Skeletal muscle atrophy during short-term disuse: Implications for age-related sarcopenia

Benjamin T. Wall, Marlou L. Dirks, Luc J.C. van Loon*

Department of Human Movement Sciences, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands

ABSTRACT

Situations such as the recovery from injury and illness can lead to enforced periods of muscle disuse or unloading. Such circumstances lead to rapid skeletal muscle atrophy, loss of functional strength and a multitude of related negative health consequences. The elderly population is particularly vulnerable to the acute challenges of muscle disuse atrophy. Any loss of skeletal muscle mass must be underpinned by a chronic imbalance between muscle protein synthesis and breakdown rates. It is recognized that muscle atrophy during prolonged (>10 days) disuse is brought about primarily by declines in post-absorptive and post-prandial muscle protein synthesis rates, without a clear contribution from changes in muscle protein breakdown. Few data are available on the impact of short-term disuse (<10 days) on muscle protein turnover in humans. However, indirect evidence indicates that considerable muscle atrophy occurs during this early phase, and is likely attributed to a rapid increase in muscle protein breakdown accompanied by the characteristic decline in muscle protein synthesis. Short-term disuse atrophy is of particular relevance in the development of sarcopenia, as it has been suggested that successive short periods of muscle disuse, due to sickness or injury, accumulate throughout an individual’s lifespan and contributes considerably to the net muscle loss observed with aging. Research is warranted to elucidate the physiological and molecular basis for rapid muscle loss during short periods of disuse. Such mechanistic insight will allow the characterization of nutritional, exercise and/or pharmacological interventions to prevent or attenuate muscle mass loss during periods of disuse and therefore aid in the treatment of age-related sarcopenia.

1. Introduction

Episodes of skeletal muscle inactivity, unloading or disuse often occur in (otherwise) healthy humans as a direct consequence of injury or illness. During disuse, rapid skeletal muscle loss ensues (Deitrick, 1948b; Gibson et al., 1987b; Ingemann-Hansen and Halkjaer-Kristensen, 1980) which leads to numerous negative health consequences such as impaired functional capacity and...
strength (Deitrick, 1948b; Gibson et al., 1987b; Ingemann-Hansen and Halkjaer-Kristensen, 1980; LeBlanc et al., 1992b; White et al., 1984b), the onset of insulin resistance (Stuart et al., 1988), a decline in basal metabolic rate (Haruna et al., 1994; Tzankoff and Norris, 1977) and accrual of body fat mass (Brooks et al., 2008; Ferrando et al., 1996, 1997). Furthermore, the extent of muscle loss that occurs during illness has previously been identified as an important predictor of the duration of hospitalization and subsequent need for rehabilitation (Christensen et al., 1982). Experimentally, muscle disuse atrophy in humans has generally been studied over a relatively prolonged period (>10 days) in young, healthy individuals to ensure measurable muscle loss in a practical research setting. However, an often underappreciated consideration is that signs of substantial muscle loss are already evident after only a few days of disuse. This is of great clinical significance since it is likely that the accumulation of (short) periods of muscle disuse atrophy that occurs throughout an individual’s lifespan contributes substantially to the etiology of age-related sarcopenia. Moreover, our understanding of the physiological mechanisms that bring about muscle atrophy over a short period of muscle disuse (<10 days) is far less comprehensive than when we consider a more prolonged period. The present review will discuss the physiological basis of skeletal muscle atrophy during prolonged and short-term muscle disuse. Particular emphasis will be placed on considering the potentially differing mechanisms which bring about short-term muscle disuse atrophy and how this relates to age-related sarcopenia.

