 141). It is unclear why there is a discrepancy between changes in muscle fiber area and muscle CSA; however, one may speculate that it is simply a methodological artifact of the histochemical method for determination of muscle fiber CSA (3). For example, Trappe et al. (127) reported changes in muscle CSA similar to that seen in single fibers. Nonetheless, rates of reduction in muscle fiber CSA, although apparently linear over 15–30 days (7, 56, 126, 127, 141), also show a plateau at longer periods of immobilization (3). A perhaps underappreciated issue with immobilization is that a muscle in an unloaded situation also shortens (13, 28). When these findings are considered in concert with the well-known changes in muscle CSA, it becomes apparent the decrements in muscle volume as a product of CSA and length changes would be more pronounced than either alone. For example, if muscle CSA declines 5% in 14 days (28, 141) and muscle length also declines by 5%, then at a first approximation, muscle volume should actually decline by ~10%. This would be true of muscles that are unloaded in a shortened position more so than those where muscle is held at a resting length.

One conspicuous difference between the results from human and animal studies is that human muscle shows much smaller differences, at least in the short-term, between fiber types in their degree of disuse atrophy (7, 56, 126, 127, 141). Rodents

Effects of Disuse on Muscle Mass

Muscle unloading results in a loss of muscle mass (i.e., atrophy) whenever muscle is not fulfilling its supportive and locomotory functions. The extent of the wasting is variable according to the different experimental regimes applied; for a review of this topic and the actual degree of disuse atrophy induced in ground-based models, see Adams et al. (3). For the leg muscles and particularly the quadriceps head-down bed rest induces the greatest rate of loss of muscle cross-sectional area (CSA) (5, 6, 12, 18, 29, 39, 90, 128); casting induces slightly less atrophy (46, 47, 56, 58, 66), whereas unilateral leg suspension (22, 28, 129) and knee immobilization induce atrophy with rates of muscle loss somewhat less than bed rest and casting (33, 48, 141). Nonetheless, based on available evidence that has accumulated to investigate why there is atrophy in all of these models, they share a common mechanism, which is a reduction in resting protein synthesis and no measurable change in proteolysis (12, 38, 48, 90).

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tend to lose greater quantities of their total muscle mass and also show muscle-specific differences (76, 124). In rodents, predominantly fast fiber-dominated muscles (extensor digitorum longus and tibialis anterior) losses are ~3 mg/day or ~1.6%/day, whereas they are ~6.2 mg/day or ~2.7%/day in slow fiber-dominated muscles (usually soleus) (15, 36, 55, 61, 70, 93, 104, 133). Of course, muscles differing with respect to their function (i.e., an extensor vs. a flexor) will also affect the degree of atrophy observed. For example, flexor muscles (e.g., soleus and gastrocnemius) atrophy far more rapidly than extensor muscles (e.g., tibialis anterior, extensor digitorum longus) (103, 132), and the same is true in humans (29). The greater atrophy seen in type I fibers in rodent muscles may be partly a result of their being larger in CSA than those of type II fibers in slow muscles like the soleus. Such dichotomies are not seen in human muscle disuse atrophy, which show far more homogeneous rates of decline in fiber CSA for type I and II fibers within the same muscle (58, 141). One possible explanation for the apparent lack of fiber-specific differences in human muscle during atrophy is that muscle biopsy samples are too small to adequately detect differences (57, 82, 83).

However, when sufficient numbers of fibers are counted to obtain reliable estimates of fiber sizes, no difference are seen in the degree of fiber atrophy among type I, IIA, and IIX fibers after 14 (141) or 21 days (58) of immobilization. In fact, even with spinal cord injury, average changes in muscle fiber size were 27, 37, and 28% for type I, IIA, and IIX fibers, respectively, between 6 and 24 wk after injury (24). The lack of differential fiber-specific atrophy in human muscle is also likely a consequence of the fact that the rates of protein turnover are much more homogeneous between fiber types than they are between fiber types in rodents. For example, Mitten dorfer et al. (87) reported that biopsies taken from the soleus, vastus, and triceps muscles of men exhibited virtually identical rates of MPS at rest and in response to feeding, a finding also reported for vastus and soleus by Carroll et al. (23). These authors observed these rates despite observing differing concentrations of phosphorylated and total eukaryotic initiation factor 4E binding protein-1 (e4EBP-1) between muscles (23). These findings are in marked contrast to data from rats (49–51, 67, 78, 81), fowl (74, 75), and lagomorphs (53, 65, 71), all of which display variations as high as twofold in rates of protein turnover between muscles composed of slow and fast fiber types. Of course, a study that examined fiber-specific changes in rates of muscle protein turnover in humans would be definitive in delineating the exact changes that occur with atrophy; this has not been done to date.

