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Review

The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy

A.J. Murton *, D. Constantin, P.L. Greenhaff

Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, The University of Nottingham, Queen's Medical Centre, Nottingham, UK

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ABSTRACT

Skeletal muscle exhibits great plasticity in response to altered activity levels, ultimately resulting in tissue remodelling and substantial changes in mass. Animal research would suggest that the ubiquitin proteasome system, in particular the ubiquitin ligases MAFbx/atrogin-1 and MuRF1, are instrumental to the processes underlying these changes. This review article therefore examines the role of proteasomal-mediated protein degradation in human skeletal muscle in health and disease. Specifically, the effects of exercise, disuse and inflammatory disease states on the ubiquitin proteasome system in human skeletal muscle are examined. The article also identifies several inconsistencies between published human studies and data obtained from animal models of muscle atrophy, highlighting the need for a more comprehensive examination of the molecular events responsible for modulating muscle mass in humans.

1. Introduction

The dynamic adaptation of skeletal muscle to changing requirements has been acknowledged for decades, but the molecular events underpinning these adaptations have only just started to become clearer. Intricately regulated anabolic and catabolic pathways are responsible for the maintenance of muscle protein content, which represents the bulk of skeletal muscle mass, and imbalance between these pathways results in a change in muscle mass that can sometimes be rapid. Classically, increases in skeletal muscle mass have been associated with individuals partaking in resistance exercise programs [1] or after the administration of anabolic agents, such as testosterone [2,3]. Conversely, a loss of muscle mass has been observed following disuse [4–6] and a number of disease states, including but not limited to; sepsis [7], cancer cachexia [8,9], AIDS [8], diabetes mellitus [9,10] and renal failure [11,12], with sometimes rapid onset and profound consequences.

In eukaryotic cells, four main mechanisms are responsible for the majority of cellular protein degradation, mediated via the actions of either cysteine-dependent aspartate specific proteases (caspases) [13,14], cathepsins [15], calcium-dependent calpains [16,17], or the ubiquitin proteasome system (UPS) [18,19]. A substantial body of evidence has accumulated implicating the UPS as the principal regulator of skeletal muscle atrophy [20]. This has led many researchers to focus on both the components and regulators of the UPS in skeletal muscle, with the hopes of providing a greater understanding of the mechanisms responsible for increased skeletal muscle protein degradation, and identifying clinically relevant therapeutic targets to ablate disease-induced muscle atrophy.

To date, the vast majority of the information about the mechanisms responsible for muscle atrophy has been obtained from cell line and small mammal (primarily rodents) based research, with limited information coming to light concerning the molecular mechanisms modulating skeletal muscle mass in humans. Whilst the similarity in organ systems, systemic physiology and genes between the rodent and human species is useful, the validity of information obtained from non-human studies needs to be questioned when considering their direct relevance to human situations of muscle hypertrophy/atrophy. Indeed, several reported observations give rise to this stance. Firstly, most studies utilise animals that are immature and still growing, where high rates of muscle protein synthesis, low metabolic stability (i.e. a weak capacity to maintain homeostasis) and a comparatively high basal metabolic rate are commonly observed [21]. In contrast, most human studies involve subjects of adult age where, in the absence of pathological events, display body weights that are effectively stable for months at a time, experience high metabolic stability, and have much lower specific metabolic rates that include lower rates of muscle protein turnover. Specific to skeletal muscle, the response of the processes governing muscle protein synthesis to insulin differs between rodents and humans, notably where insulin, even at supraphysiological doses, fails to augment muscle protein synthesis in humans without prior amino acid administration [22,23]. These observations, in conjunction with the discrepancies of both the stress response to experimental procedures and the general severity

* Corresponding author. Tel.: +44 0 115 82 30154; fax: +44 0 115 823 0142.
E-mail address: andrew.murton@nottingham.ac.uk (A.J. Murton).

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of the atrophy inducing conditions between animal models and studies conducted in informed, consenting, human adults, provides some cause for concern when translating cell-line/animal data to the human situation.

Nevertheless, findings from cell line, animal and human based research, has consistently pointed to the UPS as a pivotal component to the instigation and regulation of muscle protein breakdown. However, to date, a comprehensive examination of the literature pertaining to the regulation of the UPS in modulating human skeletal muscle mass, particularly in regard to the effects of exercise and disease, has not been undertaken. In this review article therefore, we will first outline the proposed involvement of the UPS in muscle atrophy, as discovered in cell lines and small animals. This will be followed by a detailed examination of published studies investigating the effects of exercise on the UPS pathways in human skeletal muscle. Subsequently, conditions that favour muscle atrophy will be examined and discussed, including the use of exercise to modulate the atrophic response of catabolic disease states.

2. The muscle atrophy 'program'

The increased expression of UPS constituents, including components of the 26S proteasome itself, and the prevention of increased proteolysis in atrophic conditions via the use of proteasome inhibitors [24–26], has led many to conclude that the UPS is intrinsically linked to the degradation of myofibril proteins in skeletal muscle [27]. Of note, it has been shown that the UPS is unable to degrade intact myofibrils [28], suggesting that an alternative system is responsible for initial myofibril disruption, possibly the result of increased caspase-3 [13] or calpain activity [29]. The UPS is an ATP-dependent proteolytic system that involves the degradation of target proteins, with substrates identified for degradation by the addition of ubiquitin (Ub) molecules, a process itself coordinated via the activity of a triplet of enzymes [30]. Ubiquitin transfer to targeted proteins represents a robust method for the specific targeting of protein families. In brief, Ub is first bound to the Ub-activating enzyme (E1) via a high-energy thioester bond in an ATP-dependent process (Fig. 1). Ubiquitin is subsequently transferred