2. Prolonged disuse atrophy (>10 days)

2.1. Basal muscle protein turnover

By far the most commonly employed models to study muscle disuse in humans over the past century have been (head down tilted) bed-rest (e.g. Deitrick, 1948a; Ferrando et al., 1996) and limb immobilization/suspension (e.g. Gibson et al., 1987a; Schneyder et al., 1954). Such studies have shown that a period of disuse ranging between 10 and 42 days generally leads to a rate of muscle loss of approximately 0.5–0.6% of total muscle mass per day (Brooks et al., 2008; de Boer et al., 2007a; Ferrando et al., 1995; Glover et al., 2008; Hespel et al., 2001; Jones et al., 2004; Kortebine et al., 2007; Thom et al., 2001), with a variable consequent decline in muscle strength ranging between 0.3% (LeBlanc et al., 1992a; Padon-Jones et al., 2004a) and 4.2% (Thom et al., 2001) per day. Greater relative losses of muscle strength compared with mass are likely attributed to the associated declines in neuromuscular recruitment and function that also occur with disuse (Seki et al., 2001). Given that skeletal muscle mass turns over at a relatively slow rate of ~1–2% per day, muscle disuse atrophy must ultimately be underpinned by a chronic, persistent disturbance in muscle protein balance. That is to say, for a sustained period, either muscle protein synthesis rates decline, breakdown rates increase, or a combination of both occurs. It was first demonstrated by Gibson and colleagues, utilizing contemporary stable isotope methodology, that knee immobilization for ~40 days due to cruciate knee ligament damage is accompanied by a 26% reduction in basal (fasting) muscle protein synthesis rates in young men (Gibson et al., 1987a). Disuse induced declines in basal muscle protein synthesis rates have since been replicated numerous times following 10–42 days of bed-rest (Ferrando et al., 1996, 1997, 2010; Kortebine et al., 2007; Symons et al., 2009) or lower limb immobilization (de Boer et al., 2007b; Gibson et al., 1987a, 1988; Glover et al., 2008) in younger individuals. Thus, robust evidence is available, at least in younger individuals, to show that a reduced capacity to synthesize de novo muscle proteins in the basal (fasted) state contributes to the observed loss of muscle tissue during a prolonged period of disuse.

Whether basal muscle protein breakdown rates are altered during prolonged disuse is less clear given the lack of comprehensive data. Animal models of disuse atrophy show a clear rise in muscle protein breakdown rates following the onset of disuse, which accompanies a decreased rate of muscle protein synthesis (Bodine, 2013; Lang et al., 2012; Magne et al., 2012). This parallel effect on synthesis and breakdown appears to explain the vastly greater rate of muscle loss observed in animal compared to human models of disuse (Thomson and Booth, 1990). Self-evidently, numerous differences exist between species. For instance, compared to humans, animals subjected to disuse are generally immature, metabolically unstable (i.e. homeostatic mechanisms are less tightly regulated), and in a state of stress in response to experimental interventions. Nevertheless, data from animals have led researchers to hypothesize that a rise in muscle protein breakdown may also contribute to disuse atrophy in humans. While measuring the fractional synthetic rate of infused stable isotope labeled amino acids into new muscle proteins offers a direct method for determining muscle protein synthesis rates in humans in vivo, equivalent measures for muscle protein breakdown assessment are more technically challenging and, consequently, more sparsely reported. Insight has been gained by approaches which have combined contemporary stable isotope methodology with arterio-venous balance measurements (Ferrando et al., 1996) or utilized a pulse tracer administration approach to determine fractional breakdown rates (Symons et al., 2009; Zhang et al., 1998). Such studies have concluded that muscle protein breakdown rates do not change in younger humans following 14–21 days of bed-rest. Furthermore, several studies have concluded that disuse induced impairments in muscle protein synthesis rates can quantitatively (more than) account for the observed loss of muscle mass, suggesting that muscle protein breakdown rates either do not change, or, actually adaptively decrease (Ferrando et al., 1996; Gibson et al., 1987a). Based on these data, and in contrast to similar data obtained in animal models, it has been suggested that alterations in muscle protein breakdown do not contribute to the loss of muscle tissue during prolonged disuse in humans (Phillips et al., 2009; Rennie et al., 2010). However, it is important to highlight that the quantity of available in vivo assessments of dynamic basal muscle protein breakdown rates (i.e. those data obtained from stable isotope studies) during disuse do not currently equal the insight generated on muscle protein synthesis rates.