Muscle Protein Turnover: Normally and in Disuse

Feeding is a robust stimulator of muscle protein synthesis. In the normal rested state, we know that feeding at a moderate rate doubles muscle protein synthesis, a rise mainly due to the effects of amino acids, particularly essential amino acids. The effect appears to be dose related, with a hyperbolic relationship between MPS and availability of essential amino acids (14) or protein (88), with maximal effects at ~20–25 g of protein or 8–10 g of essential amino acids. It is likely that this effect is not dependent on insulin (112), since it can be achieved when insulin concentration is clamped by the use of somatostatin and analogs (54, 113). Amino acids have a small inhibitory effect on MPB in their own right (114), but most of the effect of dietary amino acids probably results from the release of insulin, which is a potent inhibitor of MPB (26, 43, 54, 80). With normal feeding, the negative muscle net balance (i.e., MPB > MPS) observed in the postabsorptive state is rapidly reversed due for the most part to a doubling of MPS with a somewhat smaller (possibly 20–30%) diminution of MPB (Fig. 1A). However, these stereotypic responses have been shown to be perturbed when muscle is in states of disuse.

We have known for 20 years that immobilization (i.e., unloading) of a human limb results in a reduced basal-fasted rate of MPS (46). Gibson et al. (46) did not make direct measures of MPB but calculated it from the observed loss of muscle mass and the measured decline in MPS. By this means they proposed that MPB could not have increased substantially with immobilization, as suggested by results from studies of mostly static markers in hindlimb unweighting in rodents (55, 70, 76, 81, 117, 118, 132), but actually adaptively decreased. Thus, although the rates of MPS were measured only in the fasted state, rather than across the daily diurnal cycle (Fig. 1A), which would include the effects of feeding, it is highly likely that daily rates of total muscle protein synthesis, including those in the fed state, were markedly reduced, which is in fact what we recently observed (48). Recently, de Boer et al. (28) observed a decline in muscle CSA of ~10% with 21 days of limb suspension. This reduction in muscle CSA of ~0.5%/day was accompanied by reductions in the fasted state of MPS of >50%, confirming the outcome of the earlier work (see also the illustrative calculation below) (46). In immobilized human quadriceps muscle, there is also “resistance” to the anabolic effects of amino acids (48), strongly suggesting that the depressive effects of immobilization are seen across the daily diurnal span of feeding and fasting (Fig. 1C). Even if the effect on MPS were confined to the fasting state, which is not the case according to our recent data (48), the decrease in rested MPS could account for almost one-half of the observed wasting (46, 48). Thus a substantial net increase in protein breakdown is not required to account for the observed atrophy of human muscle over an extended period of immobilization. As a central hypothesis, we propose that a large part of the loss of muscle with unloading is due not only to a reduction in fasted-state synthesis but also to an induction of anabolic resistance, that is, a suppression of the normal amino acid-induced (i.e., feeding induced) increase in protein synthesis (Fig. 1C). Because we spend ~40% of our day under the influence of our last meal, it makes sense that we should expect a depression of fed-state MPS (48). The result is a disturbance of the normal rise and fall of protein net balance, mediated for the most part by reductions in MPS and not by increases in MPB. The consequences of this change are such that the fed-state gains in protein mass are less and that fasted losses remain the same (or possibly are increased early in immobilization; see Fig. 1, B and C). The implications lead us to suggest a model for what actually happens (Fig. 1C). First, assume, in steady state, that diurnal rates of human MPS and MPB are equal at ~0.05%/h or ~1.2%/day. In disuse we have shown that the fasting rate of MPS is depressed by ~60% and that the fed rate of MPS is depressed by ~50%. If the relative durations per day of postabsorptive and postprandial periods are ~9 and ~15 h, respectively, then the total diurnal average is down by 58% (i.e., to 0.03%/h or 0.72%/day). Therefore, if breakdown re-
 mains at the predisuse rate, then the rate of loss of protein = $k_{\text{breakdown}} - k_{\text{synthesis}} = 1.2 - 0.72$, or $\sim 0.5\%$/day, as observed (12, 28, 38, 90, 92). Thus there is no reason for a substantive increase in protein breakdown to see muscle mass decline. Assuming that our estimated changes in protein synthesis are correct, if proteolysis were also elevated by even 20%, then losses of muscle CSA would be closer to $\sim 0.7\%$/day and the calculated loss of muscle CSA at 23 days of immobilization would be 16.5%, not the 10% observed (28). In any case, there are no reports in humans of MPS rates, measured by dynamic methods (12, 38, 90, 116), being elevated in conditions of decreased physical mobility; if anything MPB falls or remains unchanged (38, 116). Anabolic resistance has been observed in aging (27, 44, 101, 134) and immobilization (48) and also may be pertinent in situations of transplant recipients (98) and patients with cancer (97, 98). There are those, however, who demur at our view and suggest that older persons can mount a response to protein ingestion similar to that of younger persons (69, 115) or, in a similar vein, that immobilization can be rescued with daily doses of crystalline essential amino acids, and thus immobilized muscle is not resistant to amino acid-induced anabolism (91, 92). In direct contrast to these reports (91, 92), however, are reports that muscle atrophy during short-term (28 days) (126–128) and long-term (60 days) (126–128) bed rest failed to be impacted by daily amino acid supplementation or by a daily leucine-enriched whey protein supplement, respectively. Clearly, this is an area with some important and clinically relevant discrepancies that requires further investigation.