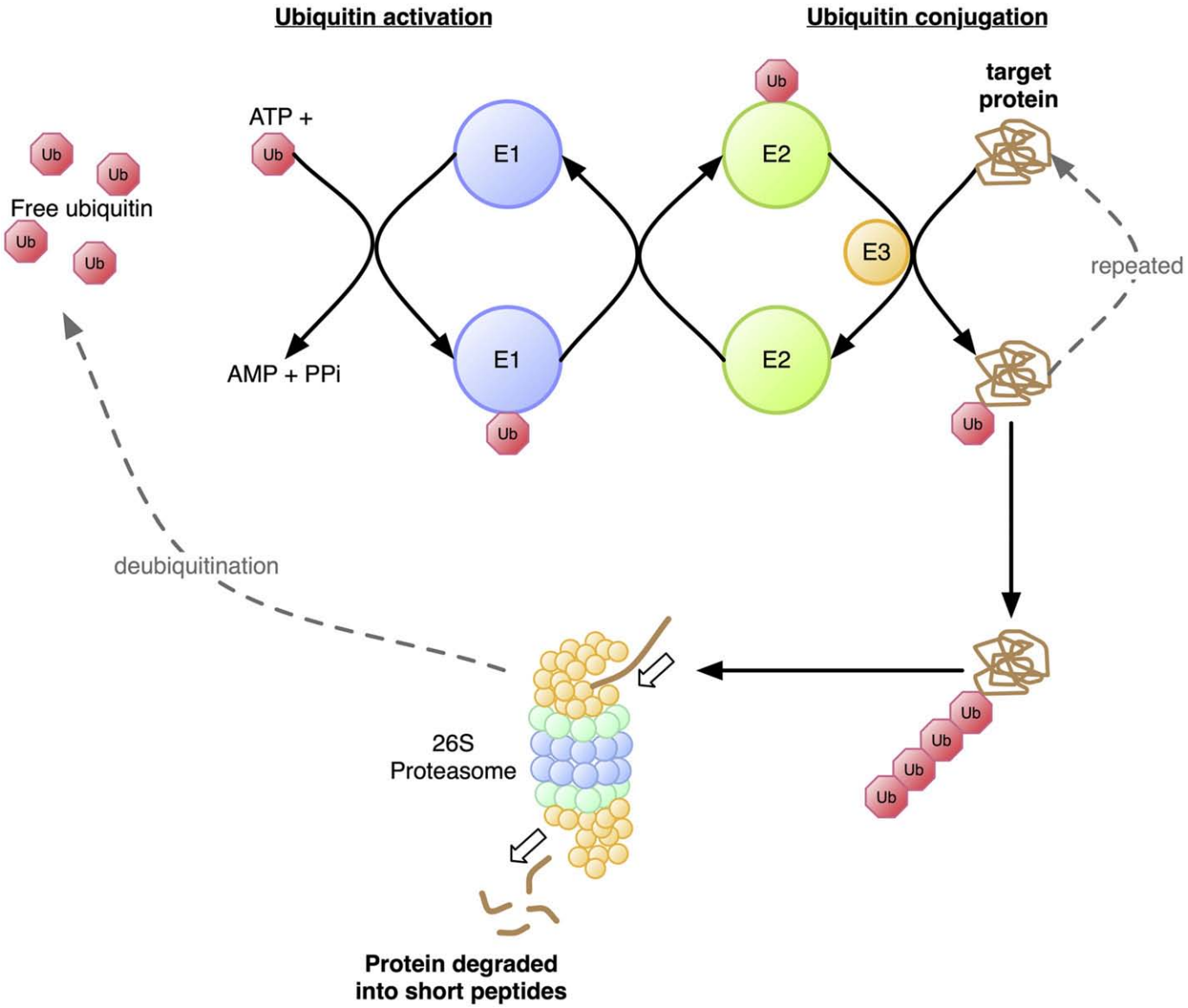

Fig. 1. UPS mediated protein degradation. Diagrammatical representation of the UPS detailing the activation and conjugation of free Ub onto Ub-ligase targeted proteins. Addition of four Ub monomers in a chain, covalently attached by lysine 76, is sufficient for entry into, and subsequent degradation by, the 26S proteasome. Ubiquitin chains are subsequently recycled via the action of the deubiquitinating enzymes.

from the Ub-activating enzyme to the Ub-conjugating enzyme (E2) via the formation of a new thioester linkage between Ub and a cysteine residue of the E2 enzyme. Generally, albeit with a few notable exceptions [31,32], the Ub monomer, catalysed by the action of an Ub-ligase enzyme (E3), is conjugated to the target protein via an isopeptide bond between the ε-amino group of a lysine residue in the target protein and the carboxy-terminal glycine residue 76 in Ub (for a review see [30]). The process is repeated until a minimum of four Ub monomers are covalently attached via lysine residue 48 of Ub to the target protein, the classical formation that is recognised by the 26S proteasome as a signal to degrade the target protein [33].

In eukaryotic cells, only one Ub-activating enzyme has been characterised and, observed in relatively high abundance, it meets the somewhat-divergent demands placed upon it by the UPS [34,35]. Indeed, even under normal physiological conditions, the UPS is constantly degrading damaged or malformed proteins [36], so as to maintain normal cell function. In humans, several dozens of Ub-conjugating enzymes are also present, in addition to hundreds of Ub-ligases [37], through which target specificity is established.

Following successful ubiquitination, and only once the criterion for recognition by the 26S proteasome has been met, proteins are unfolded and fed into the proteasome in an ATP-dependent process [19]. The 26S proteasome consists of a 20S catalytic core and 19S regulatory caps. Structurally, the 20S proteasome consists of four heptameric rings, formed from alpha subunits providing structural support [38], and the beta subunits responsible for the chymotrypsin-like, trypsin-like and caspase-like activities (for a comprehensive review see [39]). The proteasome cleaves tagged proteins into short oligonucleotides, after which the activity of tripeptidyl-peptidase II and exopeptidases result in almost complete degradation of the original protein [40].

In 2001, the mRNAs for two muscle-specific Ub-ligases were found to be elevated in the atrophied muscles of food-deprived mice [41] and limb immobilised rats [42]. These two Ub-ligases, MAFbx/atrogin-1 and MuRF1, were collectively termed 'atrogins' and increased mRNA levels for both were subsequently observed in various animal models of muscle atrophy, including but not limited to; burn injury [43], uremia [44], diabetes mellitus [44], denervation [42], unweighting [4,44], dexamethasone administration [45,46] and sepsis [49,50]. Moreover, when knocked out, MAFbx/atrogin-1$^{-/-}$ and MuRF1$^{-/-}$ mice appeared resistant to the effects of denervation-induced muscle atrophy, with a 56% and 36% respective sparing of muscle loss, compared to littermate controls [42]. Consistent with these findings, it was suggested that MAFbx/atrogin-1 and MuRF1 may, in part, be responsible for the UPS mediated muscle protein degradation observed during muscle atrophy conditions [47].

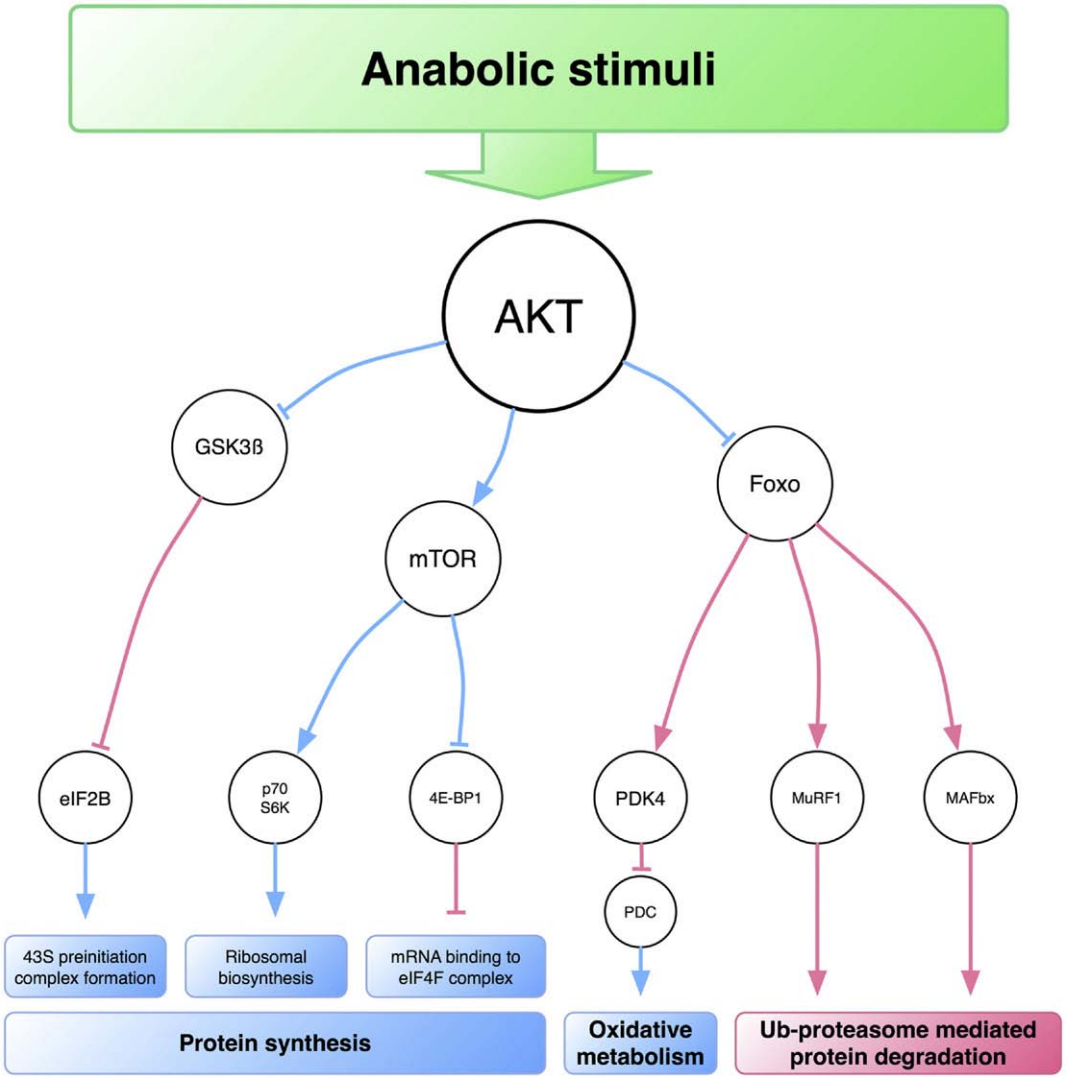

Fig. 2. AKT regulated signalling events. Representation of the effects of anabolic stimuli on AKT mediated signalling events in skeletal muscle. Blue and red colouring highlights pathways and events that are increased and decreased, respectively, following AKT activation (phosphorylation).