Protein breakdown within skeletal muscle occurs via several distinct processes; most notably caspase proteases involved in apoptosis (Wang et al., 2004), cathepsins integral to autophagy (Bech et al., 2005), the calcium-dependent calpain system (Bartoli and Richard, 2005) and the ubiquitin proteasome pathway. Though the ubiquitin proteasome pathway cannot degrade intact myofibrils (initial preprocessing of myofibrils by alternative pathways is a prerequisite for complete proteolysis), it is thought to be quantitatively the primary mediator of net skeletal muscle protein breakdown in humans (Greenhaff et al., 2008; Jago and Goldberg, 2001; Murton et al., 2008). During this process, specific proteases are tagged for degradation via a three-step, enzymatic, sequential cascade (Murton et al., 2008). The specificity for this targeting is afforded by the action of a family of ubiquitin ligases. Of these, the muscle specific ubiquitin ligases, muscle atrophy F-Box/atroglin-1 (MAFbx) and muscle-specific RING-finger protein 1 (MuRF1), have been shown to be transcriptionally up-regulated under numerous conditions associated with muscle atrophy (Lecker et al., 2004; Murton et al., 2008). Moreover, their specific knockout has been shown to induce partial resistance to muscle atrophy (Murton et al., 2008). While it was traditionally thought that MAFbx and MuRF1 acted in concert with a specific muscle ‘atrophy program’ (Lecker et al., 2004),
recent evidence has demonstrated that they can also act independently during atrophy (Baehr et al., 2011) and MuRF1 may also have a role in inhibiting muscle protein synthetic pathways (Baehr et al., 2011). Accordingly, the transcriptional regulation of these ubiquitin ligases is widely considered to be an important marker of increased ubiquitin proteasome activity, protein breakdown and muscle atrophy (Greenhaff et al., 2008; Jagoe and Goldberg, 2001; Murton et al., 2008). In the absence of direct, dynamic measures of muscle protein breakdown rates, researchers have often looked for evidence of an up-regulation of the ubiquitin–proteasome system following disuse as a proxy of increased muscle protein breakdown. For instance, increased mRNA levels of MAFbx (Jones et al., 2004; Ogawa et al., 2006) and/or MuRF1 (de Boer et al., 2007b; Jones et al., 2004; Ogawa et al., 2006), fork head box protein 01 (FOXO1; a known transcription factor for the two ubiquitin ligases) (Suetta et al., 2012), and/or the 20S proteasome α7 subunit (Jones et al., 2004), have all been reported following 10–21 days of limb immobilization or bed-rest in young subjects. Furthermore, in keeping with previous reports (Ogawa et al., 2006), it has recently been shown that the abundance of polyubiquitinated proteins within skeletal muscle increases following prolonged bed-rest (Brocca et al., 2012). The latter study also found some evidence (up-regulation of Beclin-1) of increased autophagy during bed-rest. Finally, using a gene microarray approach, a coordinated increase in the levels of protein breakdown gene clusters as a functional unit was reported after 14 days of limb immobilization in younger men (Abadi et al., 2009); although MAFbx and MuRF1 were not among those genes that were elevated. Indeed, it is important to acknowledge that not all studies show such elevations in all or any of the aforementioned protein breakdown markers during prolonged disuse (Abadi et al., 2009; Glover et al., 2010). Thus, at present, inconsistent molecular findings together with methodological limitations in our ability to accurately determine dynamic muscle protein breakdown rates make it difficult to firmly assess its contribution to muscle atrophy during prolonged disuse. Therefore, it is necessary for future research to carefully assess the impact of prolonged disuse upon muscle protein breakdown rates by employing a variety of methodologies (i.e. stable isotope methodology, molecular/enzymatic markers, etc.). Such information will allow us to better understand whether alterations in protein breakdown play any important role in prolonged muscle disuse atrophy in humans.