Recent data have suggested that there is an early increase in MPB with disuse in humans, at least raising the possibility of a biphasic rise and then fall in proteolysis (122); it also is possible that such a biphasic response exists for MPS, something that has not been tested to date. However, we have criticized the approach and validity of this work (122). One possibility is that the normal feeding-induced suppression of MPB (mediated for the most part by insulin) (26, 54) is attenuated with immobilization; support for such a thesis can be seen in human models (100). What very clearly needs to be measured in a direct and detailed fashion is how MPB and MPS change with short- and longer term durations of disuse. Given that there is evidence of reduced synthesis but no changes in proteolysis with disuse, at least to date (12, 38, 90, 109, 116), the possibility of a marked disuse-mediated atrophic response underpinned by elevated MPB seems unlikely. Interestingly, two recent reports serve as an insightful look at what happens in human skeletal muscle with disuse and also with a countermeasure (20, 116). In one study, Symons et al. (116) reported that, similar to previous studies in humans (28, 38, 46, 48, 90), MPS falls with 21 days of bed rest and provided a direct dynamic estimate of MPB showing that it is not elevated but remains unchanged. In a parallel study, Caiozzo et al. (20) reported, from the same subjects, declines in mRNA abundance of all myosin heavy chains (MHC) but stereotypical increases in type IIa MHC mRNA. Confirming previous results (25, 28), these authors also reported minor (statistically non-significant) elevations in atrogin or myostatin mRNA abundance (20), and yet this occurred in the face of no increase in MPB (116).

A potentially important and very interesting observation made by de Boer et al. (28), which we recently confirmed (48), is a sharp decline in focal adhesion kinase (FAK) phosphorylation in the early stages of immobilization. FAK has been shown in rodent and cell models to be responsive to loading and unloading (40, 41, 52) and to cyclic mechanical stretch (142), and we recently reported increases in phosphorylation of FAK in response to bouts of resistance and endurance exercise (139). As such, the acute regulation (i.e., phosphorylation) of FAK after intense resistance exercise is a potential link of the loading stimulus to metabolic effectors that induce an increase in MPS. Such a possibility is predicted by the cellular tensility model, in which focal adhesion complexes anchor cytoskeletal proteins to the extracellular matrix of the cell to transmit forces and activate relevant signaling cascades, reviewed in Ref. 60. The significance of the decrease in FAK phosphorylation remains to be elucidated completely.

To summarize, in human muscle the available evidence suggests that immobilization results in a reduced resting-fasted rate of MPS (28, 46–48) and that, at least after 14 days of immobilization, there is a reduced rate of MPS in response to amino acid provision that cannot be overcome by high doses of amino acids (AA) previously shown to maximally stimulate MPS (14). The overall result is a reduction in the basal and fed-state accretion of muscle protein, and as a result, the mass of protein declines.

**Muscle Protein Breakdown With Disuse**

Far less is known about the regulation of MPB than MPS (especially in human muscle), and our knowledge is mainly confined to measurement of changes in gene expression (mRNA) and, in some instances, protein content. Most work has focused on changes in components of the pathways thought to degrade protein. Mammalian muscle contains the three major proteolytic systems. Lysosomal cathepsin expression, although relatively low, occurs in adult muscle, cell lines, and adult human muscle satellite cells (11). Cathepsins cleave a variety of purified myofibrillar substrates in vitro (11). Where cathepsins have been identified in normal human skeletal muscle, it has been suggested that they are associated with clearance of damaged protein structures and regeneration rather than wholesale bulk degradation (138). To date, however, we have no knowledge of how this proteolytic pathway responds in human muscle to disuse in nonpathological situations.