Recent evidence has suggested that the transcriptional regulation of MAFbx/atrogin-1 and MuRF1 Ub-ligases are intrinsically linked to both cellular metabolic status and inflammatory state via a coordinated pathway of signalling events. Li and colleagues discovered that both H_2O_2 and the catabolic cytokine tumor necrosis factor-alpha (TNFα), were competent inducers of MAFbx/atrogin-1 mRNA expression in skeletal muscle [48]. Moreover, they observed that TNFα induced expression of MAFbx/atrogin-1 mRNA was blunted by the p38 MAPK inhibitors SB203580 and curcumin, suggesting that TNFα works via a p38 MAPK dependent mechanism to stimulate MAFbx/atrogin-1 expression [49]. At about the same time, Cai and colleagues, with the use of constitutively active IκK mice (termed MIKK mice), showed an activation of NF-κB leads to an elevation in MuRF1, but not MAFbx/atrogin-1, mRNA [50]. Additionally, in the tibialis anterior of MIKK mice, both fibre diameter and fibre cross sectional area were reduced to 56% and 32% respectively, compared to control mice [50]. Moreover, MIKK mice crossed with MuRF1$^{-/-}$ mice experienced a 50% rescue of protein degradation [50], thus demonstrating that NF-κB mediated protein degradation is heavily dependent on the upregulation of the MuRF1 Ub-ligase. Collectively, these findings highlight that the transcriptional regulation of MAFbx/atrogin-1 and MuRF1 are sensitive to local inflammatory mediators (TNFα) and the NF-κB inflammatory pathway itself. Furthermore, they show MAFbx/atrogin-1 and MuRF1 to be able to function independently of each other.

Transcription of the MAFbx/atrogin-1 and MuRF1 genes also appear under the control of the forkhead Foxo family of transcription factors. It had been known for some time that transgenic overexpression of Foxo1 resulted in an atrophic phenotype [51]. Likewise, elevations in the forkhead transcription factors have been observed in muscle of animals subjected to starvation, glucocorticoid administration, diabetes, uremia and cancer [44,52]. The Foxo family of transcription factors are rendered inactive as a result of phosphorylation by AKT (also known as protein kinaseB) which results in the removal of the transcription factors from the nucleus where they are subsequently sequestered in the cytoplasm [53]. AKT represents an important mediator of the protein synthesis initiation pathway and is responsible for regulating the formation of the 43S preinitiation complex via GSK3β inhibition, synthesis of ribosomal proteins, and binding of the terminal 7-methylguanosine mRNA cap to the eIF4F complex via mTOR activation (Fig. 2; for a comprehensive review see [54]). Consequently, as the Foxo1 transcription factor is also known to increase the transcription of pyruvate dehydrogenase kinase isoform 4 [52,55], a reversible inhibitor of the pyruvate dehydrogenase complex [56], and thus a regulator of the decarboxylation of pyruvate to acetyl-CoA in the TCA cycle, the regulation of MAFbx/atrogin-1 and MuRF1 mRNA expression by Foxo forkhead transcription factors appears intrinsically linked to the rate of muscle protein synthesis and cellular oxidative metabolism. In support, our group has recently shown lipopolysaccharide-induced endotoxaemia to result in the coordinated reductions of AKT protein levels and cytosolic phosphorylation of Foxo1 and Foxo3. These observations were accompanied by significant increases in MAFbx/atrogin-1 and PDK4 protein levels and significant reductions in muscle protein content and pyruvate dehydrogenase complex activity in rodent fast-twitch muscle leading to an impairment of carbohydrate oxidation [57]. To clarify, during times of increased muscle protein synthesis, it would be hypothesised that MAFbx/atrogin-1 and MuRF1 mRNA expression would be suppressed, and vice versa. However, as discussed in detail later, in humans this relationship is not consistently observed.

3. Exercise and the UPS in human skeletal muscle

3.1. Eccentric contraction induced muscle "damage" and remodelling

Exercise, an essential component of a healthy and balanced lifestyle, elicits substantial metabolic and functional demands upon skeletal muscle in humans. The nature of these demands depends greatly upon the mode, intensity and duration of the exercise [58]. A number of human studies have examined the role of the UPS in skeletal muscle hypertrophy/remodelling following eccentric and concentric contractions and, to a lesser degree, endurance exercise. While either unaccustomed eccentric or concentric exercise elevates levels of both fractional protein synthesis rates and fractional protein breakdown rates [59], the cellular and subsequent morphological changes in response to the two exercise stimuli appear somewhat divergent. Unaccustomed or intense eccentric exercise has been associated with significant muscle damage, characterised by localised muscular pain, inflammation [60], extensive sarcomere disruption, z-disk streaming [61,62] and increased serum creatine kinase activity [63] and troponin I levels [64,65]. Concomitant with a rise in myofibrillar damage and inflammation/pain, muscle strength can be reduced by 50%, with a gradual recovery over the 5–10 day period post-exercise [66,67]. Conversely, whilst a single bout of concentric resistance exercise in untrained subjects can also induce myofibrillar disruption, the degree of damage is far more limited than that of eccentric exercise [67]. However, it should be noted that mild eccentric and concentric exercise displays only limited damage and, moreover, evidence suggests that eccentric loading is important for maximising the hypertrophic response to resistance training [68,69].

Biopsies obtained form the vastus lateralis of healthy male subjects 24 h after completing an intense eccentric exercise protocol show increased proportions of myofibrillar disruption compared to samples taken immediately post exercise [70]. Thus, a progression of the exercise induced muscle damage is dependent not only on the initial mechanical stress, but also on post-exercise events [70]. Consistent with this suggestion, an elevation in the mRNA and protein expression of free Ub and components of the 20S proteasome in skeletal muscles of subjects following a single bout of eccentric exercise, in addition to increased levels of Ub conjugated proteins 48 h post exercise (Table 1), strongly suggest that the UPS may play a part in eccentric contraction induced muscle damage. A sole report demonstrating no change in the amount of Ub-conjugated proteins following eccentric exercise was potentially due to an insufficient period of time between the exercise intervention and the period of measurement (24 h; [71]).

The induction of the UPS during eccentric contraction induced muscle damage appears instrumental in the remodelling of the skeletal muscle myofilaments; tentatively instigated to limit skeletal muscle damage from future eccentric bouts. Indeed, the degree of muscle damage associated with the original bout of eccentric exercise is attenuated following a second bout performed several weeks after the first [72,73]. Thus, adaptations must have occurred to limit the muscle fibre susceptibility to mechanical stress [67]. Suggested remodelling events include the addition of sarcomeres in series, resulting in shorter average sarcomere length, altered motor until recruitment patterns [74], and an increase in desmin protein levels resulting in a putative enhancement of z-disk stability [70]. Moreover, elevated levels of skeletal muscle heat shock protein's HSP27 and HSP72 have been observed following eccentric exercise [70,75,76]. The heat shock proteins represent a family of molecular chaperones; being expressed at times of cellular stress, they are responsible for both the maintenance of protein structure and orchestrating the repair of malformed/damaged proteins. As a consequence, heat shock proteins are considered to have anti-proteolytic and anti-apoptotic effects [77]. Collectively, this appears tantamount to the skeletal muscle cell trying to preserve function during a period of increased damage/remodelling elicited by exercise, which is dependent upon intensity [78].

The role of the UPS in the initiation of the 'protective repeated bout effect' is less clear. While it had been hypothesised that a reduction in UPS mediated proteolysis of myofibrillar proteins would be expected following subsequent bouts of eccentric exercise performed several weeks apart, presumably because sarcomeric remodelling had already