2.2. Post-prandial muscle protein synthesis rates

For practical and methodological reasons, the majority of studies assessing muscle protein turnover during disuse in humans have been performed in the overnight fasted state. However, daily skeletal muscle protein turnover is a highly dynamic and sensitive process regulated in large part by nutrition (Rennie et al., 1982). Protein and/or amino acid ingestion increases muscle protein synthesis rates and inhibits muscle protein breakdown, albeit the latter to a lesser extent, resulting in a positive net protein balance (Paddon-Jones et al., 2004b; Rennie et al., 1982; Volpi et al., 1998, 1999). The post-prandial stimulation of muscle protein synthesis is mainly driven by the increase in plasma essential amino acid availability (Bohle et al., 2003; Rennie et al., 2002; Tipton et al., 1999; Volpi et al., 2003), and leucine in particular (Leenders and van Loon, 2011; van Loon, 2011; Wall et al., 2012). As such, muscle protein turnover oscillates throughout the night and day in terms of periods of negative (fasted) and positive (fed) net balance. Therefore, the magnitude and frequency of post-prandial stimulation of muscle protein synthesis is an important factor in normal skeletal muscle mass maintenance. Indeed, an impaired muscle protein synthetic response, or ‘anabolic resistance’, to dietary protein intake is now generally believed to play an important role in the development of age-related sarcopenia (Cuthbertson et al., 2005; Guillet et al., 2004; Rasmussen et al., 2006; Volpi et al., 2000), as well as other situations associated with progressive muscle loss such as cancer cachexia (Rennie et al., 1983), elective surgery (Crane et al., 1977; O’Keefe et al., 1974) and various critical illnesses (Rennie, 2009; Rennie and Wilkes, 2005). While it is clear that numerous other factors, such as tumor burden and inflammation, influence muscle loss in the aforementioned conditions (Williams et al., 2012), it is also true that they are all associated with a reduced physical activity status. Interestingly, advancing age has repeatedly been shown to progressively decrease habitual physical activity levels throughout the lifespan (Milanovíc et al., 2013; Tsunoda et al., 2013; Westerterp and Meijer, 2001). Strikingly, if physical activity is performed immediately prior to protein intake in elderly individuals, more of an ingested protein meal is subsequently used for de novo muscle protein synthesis (Pennings et al., 2011); an effect which seems to persist for as long as 24 h following exercise (Burd et al., 2011b), at least in young men. Furthermore, a higher level of physical activity stimulates both basal (Phillips et al., 1997) as well as post-prandial (Pennings et al., 2011) muscle protein synthesis rates. Taken together, these data have led us to speculate that impairments in muscle protein synthesis rates following dietary protein ingestion, such as those seen in older individuals, may be largely brought about by a decreased physical activity status (Burd et al., 2011a; Wall and van Loon, 2012).

In support of this hypothesis, it has been shown that 14 days of bed-rest reduces whole body protein synthesis rates in response to hyperaminoacidemia in young men (Biolo et al., 2002, 2004). Moreover, it has been demonstrated that the muscle protein synthetic response to amino acid administration following 14 days of leg immobilization is both delayed and of a lower magnitude in the immobilized vs control leg of healthy young males (Glover et al., 2008). These data provide compelling evidence that, aside from impairments in basal muscle protein synthesis rates, anabolic resistance to food intake contributes to muscle atrophy during a prolonged period of disuse. To add a further level of complexity, it is worthy of note that the impact of disuse on post-prandial muscle protein breakdown rates remains virtually unexplored. A possible approach which may aid the future development of this research is the application of deuterium oxide (‘heavy water’) in humans. This approach has proved capable of assessing long term changes in muscle protein metabolism such that the accumulation of environmental influences (e.g. nutrition, fasting (in)activity) over a period of days or weeks can be assessed (Robinson et al., 2011). To date, this approach has not been directed at human disuse studies.