Skeletal muscle expresses the ubiquitous calpain-1 ($\mu$-calpain) and calpain-2 (micro-calpain), as well as calpain-3 (p94) (10), a muscle-specific form that binds to the giant sarcomeric protein connectin/titin (108) and, when defectively expressed, shown in rodent and cell models to be responsive to loading and unloading (24 h) but then declined over time. Calpain p94 mRNA content was, in contrast, reduced with immobilization. Regrettably, neither protein contents nor enzyme activities were measured.

It is well accepted that in skeletal muscle the ATP-dependent ubiquitin proteasomal pathway (UPP) functions as the primary degradative system for most muscle protein (62, 77, 117, 121). Targeting of proteins to the proteasome begins with conjugation of an ubiquitin moiety by an ubiquitin carrier (E2) enzyme, which has been previously charged with ubiquitin by the
E1 ubiquitin-activating enzyme. For efficient targeting to the proteasome, at least four ubiquitin moieties must be attached, and this is accomplished by E2 in partnership with the E3 ligases, which confer specificity to the system because they recognize a limited number of substrates (62, 77, 119). The UPP cannot, however, degrade intact myofibrils (62, 77, 107, 121), and so enzymatic cleavage of “susceptible” sites in the myofibrillar lattice is thought to occur via either activated calpain (37, 137) or caspase-3 (35, 42, 135). In turn, these site-specific cleavages then result in myofibrillar proteins being exposed, partially degraded, and thus accessible for ubiquitination and thereafter degradable by the proteasome. To date, however, despite numerous published reports of changes not only in gene but also protein abundance for components of the UPP (95), we lack the corresponding kinetic measures of MPB, in any situation of disuse atrophy, in which its expression has been up- or downregulated.

Our knowledge is relatively insecure concerning the scope and regulation of proteolytic systems in human muscle. In fact, what we know is, more often than not, extrapolated almost exclusively from ex vivo studies of rodent muscle using inhibitors of one pathway or another (45, 85, 117, 121, 125). Chloroquine, an inhibitor of acidification of the lysosome, has been reported to suppress postabsorptive whole body protein turnover in humans (30) but not that of forearm muscle turnover (9), suggesting that lysosomal proteolysis does not play a quantitatively significant role in human skeletal muscle (64, 94). In addition, ubiquitinated proteins were increased in a 20-day bed rest study (89) in a model similar to that used by others in which MPB was not found to increase (38, 90). It also is important to realize that an increase in the concentration of ubiquitinated proteins can come about due to either increased rates of conjugation or decreased rates of enzymatic deconjugation. Moreover, inhibition (and not stimulation) of the proteasome can cause accumulation of ubiquitin–protein conjugates (121), which would indicate reduced but not increased proteolysis. Increases in expression of transcripts for the ubiquitin ligases atrogin-1 and cbl-b also have been reported in humans during bed rest (89), but the authors proposed that their role in disuse atrophy may not be through the bulk degradation of myofibrillar proteins but, rather, via suppression of protein synthetic capacity by enhanced degradation of growth-promoting proteins. Evidence supporting such a hypothesis has been presented in the form of modulation of eukaryotic initiation factor-3 subunit f (eIF3-f) by MAFbx under proatrophic conditions (72). These data provide a link between the increased ubiquitin ligase expression seen in models of atrophy and downregulation of MPS (72). It also is possible that muscle protein degradation in disuse atrophy is linked to degradation of factors involved in myogenesis and cell cycle regulation; anti-atrophy interventions have consistently been shown to be associated with increased myogenic regulatory factor expression (2, 4, 56).