Table 1
Exercise and the UPS

	Measured component	Time point(s) measured	Intervention		Study
Eccentric exercise	***Ubiquitin***				
	↑ Ub mRNA and protein	6 and 24 h	KE — 7 sets×10 reps at 150% of 1-RM		[64]
	↑ Ub mRNA and protein	48 h	KE — 7 sets×10 reps at 150% of 1-RM		[76]
	↑ Ub	48 h	Dynamoter — 5 sets×53 reps		[168]
	↔ Ub-conjugated proteins	24 h	LP — 3 sets×12 reps at 120% of 1-RM KE — 10 sets×10 reps at 120% of 1-RM		[71]
	↑ Ub-conjugated proteins	48 h	Dynamoter — 5 sets×53 reps		[168]
	20S proteasome				
	↑ 20S mRNA and protein	48 h	KE — 7 sets×10 reps at 150% of 1-RM		[76]
	↑ HC2 and HC3 mRNA	6 and 24 h	KE — 7 sets×10 reps at 150% of 1-RM		[64]
	↑ 20S protein	24 h	KE — 7 sets×10 reps at 150% of 1-RM		[64]
	Ub-conjugating enzymes				
	↑ E2 mRNA and protein	6 and 24 h	KE — 7 sets×10 reps at 150% of 1-RM		[64]
	↑ E2 mRNA and protein	48 h	KE — 7 sets×10 reps at 150% of 1-RM		[76]
	Ub-ligase				
	↓ MAFbx/atrogin-1	3, 6 and 24 h	0.55 m step down every 2 s for 12 min		[97]
Resistance exercise	***Ub-ligases***				
	↑ MuRF1 mRNA	1–4 h	KE — 3 sets×10 reps at 70% of 1-RM	*	[91]
	↑ MuRF1 mRNA	2 h	LP — 4 sets×10 reps at 80% of 1-RM		[94]
	↑ MuRF1 mRNA	4 h	KE — 3 sets×10 reps at 70% of 1-RM		[93]
	↑ MuRF1 mRNA	4 h	KE — 3 sets×10 reps at 65% of 1-RM		[92]
	↑ MuRF1 mRNA	24 h	KE — 3 sets×10 reps at 70% of 1-RM	*	[91]
	↔ MuRF1 mRNA	24 h	KE — 3 sets×10 reps at 65% of 1-RM		[92]
	↔ MuRF1 mRNA	48 h	LP — 4 sets×10 reps at 80% of 1-RM		[94]
	↑ MAFbx/atrogin-1 mRNA	Immediately	LE — 10 sets×10 reps at 80% of 1-RM		[95]
	↑ MAFbx/atrogin-1 mRNA	1–4 h	KE — 3 sets×10 reps at 70% of 1-RM	*	[91]
	↓ MAFbx/atrogin-1 mRNA	3 h	LP — 8 sets×5 reps at 80% of 1-RM		[98]
	↔ MAFbx/atrogin-1 mRNA	3 h	LE — 8 sets×5 reps at maximal effort		[96]
	↔ MAFbx/atrogin-1 mRNA	4 h	KE — 3 sets×10 reps at 70% of 1-RM		[93]
	↔ MAFbx/atrogin-1 mRNA	4 h	KE — 3 sets×10 reps at 65% of 1-RM		[92]
	↓ MAFbx/atrogin-1 mRNA	6 h	0.55 m step up every 2 s for 12 min		[97]
	↔ MAFbx/atrogin-1 mRNA	8–24 h	KE — 3 sets×10 reps at 70% of 1-RM	*	[91]
	↔ MAFbx/atrogin-1 mRNA	24 h	KE — 3 sets×10 reps at 65% of 1-RM		[92]
	↓ MAFbx/atrogin-1 mRNA	24 h	LE — 10 sets×10 reps at 80% of 1-RM		[95]
	↓ MAFbx/atrogin-1 mRNA	48 h	LP — 4 sets×10 reps at 80% of 1-RM		[94]
	↔ MAFbx/atrogin-1 mRNA	72 h	LE — 10 sets×10 reps at 80% of 1-RM		[95]

Data obtained from human studies investigating changes in skeletal muscle UPS components following acute eccentric or acute resistance exercise bouts in non-previously resistance trained individuals. Time denotes period(s) elapsed from completion of exercise bout and recorded measurement. KE = Knee extension, LE = Leg extension, LP = Leg press, 1-RM = 1-Rep max. * = previously endurance trained.

occurred, contrasting observations have thus far been reported. For example, Willoughby et al. [64] showed greater increases in muscle Ub protein content in healthy volunteers following the first, rather than a second eccentric exercise bout conducted 3 weeks apart. Conversely, increased Ub-conjugation in skeletal muscle samples of the vastus lateralis have been observed on the second of two exercise sessions (involving eccentric leg press and knee extension exercises) separated by >5 weeks [71]. Thus, further work is required to rationalise these two observations and determine the involvement of the UPS in this adaptive response. It also seems that to provide meaningful information these experiments need to be performed in conjunction with measurements of muscle protein turnover.

The signal(s) responsible for instigating increased skeletal muscle UPS-mediated proteolysis following eccentric exercise are currently unknown, but the cytokines have garnered much attention. Exercise induced muscle damage is known to stimulate an acute phase inflammatory response resulting in the infiltration of neutrophils and macrophages into skeletal muscle [60]. Indeed, a single bout of eccentric exercise is known to increase macrophage counts per surface unit of tissue in skeletal muscle compared to controls [71]. Moreover, downhill running, which requires extensive eccentric contractions of the vastus lateralis muscle, results in localised elevations of TNFα and interleukin-6 (IL-6) mRNA and induces substantial muscle damage [79]. Interestingly, contracting skeletal muscle is one of the major sites of IL-6 production [70], however, the source of the elevated TNFα is less clear but is most likely to be macrophage-derived. Given that both TNFα and IL-6 are capable of inducing muscle atrophy [49,80–83] and of activating the UPS [49,84,85], it is tempting to suggest that they may be involved in the instigation of the remodelling process following eccentric damage. However, no evidence in humans yet exists to confirm these postulations. Moreover, given that eccentric exercise contractions often result in only a small percentage of severely damaged fibres and yet global reduced rates of glucose uptake to the order of 20–30% are observed, a systemic factor may be responsible for the events that follow eccentric-induced muscle damage [86–88].

3.2. Resistance training induced muscle mass gains

Heavy resistance training can induce substantial increases in muscle mass, predominantly the result of increases in fibre cross-sectional area (hypertrophy) as opposed to fibre number (hyperplasia). Moreover, resistance training can increase the cross-sectional area of all three major fibre types (I, IIa and IIb), with a 20-week training program sufficient to increase the cross-sectional areas of type IIab and IIb fibres by 47%, type IIa by 39% and type I by 17% [89]. The hypertrophic response to strength training appears predominantly the result of enhanced rates of muscle protein synthesis simultaneous to increases in muscle protein breakdown, albeit the change in the latter being of smaller magnitude than the former thus leading to an overall positive accumulation of muscle protein content [90]. Two groups have reported an elevation of MuRF1 mRNA levels up to 24 h [91–94], and MAFbx/atrogin-1 mRNA levels up to 4 h [91,95], following the induction of an acute bout of resistance exercise (Table 1). However, mRNA quantification of the atrogenes a few hours subsequent to these measurements have shown either reduced or basal levels [91–98]. Collectively, these

observations are in accordance with findings of a 31% increase in the fractional breakdown rate of skeletal muscle proteins 3 h post-resistance exercise, declining to an 18% increase by 24 h, and a return to resting values by 48 h [59]. The transient elevation of the fractional breakdown rate concomitant to an increase in MuRF1 and MAFbx/atrogin-1 mRNA levels is consistent with the suggestion that the enhanced leg proteolysis observed is in part MAFbx/atrogin-1 and MuRF1 dependent. However, measurements of the protein expression for the two atrogins are currently lacking and would represent a necessary prerequisite, in conjunction with the simultaneous determination of muscle protein breakdown, in confirming this hypothesis.

The above findings are largely based on studies involving a single bout of resistance exercise in previously untrained, recreationally active individuals. The response to a regular and progressive resistance exercise protocol is likely to differ. Chronic resistance exercise is known to elicit a significant increase in the proportion of type IIa fibres [89]. Moreover, greatest increases in MAFbx/atrogin-1 mRNA levels appear to occur in slow-twitch fibres following resistance exercise in humans (4 h and 24 h post-exercise; [92]), suggesting that the purported rise in MAFbx/atrogin-1 and MuRF1 mediated protein breakdown following resistance exercise represents a remodelling stimulus driving a change in fibre-type expression to that of a faster phenotype. Despite the extensive remodelling that occurs following a chronic resistance exercise regime, levels of MAFbx/atrogin-1 protein and MuRF1 mRNA remain elevated [99]. The consequences of the sustained elevation of the atrogins in response to chronic resistance exercise remains unknown, although suggestions levied of an increased basal rate of protein breakdown in the context of increased protein turnover remain speculative in the absence of measures of muscle protein turnover itself.