2.3. Prolonged disuse atrophy in the elderly

While the loss of muscle mass during disuse presents numerous health complications to any individual, the elderly are of particular relevance. That is to say, for an already compromised elderly individual with a low physical activity status, just a small loss of muscle mass during a prolonged period of disuse may be detrimental for metabolic health and/or functional capacity. The magnitude of muscle loss that occurs during illness has been shown to be a clear indicator for the time required for hospitalization as well strongly influencing the time period required for rehabilitation in elderly individuals (Christensen et al., 1982). Furthermore, muscle loss in an older adult represents an important risk factor for falls and fragility fractures (Mital et al., 2012). Indeed, it has been reported that 71% of hip fracture patients were classified as sarcopenic (Fiatarone Singh et al., 2009). Hip fractures lead to dramatically impaired mobility and poorer quality of life even after two years of recovery (Kim et al., 2012). Moreover, hip fractures have a strong association with overall mortality (Browner et al., 1996; Center et al., 1999; Cornwall et al., 2004), with 34% of elderly hip fracture patients dying within 1 year of surgery (Holt et al., 2012) and
this number increases with advancing age (Holt et al., 2012). Thus, muscle mass maintenance in older individuals is a vital consideration to support healthy aging and reduce the burden on our health care systems. The muscle loss associated with a period of prolonged disuse could likely lead to the loss of independence, institutionalization and development of metabolic disorders such as obesity and type 2 diabetes. This underlines the importance of understanding the mechanisms underlying muscle disuse atrophy with specific reference to the elderly if we are to develop successful interventions. It could be speculated that anabolic resistance to food intake that has been seen in normal aging (Cuthbertson et al., 2005; Guillet et al., 2004; Rasmussen et al., 2006; Volpi et al., 2000), and induced by prolonged disuse (Biolo et al., 2002, 2004; Glover et al., 2008), may converge to become particularly significant in this population. In agreement, a recent study by Drummond and colleagues demonstrated a profound ~35% decline in the muscle protein synthetic response to essential amino acid ingestion in elderly individuals following 7 days of bed-rest (Drummond et al., 2012). Interestingly, while basal muscle protein synthesis rates also showed a (non-significant) decline, the authors concluded that the anabolic resistance to food intake was likely the key factor bringing about muscle disuse atrophy (Drummond et al., 2012). This highlights the need for future work to address the possibility of restoring (or maximizing) the anabolic response to food ingestion during disuse, particularly in the older and more clinically compromised populations.

3. Short-term disuse atrophy (<10 days)

Although a single bout of prolonged disuse provides an acute metabolic and functional challenge to elderly individuals, perhaps of more concern in the long-term are successive periods of short term muscle disuse. The average length of hospitalization for elderly patients admitted with acute illness is 5–6 days (Fisher et al., 2010). Moreover, periods of illness, minor injury and reduced physical activity which do not require hospitalization but necessitate inactive home recovery are likely to be short and typically last less than one week. As such, throughout the life-span, an individual will experience many periods of short-term disuse (<10 days). We (Wall and van Loon, 2012, and others (English and Paddon-Jones, 2010; Mithal et al., 2012), have hypothesized that these short periods of disuse will lead to substantial muscle tissue loss over the lifespan and, as such, represents an important consideration in the etiology of age-related sarcopenia. In support, we have recently generated pilot data fromelderly volunteers (n = 8) demonstrating that only 5 days of limb immobilization already leads to a 1.5% loss of quadriceps cross sectional area. When extrapolating this to a whole body level, merely 5 days of bed-rest would result in ~1 kg of muscle tissue lost. Structured and prolonged resistance-type exercise training is effective for muscle mass gain in the elderly (Evans, 1986; Fiararone et al., 1990) and so should be considered vital in the recovery from a period of disuse. However, current clinical practice does not mandate such a rehabilitation program following a period of disuse, and elderly individuals generally show low adherence to non-supervised, structured resistance-type exercise training (Dunstan et al., 2006; Kohler et al., 1994; Miller et al., 2008).