Interestingly, in a number of situations decreases in MPB have been demonstrated to be dissociated from proteasome subunit mRNA expression in subjects on a low-protein diet (17). Furthermore, a mismatch has been observed in the insulin-induced suppression of protein breakdown and changes in ubiquitin ligases and proteasome subunit C2 protein levels (54). There also are a number of published reports in humans in various disease- and pharmacologically based states showing that, even in the face of elevations in transcript abundance for elements of the UPP, there is no measurable change in proteolysis or muscle mass in disease (16, 17, 63, 102) and disuse (20, 116). It is difficult to reconcile such large increases in the expression of genes coding for UPP proteins with a lack of apparent increments in proteolysis; however, it may be that the UPP is selectively degrading only a specific population such as peripheral cellular structures, as suggested recently by the data of Urso et al. (130, 131). Alternatively, the large changes in expression of genes coding for UPP proteins may not be resulting in proportional changes in protein content of UPP proteins. Another final possibility is that the kinetics of the UPP, which are still largely unknown, are altered and that even changes in protein abundance do not translate into changes in proteolytic activity due to unrecognized modes of regulation. Thus there are a number of situations in which UPP mRNA changes are discordant with actual measured rates of proteolysis. In our view, static measurements of mRNA-encoding subunits of various proteolytic proteins (20), although informative in their own right, are far removed from the actual measured proteolytic rate (116), and thus these measures are difficult to interpret in terms of their significance.

Thus a lacuna in our understanding of how disuse affects muscle protein turnover in humans is what happens to protein breakdown. We argue that, based on estimated rates (see above), it appears unlikely that increased breakdown is playing a role in the decline in muscle tissue mass, and there also is tracer-based kinetic evidence to support this contention, at least between 10 and 42 days (38, 90). The indirect evidence of enhanced proteolysis, at least insofar as increases in MAFbx and MuRF-1 mRNA are concerned, observed by de Boer et al. (28) shows that early (between 0 and 10 days) after immobilization, proteolysis may be upregulated (122), and with longer periods (at least >30 days), there is a decline. If the anabolic resistance we have seen occurs early in immobilization and proteolysis is also elevated (Fig. 1, B and C), then this may explain why loss of muscle mass is more rapid early (1–30 days; see above) and then subsequently reaches a plateau with longer periods of disuse (i.e., 90–120 days) (3). However, even in patients with spinal cord injuries that completely or partially block neural impulses to muscle there is preservation of muscle mass, which is dependent on the level and severity of the injury. Mild electrical stimulation (47) as well as high-intensity and -load programs of resistance exercise (5, 22, 129) have been shown to be effective in limiting human muscle wasting with disuse.

Inherent Species Differences: A Tale of Rodents and Men

When considering the relevance of animal models to human muscle protein turnover, species differences in protein metabolism should be taken into account. Total protein turnover in adult rats is 3- to 4-fold higher than in adult humans, whereas adult rat muscle protein synthetic rates are ~2.5-fold greater (136). Therefore, it is not inconceivable that the response of rodent muscle to reduced loading may differ in terms of scale, pattern, and the relative contribution of each arm to turnover from that of humans. This is supported by the more rapid and severe atrophy observed in rodent models, as discussed above. Indeed, Thomason et al. (123) reported a depression in soleus muscle protein synthesis as early as 5 h after the onset of
hindlimb unweighting, a phenomenon unlikely to be paralleled in the human situation, where we spend ~8 h of each day unloaded while sleeping and resting fasted and fed rates of MPS are unchanged.

Interestingly, in a recent publication (25) it was observed that aside from increases in MAFbx/atrogin-1, there were very few changes in proteolytic gene expression in short-term immobilized human skeletal muscle. A number of other reports, all using rodent models, have confirmed that hindlimb unloading, casting, and denervation result in elevations of mRNA transcripts for components of the UPP (70, 84, 85, 117, 133). These findings have led to the conclusion that muscle disuse leads to elevations in MPB. We (96) recently commented that the use of static measurements of proteolytic pathway components to provide “proof” that proteolysis is the main mechanism for muscle loss is potentially flawed. Specifically, we pointed out that in animal studies static measurements of concentrations of molecules thought to be markers of muscle proteolysis have been taken in conditions, both physiological and pathophysiological, in which there is disuse atrophy resulting from immobilization. However, few have ever been shown to be coincident with the appropriate magnitude of change in dynamically measured MPB. Thus our conclusion is that static markers (mRNA and/or protein abundance) are not often related to dynamic changes in protein breakdown and so lack meaning when measured in isolation, and so such an approach is flawed. Instead, we propose that changes in proteolytic components reflect “trimming” or modification of components of the connective tissue matrix or the proteome that have specific metabolic effects unrelated to the changes in bulk protein turnover leading to net protein loss.