Increases in muscle protein synthesis are observed hours after a bout of resistance exercise paralleled by increases in the phosphorylation state of key components of the insulin-signalling pathway, in most cases including AKT [100]. However, two recent reports have suggested that the association between factors of translation initiation and muscle protein synthesis in the human may be complex, where changes in phosphorylation state of key members of translation initiation, including AKT, GSK3β and mTOR, appear not to mirror changes in leg protein synthesis [101,102], reinforcing the requirement of measures of muscle protein turnover in study designs. What is also not clear is the impact of Foxo inhibition (phosphorylation) by AKT on MAFbx/atrogin-1 and MuRF1 transcription in human skeletal muscle under conditions of resistance exercise. While reports in animals and C2C12 myotubes would suggest a pivotal role for this pathway in the control of MAFbx/atrogin-1 and MuRF1 expression [103,104], and by association, muscle protein breakdown in the initial hours following resistance exercise, its role in humans would appear modest. Indeed, given the discord reported between the mRNA levels of the two atrogenes [91–94] and the independent but contradictory reports of transient increases in expression of both Ub-ligases in the face of AKT activation following resistance exercise, other regulatory mechanisms are likely to dominate in human skeletal muscle. Intriguingly, an early increase in p38 MAPK by resistance exercise has been observed [95] which, in conjunction with localised muscle inflammation and NF-κB activation [105], could represent the primary elements responsible for exercise-induced expression of the atrogenes and thus, the early protein breakdown component of exercise.

However, skeletal muscle contraction also induces a marked elevation in plasma IL-6 [106]. Moreover, the degree of IL-6 expression is dependent on the intensity, duration and mode of the exercise [107] and appears muscle derived [108]. Interestingly, exercise also results in increased circulating levels of anti-inflammatory cytokines and the inflammatory cytokine inhibitors, IL-1 receptor antagonist and soluble TNFα receptors [106,109,110]; only very intense exercise stimulates TNFα expression [111]. In addition, IL-6 is typically the first cytokine to appear in the circulation during exercise and shows the most marked expression amongst the elevated cytokines. Intriguingly, treatment of cachectic murine colon adenocarcinoma with anti-mouse IL-6 antibody was successful at ablating the skeletal muscle atrophy commonly observed [112]. Furthermore, in C2C12 myotubes, IL-6 shortens the half-life of long-lived proteins [113]. Concordantly, transgenic mice overexpressing IL-6 experience profound muscle atrophy and elevated Ub gene expression, which is preventable by the administration of an IL-6 receptor antagonist [85]. Therefore, the exercise-induced expression of IL-6 could, tentatively, represent an alternative stimulus following resistance exercise for the early elevation in the UPS.

4. UPS in muscle atrophy conditions in humans

Skeletal muscle comprises ~40% of the total body mass of an average adult human and has energy requirements even at rest. Moreover, the inherent structure of skeletal muscle represents a substantial reservoir of protein that can be utilised by hepatic tissue during times of stress [114]. During periods of prolonged inactivity or disease, a substantial reduction in muscle mass is commonly observed, reducing total muscular energy requirements and, during disease, providing substrates for hepatic gluconeogenesis and acute phase protein production [114]. Despite these beneficial features, a dramatic loss of skeletal muscle mass can be debilitating, resulting in prolonged times to recovery, increased risk of subsequent injury and a severe burden on health care provisions. Pathological and environmental states that can induce skeletal muscle atrophy can be broadly divided into those with and without an inflammatory stimulus. Examples of the former include bed rest/immobilisation, spinal cord injury and fasting; whilst states with an inflammatory element include cancer, AIDS, sepsis and perhaps sarcopenia in the frail elderly. Despite both cohorts experiencing skeletal muscle atrophy, the mechanistic events between groups most likely differ and are examined in detail below.

4.1. Non-inflammatory skeletal muscle atrophy

Muscle disuse through limb immobilisation, bed rest or spinal cord injury represent the majority of non-inflammatory related atrophy seen in the clinical setting, and the degree of muscle loss can be appreciably large. Indeed, two weeks of leg-immobilisation has been shown to result in an almost 5% reduction in quadriceps lean mass and a 27% fall in isometric strength [4]. Likewise, a 20% reduction in type I muscle fibre cross sectional area and a 30% reduction in type II fibre area, has been observed following 60-days bed rest [115]. While elements of the UPS, notably levels of ubiquitinated proteins [116] and HC6 mRNA [4], appear elevated during disuse, a consistent elevation of MAFbx/atrogin-1 and MuRF1 mRNA has not been observed in human skeletal muscle in the studies performed to date (Table 2). While some reports have detailed an increase in MAFbx/atrogin-1 [4,116,117] mRNA to disuse stimuli in humans, a number have reported no change or a reduction in the mRNA levels of MAFbx/atrogin-1 [6] or MuRF1 [4,6,115,116]. In comparison, a classical elevation of the atrogenes has been observed in animals following denervation [42], hind limb suspension [42], or space-flight [118]. Numerous explanations exist for the disparity between animal models of disuse, where large and consistent elevations in expression of the atrogenes have been observed, and humans, where variable findings in regard to MAFbx/atrogin-1 and MuRF1 levels have thus far been reported. Most notable is the generally greater severity of the procedures performed on animals to induce muscle disuse compared to that employed in human volunteers/patients. Furthermore, with the desire to limit invasive procedures in humans, the number of muscle biopsies performed to obtain tissue is usually minimised; as a consequence, a comprehensive examination of the temporal expression of the atrogins in

Table 2
The UPS in inflammatory and non-inflammatory states characterised by muscle atrophy

	Measured variable	Condition	Muscle	Study
Non-inflammatory	***Ubiquitin***			
	↑ Ubiquitinated proteins	20-day bed rest	VL	[116]
	↓ Ubiquitylation rate	Primary operative fracture repair (within 8 h of fracture)	Various	[169]
	20S Proteasome			
	↑ HC6 mRNA	14–day leg immobilisation	VL	[4]
	↓ TPP II mRNA	10–21 day immobilisation	VL	[6]
	Ub-conjugating enzymes			
	↓ E2D3 Conjugating enzyme	Ankle fracture (4–11 days immobilisation)	GAS	[117]
	↓ E2N	Ankle fracture (4–11 days immobilisation)	GAS	[117]
	Ub-ligases			
	↑ Cbl-b mRNA	20-day bed rest	VL	[116]
	↔ E3 ligase mRNA	14-day leg immobilisation	VL	[4]
	↔ MAFbx/atrogin-1 mRNA	0–10 day immobilisation	VL	[6]
	↑ MAFbx/atrogin-1 mRNA	Ankle fracture (4–11 days immobilisation)	GAS	[117]
	↓ MAFbx/atrogin-1 mRNA	10–21 day immobilisation	VL	[6]
	↑ MAFbx/atrogin-1 mRNA	14-day leg immobilisation	VL	[4]
	↑ MAFbx/atrogin-1 mRNA	20-day bed rest	VL	[116]
	↑ MuRF1 mRNA	0–10 day immobilisation	VL	[6]
	↓ MuRF1 mRNA	10–21 day immobilisation	VL	[6]
	↔ MuRF1 mRNA	14-day leg immobilisation	VL	[4]
	↔ MuRF1 mRNA	20-day bed rest	VL	[116]
	↔ MuRF1 protein	60-day bed rest	VL	[115]
	↑ MuRF1 protein	60-day bed rest	SOL	[115]
Inflammatory	***Ubiquitin***			
	↑ Ubiquitin protein	Critical illness; 1–25 days after ICU admission (APACHE 8–31)	TA	[148]
	↑ Ubiquitin mRNA	Cancer patients pre-op	RA	[170]
	↑ Ubiquitin mRNA	Cancer patients pre-op	RA	[139]
	↑ Ubiquitin mRNA	Cancer patients pre-op	RA	[140]
	↑ Ubiquitin mRNA	Septic patients undergoing abdominal surgery	RA	[147]
	↑ Ubiquitin mRNA	Severe head trauma (Glasgow score 3–8)	VL	[150]
	↑ UbB polyubiquitin mRNA	Multiple injury; 8–12 days after trauma (APACHE II 15±3)	VL	[149]
	20S Proteasome			
	↑ HC3, HC5, HC7 and HC9 mRNA	Cancer patients pre-op	RA	[170]
	↑ HC2 and HC5 mRNA	Cancer patients pre-op	RA	[138]
	↑ HC2 and HC8 mRNA	Severe head trauma (Glasgow score 3-8)	VL	[150]
	↔ HC8 protein	COPD patients undergoing thoractomy for lung cancer	D	[153]
	↑ HC3 mRNA	Septic patients undergoing abdominal surgery	RA	[147]
	↑ Chymotrypsin-like activity	COPD patients undergoing thoractomy for lung cancer	D	[153]
	↑ Chymotrypsin-like activity	Cancer patients pre-op	RA	[140]
	↑ Trypsin-like activity	Cancer patients pre-op	RA	[140]
	↑ Peptidylglutamyl peptide-hydrolyzing activity	COPD patients undergoing thoractomy for lung cancer	D	[153]
	↑ Peptidylglutamyl peptide-hydrolyzing activity	Cancer patients pre-op	RA	[140]
	Ub-conjugating enzymes			
	↑ E214K	Severe head trauma (Glasgow score 3–8)	VL	[150]
	Ub-ligases			
	↑ MAFbx/atrogin-1 mRNA	COPD patients undergoing thoractomy for lung cancer	D	[153]
	↑ MAFbx/atrogin-1 mRNA	Acute quadriplegic myopathy	VL	[151]
	↑ MAFbx/atrogin-1 mRNA	COPD patients	VL	[152]
	↑ MAFbx/atrogin-1 mRNA	ALS patients	B/VL	[126]
	↔ MAFbx/atrogin-1 protein	COPD patients	VL	[152]
	↑ MAFbx/atrogin-1 protein	ALS patients	B/VL	[126]
	↑ MuRF1 mRNA	COPD patients	VL	[152]
	↔ MuRF1 mRNA	COPD patients undergoing thoractomy for lung cancer	D	[153]
	↔ MuRF1 mRNA	ALS patients	B/VL	[126]