Indeed, following a period of bed-rest, it has been demonstrated that elderly individuals reduce their habitual daily activity and, even with structured, supervised training, spend more of their day completely inactive (Kortebein et al., 2008). Moreover, it has been shown in animals (Brooks and Faulkner, 1990; Zarzhovsky et al., 2001) and humans (Hvid et al., 2010; Suetta et al., 2009, 2013) that the elderly display a marked reduction in their ability to regain lost muscle tissue following a period of disuse, even with an intensive, supervised, resistance-type exercise training schedule. Thus, even optimistically assuming an ~80% recovery of muscle mass following each period of disuse in an elderly person, at least ~400 g of muscle tissue would be lost following only two short periods of illness or injury per year. This equates to 0.8% muscle loss per year, and therefore contributes largely to the estimated 1–2% yearly muscle loss from the age of 50 onwards (Buford et al., 2010; Nair, 2004); or, even more strikingly, would entirely account for recent (more conservative) estimates that sarcopenia occurs at a rate of ~0.5–1.0% muscle loss per year (Mitchell et al., 2012). Thus, it is clear that accumulated periods of muscle disuse atrophy play an important role in the development of age-related sarcopenia.

Compared to a more prolonged period, relatively few studies have addressed the early phase of muscle disuse. Those studies which have investigated short-term disuse have generally been in an effort to better understand the cellular and subcellular processes which precede measurable muscle atrophy at either the fiber or whole muscle level (Chen et al., 2007; Gustafsson et al., 2010; Reich et al., 2010; Tesch et al., 2008; Urso et al., 2006). Consequently, physiological measurements of functional capacity, muscle mass and muscle protein turnover in this early phase of disuse are sparse. However, there are indications that the rate of muscle loss may be greater in early stages of disuse. For instance, it has previously been shown that, following two weeks of limb immobilization, the greater part of the total muscle atrophy had already occurred after the first week (White et al., 1984a). Furthermore, profound muscle atrophy (3–12%) has been observed at the whole muscle level by magnetic resonance imaging after 7 days of bed-rest (Ferrando et al., 1995), by dual energy X-ray absorptiometry after 10 days of bed-rest (Kortebein et al., 2007) or by ultrasonography after 10 days of limb immobilization (Tom et al., 2001). While assessments of whole limb or body muscle mass over only a few days of disuse are currently lacking, it has recently been shown that only 4 days of limb immobilization leads to a ~10% decrease in mean muscle fiber cross sectional area in both young and elderly men (Suetta et al., 2012). Interestingly, studies employing much longer durations of disuse (17 weeks), have shown that the rate of muscle loss is only around 0.1% of inactive muscle tissue per day (LeBlanc et al., 1992a) as opposed to the 0.5–0.6% that can be expected during periods of disuse ranging between 10 and 42 days (Phillips et al., 2009; Wall and van Loon, 2012). Based on these data, we have recently proposed that substantial muscle atrophy likely occurs during short-term disuse, with rates of muscle loss much higher than typically observed during more prolonged disuse (Wall and van Loon, 2012). This contention suggests that the mechanisms responsible for the early loss of muscle during disuse must differ from those bringing about muscle loss during prolonged disuse.