Some have argued that spinal isolation and denervation models used in rodents (84, 104, 105, 140) may well induce more “severe” atrophic changes than those seen with hindlimb suspension (4, 21, 55, 59, 93, 117, 120, 132). However, a comparison of the general programs of gene transcription between these two models reveals they are similar in many respects (104, 110). Notably, Stevenson et al. (110), who used a model of hindlimb suspension, reported a robust activation of proteosomal components, as well as MAFbx/atrogin-1, MuRF-1, and lysosomal proteins. Sacheck et al. (104), using models of spinal isolation and denervation, made similar observations citing the rise in proteolytic components as the reason for atrophy in these models in a manner similar to that seen in uremia and sepsis. Simply put, do elevations in genes for these pathways indicate that proteolysis is the dominant process? Seemingly convincing evidence would appear to come from studies of cells or mice (106, 111); however, at no point in any of these studies have the processes of protein synthesis and breakdown ever been measured simultaneously with static measurements of genes or proteins.

Contributing to the confusion is the fact the measures of UPP components, almost exclusively mRNA abundances for proteins involved in proteolysis, appear to align with ex vivo measurements of MPS and MPB with the use of isolated muscle strips from rodents (84, 85, 117, 140). There are several problems with measurements of muscle protein kinetics with isolated muscles or muscle strips, however, particularly from young growing rodents. For example, the results of studies using isolated rodent muscles taken from animals that have experienced some form of disuse atrophy are, we propose, unsatisfactory. This is mainly because of the difficulty of getting the isolated muscle preparations to behave physiologically, in vitro, in terms of MPS and MPB. For example, none of the ex vivo preparations are even able to sustain net positive protein balance even in nonpathological conditions. In fact, muscle preparations from normal young rats display much lower synthetic rates and higher breakdown rates than those observed in vivo (136). In addition, rodents are inherently metabolically unstable because they spend a far greater proportion of their life span growing. During growth in rodents, skeletal muscle MPS is still insulin sensitive (79) as it is in humans during growth, but this stimulation is lost in adulthood (54). On the basis of these criticisms, it would appear that isolated rodent muscle preparations are inevitably predisposed to show an increase in MPB in many situations. In fact, even a decline in protein synthesis would be registered as an increase in breakdown in an ex vivo preparation in which tyrosine release into the perfusion bath is the method of measuring proteolysis, since synthesis cannot recapture amino acids arising from proteolysis (84, 85, 117, 140).

Almost 20 years ago, Thomason and Booth (124) stated in their review that it is extremely difficult to obtain valid estimates of the true rates of protein degradation in the unweighted soleus muscle. They proposed estimating breakdown from a first-order model of the decline in synthetic rates with hindlimb unloading in adult rats (123) as an alternative to in vitro amino acid-release rates of muscle preparations and in vivo estimates obtained by subtracting growth rates from in vivo protein synthesis rates, which were performed on growing rats (124). With this approach degradation is estimated to gradually increase, reaching a peak by 15 days, but thereafter declines to below-baseline rates. When measuring MPS in vivo, combined with estimates of MPB by difference from rates of muscle loss, hindlimb suspension can be seen to increase MPB. Thus there appears to be marked difference in the importance of changes in MPS and MPB in rat and human muscle. Similar criticisms also have been made when comparing rats and humans in the field of aging (31, 32).

By contrast to rats, in vivo human studies have a number of advantages. For example, the muscle is, at least during a stable isotope infusion, being perfused by an intact circulatory system. The muscle in this model can sustain a net positive protein balance and behaves normally in the face of hyperaminoacidemia. In this model there is the ability to combine direct measurements of protein turnover with simultaneous measures of protein and gene expression from the same piece of muscle that gave rise to the phenotypic response of MPS and MPB. Thus all of these conditions would, we propose, yield a more optimal experimental protocol from which to obtain a comprehensive description of how disuse affects muscle turnover leading to atrophy.

Conclusion

In our view, the discrepancy between much of the accepted descriptions, derived from animal models, of how disuse atrophy is mediated may be due to a combination of both methodological and inherent species-specific differences in how muscle proteins turn over. Clearly, to resolve some of the reported inconsistencies we highlight above, more human studies are required with simultaneous measurements of MPS,
MPB, and cotemporal measurement of static markers of breakdown. It is imperative that these studies include examination of dynamic measures of muscle protein turnover and putative metabolic controllers early after immobilization (before 10 days) in the fed and fasted states. Factors such as sex, age, and comorbidities will obviously affect the responses, but unless we have a clear idea of the basic responses to immobilization per se, the effects of such factors will not be easily teased out and therapeutic goals will remain largely unattainable.

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Review

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