Data obtained from human studies investigating changes in skeletal muscle UPS components following non-inflammatory and inflammatory states of muscle atrophy. COPD = Chronic obstructive pulmonary disease, ALS = Amyotrophic lateral sclerosis; VL = Vastus lateralis, GAS = Gastrocnemius, SOL = Soleus, TA = Tibialis Anterior, RA = Rectus abdominus, B = Bicep, D = Diaphragm.

response to disuse in the human, particularly in the first few days after onset, is currently lacking. A lone report examining temporal levels of MAFbx/atrogin-1 and MuRF1 mRNA reinforces the need for such work to be undertaken as a matter of course. de Boer et al. [6] showed 10 days limb immobilisation to result in elevated mRNA levels for MuRF1, but not MAFbx/atrogin-1, in the vastus lateralis of healthy human volunteers; however, 11 further days of limb immobilisation saw a subsequent fall in mRNA levels for both atrogenes. Given these shortcomings which, in conjunction with a general absence of data on the protein expression of the two atrogins (with the exception of [115]), it is not currently possible to discern the functional relevance of MAFbx/atrogin-1 and MuRF1 to the human condition of muscle disuse atrophy. The lack of protein levels of the atrogins is particularly pertinent given the recent observations of Vary et al. [119] who demonstrated acute alcohol intoxication in rodents to increase MAFbx/atrogin-1 and MuRF1 mRNA levels without resulting in a concomitant rise in muscle proteolysis. Such observations could suggest that MAFbx/atrogin-1 and MuRF1 do not necessitate muscle protein breakdown; but without direct measures of MAFbx/atrogin-1 and MuRF1 protein levels, it is difficult to ascertain the true significance of such findings. However, it should be noted that a few reports of muscle proteolysis occurring independently of changes in MAFbx/atrogin-1 and/or MuRF1 mRNA levels have recently been published, which would question the role of the atrogins in muscle proteolysis [50,119–121]. Further work is required to rationalise these important observations.

The Ub-ligases Cbl-b, Siah-1A and Nedd4, originally identified in animals following unloading [118,122], have been tentatively linked to disuse atrophy. Preliminary evidence from humans has shown a statistically significant, albeit small (~2.5-fold increase compared to controls) rise in Cbl-b, but not Siah-1 A, mRNA levels following disuse atrophy [116]; whilst the involvement of Nedd4 in muscle atrophy remains to be examined in human skeletal muscle. Thus, an examination of the involvement of Cbl-b and Nedd4 in the induction of disuse atrophy is warranted. Interestingly, a target of Cbl-b Ub-ligase activity includes growth factor receptors, possibly including IRS-1 [116]. Furthermore, with a bona-fide target of Nedd4 being Notch1, a protein associated with satellite cell proliferation and differentiation [122], the further study of both Ub-ligases appears judicious.

The advent and adoption of microarray technology has allowed the rapid identification of global changes in gene expression following an applied intervention and represents a valuable approach, particularly when integrated with functional measurements in a time-dependent experimental paradigm. However, to date, only a handful of human studies investigating muscle atrophy events have adopted the microarray approach. Despite this, several novel observations related to proteins associated with the UPS have been observed in three differing models of human muscle disuse atrophy. Firstly, in the immediate days following spinal cord injury (2 and 5 days from injury) increased expression changes in Ub E3-ligase C and the 26S proteasome non-ATP-ase regulatory subunit 11 has been revealed using the Affymetrix GeneChip approach [123]. Furthermore, increases in the Ub-conjugating enzyme, UBE2E, as well as the Ub-ligase, FBXO9, have been identified in muscle biopsies following limb immobilisation [124]. These immobilisation-induced changes were in addition to elevations in USP-6, a deubiquitinating-enzyme responsible for cleaving Ub from Ub tagged proteins, thereby purportedly allowing efficient recycling of Ub monomers during catabolic states, and SUMO-1, a small Ub modifier, previously postulated to prevent UPS-mediated protein degradation of conjugated proteins [124,125]. Lastly, 4–11 days of cast mediated immobilisation following ankle fracture resulted in 227 genes being differentially expressed compared to muscle from healthy control subjects prior to cast mediated immobilisation, with all bar 26 genes downregulated [117]. Interestingly, 96% of the transcriptional changes that occurred following limb immobilisation in ankle fracture patients were also observed in the contralateral leg of the patients, but these changes were not observed in the non-immobilised limb from cast-immobilised healthy volunteers [117]. Although perplexing, it is possible that the observed transcriptional changes in the contralateral leg of ankle fracture patients may be due to increased bed rest/decreased mobility following ankle fracture, highlighting the additional complexity of studying patient populations where a component of the observed muscle atrophy could be due to decreased activity levels [117]. However, elevated levels of MAFbx/atrogin-1 was only observed in samples isolated from patients with ankle fractures, an observation that is supportive of its role as a regulator of muscle atrophy; although in contrast, other members of the UPS such as E2D3 and E2N were downregulated in the muscle of ankle fracture patients when compared to healthy control subjects prior to cast mediated immobilisation [117].

With two notable exceptions [115,126], the general absence of protein measurements for the two atrogins is concerning and introduces compounding issues in the interpretation of current mRNA data for the role of the UPS in muscle atrophy. Interestingly, it has been suggested that the loss of lean body mass observed following disuse induced muscle atrophy in the human is primarily the result of a decrease in muscle protein synthesis [127]. Reductions in muscle protein synthesis following disuse atrophy have been reported by several groups, despite the maintenance of adequate nutritional intake [128,129]. de Boer et al. [6] detected a 50% reduction in myofibrillar protein synthesis after 10 days of limb suspension, which was similarly suppressed 11 days later. They approximated that if daily mixed muscle protein synthesis fell by 50% to 1.0–1.25% per day, protein breakdown rates would not need to be elevated above the basal rate of 2.0–2.5% per day to account for the 0.5% fall per day in cross sectional area. However, given the existence of the purported AKT/Foxo/atrogene axis [103,104], this observation of a marked reduction in muscle protein synthesis during human limb immobilisation cannot be easily reconciled with the proposal of unchanged muscle protein breakdown and moreover, is not in concordance with experimental observations of increased muscle protein breakdown in animals following either denervation [130], hindlimb suspension [131] or limb immobilisation [132]. In reality, evidence of changes in muscle protein degradation in humans over the time-course of muscle disuse remains to be demonstrated and represents an essential component in the determination of the true significance of UPS-mediated degradation during immobilisation induced atrophy.