3.1. Alterations of muscle protein turnover

To date, no human study has employed stable isotope methodology to assess dynamic muscle protein turnover following a period of disuse of less than 7 days. Thus, our understanding of how disuse modulates muscle protein synthesis and breakdown rates at this early stage is in its infancy and limited only to static molecular/enzymatic markers. Whether the decline in basal muscle protein synthesis rates that is clearly evident following 10–42 days of bed-rest (Ferrando et al., 1996, 1997, 2010; Kortebein et al., 2007; Symons et al., 2009) or lower limb immobilization (de Boer et al., 2007b; Gibson et al., 1987a, 1988; Glover et al., 2008) also occurs in the first few days of disuse is an important issue, but currently somewhat unclear. For instance, a microarray approach revealed a coordinated decline in the skeletal muscle mRNA levels of genes involved in protein synthetic pathways at 14, but not at 2 days of limb immobilization in young men (Abadi et al., 2009), suggesting that a decline in protein synthesis may only occur later on into disuse. In agreement, data from a recent microarray
an analysis reported that no genes implicated in muscle protein synthesis pathways were differentially expressed following 2 days of limb immobilization (Reich et al., 2010). It is important to note, however, that muscle protein synthesis is generally considered to be regulated post-transcriptionally by the phosphorylation of mammalian target of rapamycin (mTOR) and its subsequent downstream activation of P70S6 kinase (p70S6K/S6K1) and 4E-BP1 (Kimball et al., 2002; Kimball and Jefferson, 2001; Welle et al., 1999). By way of example, pharmacological blockade of the mTOR pathway has been shown to completely abolish the normally robust acute rise in muscle protein synthesis seen in the hours following amino acid administration (Dickinson et al., 2011) or muscle contraction (Drummond et al., 2009) in humans. As such, more rapid changes in the signaling proteins controlling translation are likely to be more informative of alterations in muscle protein synthesis rates compared to modifications in gene expression, which would be more meaningful after a longer adaptive period. With this in mind, it has also been shown that 2 days of disuse in young men leads to a profound reduction in the muscle phosphorylation status of Akt (protein kinase B) (Urso et al., 2006), a key upstream regulator of the mTOR/p70S6K pathway (Bohle et al., 2001). In agreement, recent data show that both Akt and p70S6K phosphorylation are suppressed between 1 and 4 days following the initiation of disuse in younger adults (Suetta et al., 2012), and in vivo muscle protein synthesis rates have been shown to decline over a 24 h inactive period following surgery (Tjader et al., 1996). Thus, while the current understanding of the time-course with which muscle protein synthesis becomes blunted with disuse is incomplete, the clearest data to date suggest a rapid suppression (i.e. within 2–4 days of disuse) which is then maintained indefinitely.

An increase in muscle protein breakdown following 3 days of limb immobilization has been inferred using a rise in muscle interstitial 3-methylhistidine concentrations as a marker of proteolysis (Tsesc et al., 2008). While this approach is not conclusive (Rennie et al., 2008), several studies have also employed a microarray approach to examine limb immobilization (2–3 days) induced changes in mRNA levels across almost the entire human genome (Abadi et al., 2009; Reich et al., 2010; Urso et al., 2006). Interestingly, a common finding of these studies was that the ubiquitin proteasome system was the most (or at least amongst the most) enriched pathways following short-term disuse (Abadi et al., 2009; Reich et al., 2010; Urso et al., 2006). However, protein breakdown as an entire functional unit was not necessarily up-regulated in these array analyses (Abadi et al., 2009). This suggests that muscle protein breakdown may indeed be elevated early on in disuse, but that it is primarily mediated by the ubiquitin proteasome pathway. In line, a previous study has shown that, following 48 h of disuse in young men, the ubiquitination of high molecular weight proteins is increased in parallel with an increased mRNA level of both MAFbx and MuRF1 (Abadi et al., 2009). These data suggest that the ubiquitin proteasome system actively contributes to muscle protein breakdown of larger muscle proteins (for example the myofibrillar proteins) (Murton et al., 2008). In keeping with this, other studies have also shown an increased mRNA expression of MAFbx and/or MuRF1 following 2 (Reich et al., 2010; Suetta et al., 2012), 3 (Gustafsson et al., 2010) or 4 (Suetta et al., 2012) days of limb immobilization in human volunteers. However, at present, data showing corresponding changes in the protein content of these ubiquitin ligases have not been reported (Reich et al., 2010). Few studies have addressed markers of muscle protein breakdown at multiple time points during disuse. However, two studies have reported that early indications (2 days) of an up-regulation in the ubiquitin proteasome system (ubiquitination of high molecular weight proteins and/or increased mRNA levels of MAFbx/MuRF1) during disuse do not persist to the same extent after 2 weeks (Abadi et al., 2009; Glover et al., 2010).

Thus, available evidence suggests that short-term disuse quickly induces a decline in rates of muscle protein synthesis and a parallel rapid, and possibly transient, rise in muscle protein breakdown. The combination of decreased synthesis and increased breakdown of proteins within skeletal muscle may explain the apparently greater loss of muscle mass during this early phase of disuse (see Fig. 1). We propose that the entire withdrawal of muscle contraction represents a significant homeostatic disturbance which leads to the initiation of the skeletal muscle remodeling process. Specifically, opposing changes in the rates of muscle protein synthesis and breakdown allow a rapid and transient rate of muscle atrophy to occur in the first few days of disuse while the muscle attempts to restore homeostasis. Thereafter, when disuse is maintained, muscle protein breakdown rates return back to near basal levels and a more modest rate of gradual muscle loss is facilitated primarily by a decline in the basal and post-prandial rate of muscle protein synthesis. This hypothesis highlights the clinical relevance of studying disuse atrophy over a shorter period.

4. Studying muscle disuse atrophy in elderly/compromised populations

An important consideration in the study of muscle disuse atrophy in humans is the (sub)population examined. The vast majority of our mechanistic insight into muscle loss during disuse has been obtained in young, healthy subjects (for an overview of all key papers see Wall and Van Loon, 2012), while relatively few studies have imposed a period of experimental disuse in older volunteers (see Table 1 for a summary of the key studies). Data directly assessing disuse induced changes in muscle protein turnover in elderly individuals are confined to only four studies (Drummond et al., 2012; Deutz et al., 2013; Ferrando et al., 2010; Kortebein et al., 2007) and disuse induced changes in myocellular signaling in older adults to a single recent study (see Table 1; Suetta et al., 2012). Furthermore, the specific impact of disuse on the skeletal muscle morphology and metabolism of more compromised elderly populations, such as those classified as frail, or surgery, hip-fracture or critically ill patients, has not yet been comprehensively addressed. Nevertheless, those studies carried out in older subjects have already allowed interesting considerations about potential interactions between aging and disuse to emerge; such as altered sensitivity of senescent muscle to disuse atrophy (Hvid et al., 2010; Kortebein et al., 2007; Suetta et al., 2012), anabolic resistance to dietary protein as a key causative factor (Drummond et al., 2008), and the reduced ability of older people to regain lost muscle tissue during the recovery from disuse (Hvid et al., 2010; Suetta et al., 2009, 2013). Bed-rest studies performed in elderly volunteers have also shown great promise in demonstrating the efficacy of new nutritional strategies aimed at attenuating detriments in muscle mass/function in a highly relevant populations (Deutz et al., 2013; Ferrando et al., 2010). Finally, research on muscle disuse atrophy in female populations is virtually non-existent. It is known that men experience greater muscle loss during aging than do women (Forbes and Reina, 1970; Hughes et al., 2002; Zamboni et al., 2003); though due to a longer life expectancy and lower initial muscle mass/strength, sarcopenia is often viewed as a greater health concern in females. An intrinsic sexual dimorphism in terms of normal muscle protein turnover rates do not seem to fully explain the differing rates of sarcopenia (Smith et al., 2012), but whether an altered frequency/severity of periods of disuse occur in males compared to females requires further investigation. As such, it is of great importance that future studies address muscle disuse atrophy during short periods of bed-rest or immobilization in both young and older volunteers, as well as paying particular attention to the (clinically relevant) sub-population studied, in order to...
better define the contribution of disuse to the etiology of sarcopenia.

5. Conclusions

The undesirable consequences of muscle disuse atrophy are plentiful and well documented. During prolonged (>10 days) disuse, skeletal muscle atrophy is likely to be primarily brought about by declines in both post-absorptive and post-prandial muscle protein synthesis rates; while changes in muscle protein breakdown rates do not seem to contribute substantially. However, during short-term disuse (<10 days), it is probable that a rise in muscle protein breakdown and a simultaneous decline in muscle protein synthesis converge to rapidly initiate the skeletal muscle atrophy process. The relevance of muscle disuse atrophy to the elderly needs to be carefully addressed. Acute bouts of disuse can lead to older individuals falling below a critical threshold in terms of metabolic and functional health, thus increasing the potential for falls, fractures and all-cause mortality. Furthermore, successive short periods of disuse throughout the life-span likely play an important role in the development of age-related sarcopenia. For these reasons, it is vital that disuse atrophy is considered within the context of age-related sarcopenia, and that future studies address the impact of short periods of muscle disuse on the physiological and molecular regulation of muscle protein turnover in both young and older adults. Such information will be of great significance in the physiological understanding and the successful prevention and treatment of age-related sarcopenia.

References