Despite the aforementioned scepticism held by some in regard to the role of muscle protein breakdown in human muscle disuse, confocal microscopy of vastus lateralis muscle samples taken after 60-days bed rest has revealed MuRF1 immunoreactivity in the cytosolic/myofibrillar compartment of myofibres, with strong cytosolic immunofluorescence in subpopulations of atrophic myofibres [115]. Furthermore, work emanating from our group has shown the suppression of limb protein breakdown that occurs following intravenous amino acid infusion during periods of elevated serum insulin (30 mU l^{-1}), occur in conjunction with a fall in MAFbx/atrogin-1 and proteasome subunit HC2 protein levels, suggesting that MAFbx/atrogin-1 may in part be responsible for increased muscle protein breakdown in human muscle [102]. Notwithstanding where measurements of muscle protein breakdown remain outstanding, such observations would be in agreement with suggestions that the atrogins play a role in the loss of muscle mass during disuse, possibly by regulating cellular signalling mechanisms. Ubiquitin tagging and/or UPS-mediated degradation has long been considered a viable mechanism in the regulation of distinct pathways, as observed in the regulation of NF-κB activity by IκB (for a review see [133]). Interestingly, reduced muscle protein synthesis following amino acid deprivation has been observed in wild-type but not $MuRF1^{-/-}$ mice implicating MuRF1 as a negative regulator of muscle protein synthesis [134]. This is further reinforced by reports of $MuRF1^{-/-}$ $MuRF2^{-/-}$ mice displaying significant muscle hypertrophy and elevated levels of p70S6K in its active form, but notably, without changes in total levels of multiubiquitinated proteins and thus, presumably, degradation [137]. These findings, in conjunction with the currently known targets of the atrogins, which in skeletal muscle include MyoD [135] and eIF3f [136] for MAFbx/atrogin-1, and myosin heavy chain [47], creatine kinase [134] and possibly the myofibrillar proteins titin, nebulin and myosin light chain-2 [137] for MuRF1, suggest that in addition to proteolysis of myofibrillar proteins, the atrogins may also be responsible for suppressing anabolic and hyperplastic signals, including muscle protein synthesis. While the extent to which these findings are applicable to human skeletal muscle is unknown, it is intriguing to note that such observations are compatible with current reports in humans, although they do not explain why elevations in MAFbx/atrogin-1 and MuRF1 are not consistently observed in all models of disuse, though this could simply be due to the timing of muscle sampling failing to coincide with transient elevations of the atrogenes and/or that only transcriptional changes in the atrogenes have mainly thus far been examined. Work examining the temporal expression of the atrogins (mRNA and protein) during disuse in conjunction with measurements of protein degradation in humans should resolve this conundrum.

4.2. Inflammatory-mediated atrophy

Many disease states that are characterised by systemic inflammation are also associated with a muscle mass deficit, which can have a

rapid onset. Moreover, a number but albeit not all, inflammatory disease states also display increased levels of both UPS components and the two atrogenes (Table 2). Interestingly, mRNA levels for proteasomal subunits and Ub, in addition to proteasomal chymotrypsin-like activity measured in muscle samples of patients suffering from cancer, appear greatest in individuals experiencing significant weight loss [138–140]. Moreover, mRNA levels of proteasomal subunits HC2 and HC5 in cancer cachectic patients appeared dependent on the degree of weight loss [138]. Patients experiencing a weight loss greater than 10%, where muscle protein content starts to become significantly depressed, show a parabolic relationship between the two subunits and weight loss; with HC2 and HC5 mRNA increasing maximally for patients with 12–19% weight loss and declining levels thereafter [138]. Likewise, the Ub-conjugating enzyme $E2_{14K}$ also showed a similar parabolic relationship with weight loss as the two proteasomal subunits [138]. Importantly, significantly higher circulating levels of both IL-6 and C-reactive protein (CRP), an acute phase protein commonly employed as a marker for the magnitude of systemic inflammation, have been observed in weight-losing cancer patients compared to cancer patients without weight loss [141]. Furthermore, plasma levels of CRP and IL-6 appeared to correlate with skeletal muscle Ub mRNA levels in weight losing cancer patients [142]; while gastrointestinal cancer patients receiving the anti-inflammatory compound, ibuprofen, in conjunction with an appetite stimulator (megestrol acetate), demonstrated significant improvements in weight, mid-upper arm circumference and quality of life scores compared to patients receiving megestrol acetate alone, independent of changes in appetite [143]. Collectively, these findings would suggest that in cancer cachexia, the UPS is actively involved in the initial stages of muscle protein loss and furthermore, implicate the associated inflammatory response as a potential regulator of the catabolic processes underlying cancer cachexia.

Inflammatory states result in the systemic production of a multitude of inflammatory and anti-inflammatory cytokines. Moreover, the cytokine response observed differs from that elicited by exercise, where increases in TNFα and IL-1β are generally not observed [144]. Interestingly, numerous reports have demonstrated that the cytokines are capable of eliciting UPS-mediated protein degradation in skeletal muscle (for a review see [145]). Thus, a purported cytokine induction of the UPS could be responsible for the observed differences between disuse and inflammatory mediated muscle loss. However, determining causality for the cytokines in inflammatory states, especially in humans, remains particularly problematic.

Induction of the UPS has also been documented in the skeletal muscles of patients admitted to Intensive Care Units (ICU), a subpopulation of patients that often present with pronounced inflammatory responses [146]. In muscle samples from septic patients obtained during abdominal surgery, an elevation in mRNA levels for Ub and proteasomal subunit HC3 was detected [147]. In a subsequent study which utilised patients representing a larger spectrum of admissions to the ICU, including individuals suffering from sepsis, vascular disease or road traffic accident trauma, a mean daily decrease in cross sectional area of 4% for type II and 3% for type I fibres was observed in addition to an overall increase in Ub protein levels [148]. Furthermore, a separate study of ICU patients detailed a significant positive correlation between the levels of UbB polyubiquitin mRNA and the rate of protein degradation 8–12 days after admission, as determined by the rate of intracellular phenylalanine appearance by the isotope dilution technique [149], with the authors concluding that the rate of proteolysis after trauma is mainly regulated at the level of transcription. Additionally, these findings are in concordance with the work by Mansoor et al. [150], who demonstrated significantly increased mRNA levels of Ub, $E2_{14K}$ and HC2 in head trauma patients presenting with negative nitrogen balance and indices of increased protein breakdown (endogenous leucine production). Lastly, increased levels of MAFbx/atrogin-1 mRNA has been observed following acute quadriplegic myopathy, a subacute muscle disorder characterised by generalised progressive muscle weakness and atrophy that is predominantly associated with a patient history of sepsis, multiple organ failure, surgery and intensive care admission [151]. Collectively, these findings highlight the catabolic consequences of trauma in its various forms and demonstrate evidence that the reductions in muscle protein content are, at least in part, mediated via the actions of the UPS. However, a spectrum of critically ill patients will likely contain a host of different clinical presentations, each able to modify the catabolic response. Such possible influences include massive systemic inflammation, starvation, immobilisation, insulin resistance and denervation. This represents a limitation in the ability to determine the primary initiator of the catabolic response when utilising patient populations, if indeed such a thing exists.

Unfortunately, the number of studies that have examined the transcriptional changes of the two atrogenes in human inflammatory states is severely limited. While elevations in MAFbx/atrogin-1 mRNA have been observed in amyotrophic lateral sclerosis patients [126], conflicting results have been observed in chronic obstructive pulmonary disease (COPD) sufferers, where reports of both an elevation [152] and no significant change [153] in the level of the Ub-ligase have been reported (Table 2). However, where elevations in MAFbx/atrogin-1 mRNA have been reported, they do not appear to have led to increased levels of the MAFbx/atrogin-1 protein [152]. Furthermore, elevations of MAFbx/atrogin-1 mRNA were also not observed in the muscles of either old adults (>60 yrs; [154]) or very old women (~85 yrs; [93]). Similarly, inconsistent findings for MuRF1 mRNA levels have been observed in COPD sufferers, with both increased [152] and basal [153] levels of MuRF1 mRNA reported. Increased MuRF1 mRNA levels have been observed in elderly women (~85 yrs; [93]), but no change recorded in either amyotrophic lateral sclerosis patients [126]) or elderly adults (>60 yrs; [154]). However, given that the age-dependent loss of skeletal muscle mass (sarcopenia) is thought to be predominantly due to the resistance of skeletal muscle protein synthesis to circulating amino acids [155], it is not surprising that a robust induction of MAFbx/atrogin-1 and MuRF1 was not observed in elderly individuals. Taken together, it is difficult from the limited evidence available to determine if MAFbx/atrogin-1 and MuRF1 have an active role in the UPS-mediated degradation of muscle proteins during inflammatory diseases. Given the robust induction of the UPS in ICU and cancer cachectic patients, it may be that the study of more acute and extreme inflammatory states, as seen in sepsis [7] and following burn-injury [156], may provide greater insight into the purported roles of the two atrogins in humans. It is noteworthy that both sarcopenic and COPD patients have been reported to present with systemic low grade inflammation [157–159] and, arguably in COPD patients, the localised levels of cytokines in the hindlimb muscles may not be notably elevated compared to controls [160]. Indeed, components of the UPS in chronic conditions such as Cushing's syndrome and Duchenne muscular dystrophy are known to be unchanged compared to acute catabolic states [161].

Interestingly, where increases in atrogene mRNA levels have been reported in the skeletal muscle of COPD sufferers, a parallel increase in skeletal muscle cytoplasmic protein content of phosphorylated AKT simultaneous to an increase in both Foxo1 mRNA expression and Foxo1 protein levels present in the nucleus have also been recorded [152]. Likewise, enhanced MAFbx/atrogin-1 mRNA and protein levels in amyotrophic lateral sclerosis sufferers occurred in conjunction with a fall in AKT phosphorylation, but no change in the nuclear protein content of Foxo1 or Foxo3 [126]. Therefore, in contrast to a proposed regulation of MAFbx/atrogin-1 and MuRF1 transcription by an AKT-forkhead transcription factor axis (Fig. 2) [103,104], the expression of the two atrogenes appears to be dependent on another mechanism, at least in COPD or amyotrophic lateral sclerosis patients. The observation of a discord in the relationship between AKT and Foxo proteins is not without precedent. In human primary breast tumours lacking a

detectable phosphorylated AKT protein, Foxo3 was predominantly confined to the cytoplasm as opposed to the nucleus [162]. Reasons for this remain unclear.

5. Involvement of the UPS in exercise rehabilitation

For numerous years, exercise has been prescribed as an essential element of the rehabilitation program for individuals following muscle atrophy. A limited number of studies have examined the changes that occur in the UPS following the induction of exercise-mediated rehabilitation. For example, our laboratory has shown 6 weeks of resistance exercise training to be sufficient to reverse the ~5% fall in quadriceps lean mass observed after two weeks leg immobilisation ([4]; Fig. 3). Moreover, 24 h following cast removal and the first exercise session, mRNA levels of both atrogenes were decreased, returning to basal levels when compared to immediately

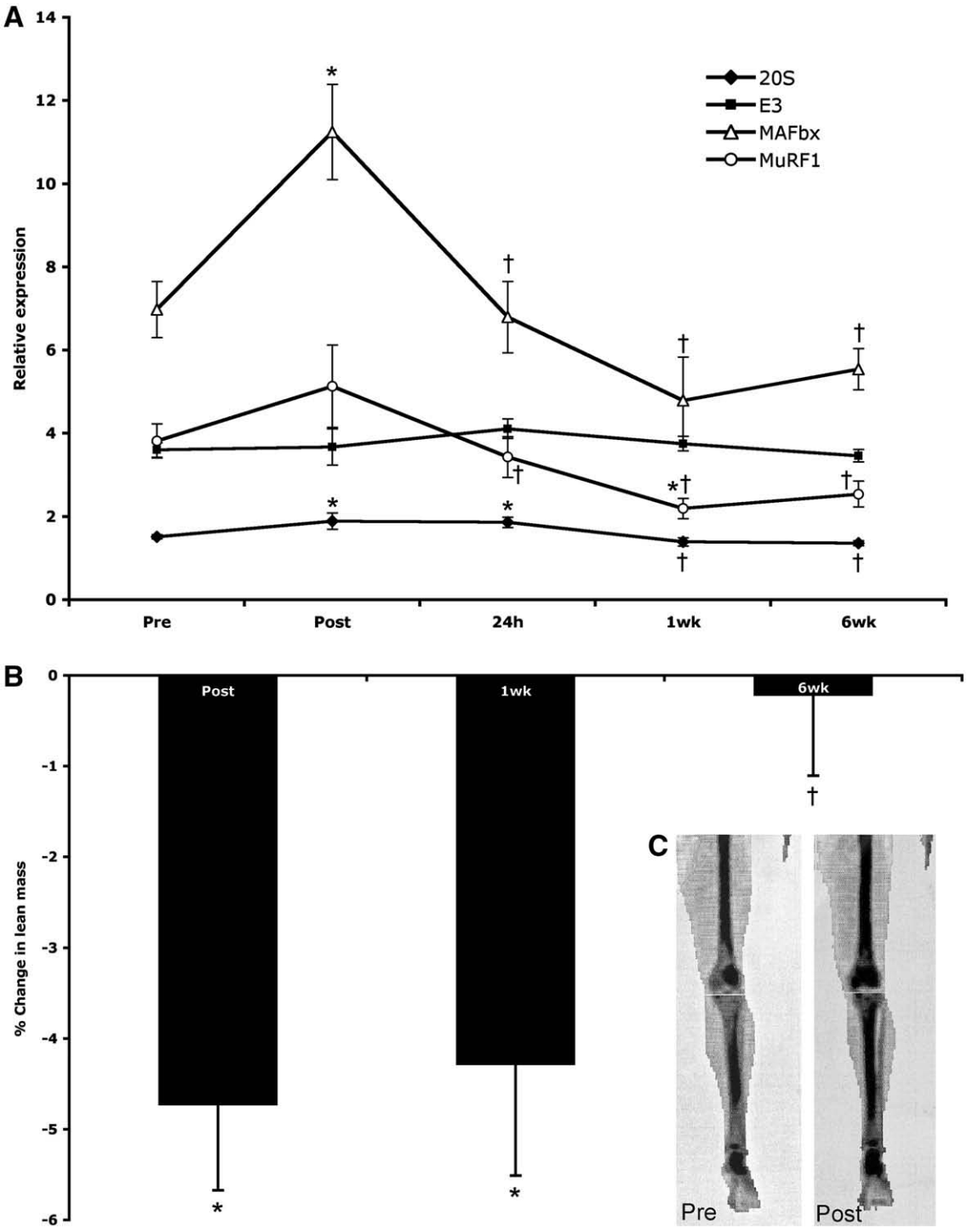

Fig. 3. Effect of leg immobilisation and rehabilitation on the UPS and muscle mass. Two weeks leg immobilisation and 6 weeks isokinetic rehabilitation exercise in healthy young men on: (A) mRNA expression of 20S (closed diamonds), ubiquitous E3 ligase (closed circles), MAFbx (open triangles), and MuRF1 (open circles, $p=0.1$ for pre- vs. post-immobilisation MuRF1 mRNA levels); (B) Change in quadriceps lean mass compared to basal. Bars/lines show mean±SEM; * significantly different from basal; † significantly differently from immediately post-immobilisation; significance defined at $p<0.05$. (C) Representative DEXA images localised to left leg pre- and post-immobilisation. Adapted from [4] and used with permission.

post-immobilisation. In addition, immobilisation induced mRNA increases in 20S proteasome subunit HC6, returned to normal levels 1 week from the start of the exercise regime [4]. Likewise, a combination of resistance exercises and aerobic exercises performed on opposing days, at an interval of once every 3 days, was able to prevent the increased protein expression of MuRF1 in soleus muscle and maintain fibre cross sectional area following 60-days bed rest [115]. These observations, in conjunction with reports that passive leg cycling in spinal cord injury patients significantly reduced mRNA levels of Ub, E2 and 20S [163], demonstrate that exercise is sufficient to blunt the UPS in human skeletal muscle following disuse atrophy. Whilst the significance of the exercise induced depression of UPS components on protein content is unknown, it is interesting to note its apparent relationship with the restoration of muscle mass.

Conversely, exercise (cycle ergometry) has been shown to exacerbate the increased expression of UPS components in COPD patients [164]. Such changes were not observed in healthy controls undergoing the same training protocol [164]. Interestingly, the pathophysiology of COPD could induce mild oxidative stress during periods of exercise [165] and such conditions are known to be capable of increasing the expression of UPS components [166]. However, it should be noted that it is unequivocal that endurance training improves exercise capacity, muscle force, quality of life and functional status of COPD sufferers [167].

6. Conclusions

In comparison to the wealth of knowledge from animal models concerning the involvement of the UPS in skeletal muscle atrophy and/or remodelling, studies in humans are at a comparatively early stage, with most studies based predominantly upon the examination of transcriptional events. However, initial findings would suggest that significant differences exist in the molecular events that underpin UPS mediated protein degradation in animals and humans in response to diverse stimuli. This is most notable in non-inflammatory conditions of muscle atrophy, where the role of the UPS and that of the atrogins, appear particularly exaggerated in animals compared to that observed in humans, although this could merely be a reflection of the disparity between the severity of animal and human models of muscle disuse. Thus, in more global terms, the role of muscle protein breakdown in these conditions remains equivocal. Furthermore, several discrepancies in the proposed regulation of the atrogenes between animals/cell lines and that of humans have started to emerge, suggesting alternative regulatory pathways that have yet to be described. Collectively, these findings highlight the pivotal importance for detailed human studies in determining the pathways regulating skeletal muscle mass in conditions of health and disease, utilising temporal measures of muscle protein turnover and biomolecular techniques across the spectrum of transcriptional, translational and post-translational events.

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