The Journal of Physiology

CROSSTALK

CrossTalk opposing view: The dominant mechanism causing disuse muscle atrophy is proteolysis

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Skeletal muscle adapts to a prolonged reduction in physical activity by decreasing muscle fibre size and protein content. This physiological response is commonly termed disuse atrophy. It is most clearly stimulated by conditions that reduce mechanical loading of postural or respiratory muscles in the absence of disease and neural deficits. Examples of such conditions include bed rest, joint immobilization, limb suspension, microgravity and mechanical ventilation. In muscle disuse, as in all atrophic processes, the rate of proteolysis exceeds the rate of protein synthesis causing net loss of protein. A dispassionate review of the literature shows that both aspects of muscle protein homeostasis are disrupted by prolonged disuse. Proteolysis increases and synthesis declines. These changes have additive effects, contributing jointly to protein loss and muscle atrophy. The relative magnitudes of these contributions have not been measured directly and are not known. Therefore, like most scientists, we are resolutely ecumenical on the issue of proteolysis *vs.* synthesis. In contrast, a few individuals fervently argue that disuse atrophy is caused by decreased protein synthesis and that proteolysis is unimportant. This viewpoint has created unnecessary controversy in the literature and heated discussion at scientific conferences. This controversy is the focus of our CrossTalk debate.

To be clear, the 'dominant mechanism' of disuse atrophy is not known. Rates of proteolysis *vs.* protein synthesis cannot be quantified using existing methods nor can differences in these rates be measured directly. Attempts to estimate these values must be based on first principles, must involve untested assumptions, and cannot be validated experimentally. This makes it impossible to assign 'dominance' with scientific rigor. Instead of speculating on this issue, we will present evidence for the biological importance of proteolysis in disuse atrophy. The extent of peer-reviewed research, the diversity of experimental approaches, and the level of mechanistic detail make a compelling case for our position that proteolysis is essential for disuse atrophy.

Disuse stimulates proteolysis

For almost four decades, we have known that disuse promotes atrophy of skeletal muscle by stimulating protein breakdown. This process involves the co-activation of multiple proteolytic mechanisms that have complementary roles in degrading muscle protein. The ubiquitin–proteasome pathway is the dominant mechanism of protein breakdown during muscle atrophy (Bodine, 2013). Disuse activates this pathway in muscle, as shown by three lines of evidence: First, muscle increases the expression of key gene products that regulate pathway activity. Mechanical unloading stimulates a rise in mRNA for the polyubiquitin gene, E2 ubiquitin conjugating proteins, E3 ubiquitin ligases, and subunits of the proteasome complex (Taillandier *et al.* 1996; Ikemoto *et al.* 2001; Jones *et al.* 2004; Nikawa *et al.* 2004; Hussain *et al.* 2010). Gene targets of particular interest are MAFbx/atrogin1 and MuRF1, two muscle-specific E3 ligases that regulate muscle atrophy in a variety of experimental models (Bodine *et al.* 2001). Like virtually all conditions of muscle wasting, MAFbx/atrogin1 and MuRF1 mRNA are increased in disuse atrophy. This response has been documented in diaphragm of mechanically ventilated humans and rodents (DeRuisseau *et al.* 2005; Levine *et al.* 2008, 2011; Hussain *et al.* 2010), limb muscles of humans after bed rest, lower limb suspension, or knee immobilization (Jones *et al.* 2004; de Boer *et al.* 2007; Abadi *et al.* 2009; Gustafsson *et al.* 2010; Reich *et al.* 2010; Wall *et al.* 2014), and limb muscles of rodents after hindlimb unloading, limb immobilization, or spaceflight (Nikawa *et al.* 2004; Senf *et al.* 2008; Andrianjafiniony *et al.* 2010). Second, upregulation of these pathway elements has the predicted outcome: ubiquitin conjugation to muscle proteins is increased in both humans and rodents (Ikemoto *et al.* 2001; DeRuisseau *et al.* 2005; Abadi *et al.* 2009; Ferreira *et al.* 2009; Levine *et al.* 2011; Brocca *et al.* 2012). Third, unloading increases the proteolytic activities of the 20S- and 26S-proteasome complexes. This occurs in human and rodent muscles (Ikemoto *et al.* 2001; Shanely *et al.* 2002; Levine *et al.* 2011), facilitating breakdown of ubiquitin-conjugated proteins.

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DOI: 10.1113/jphysiol.2014.279406

Muscle proteases and autophagy are also activated during disuse atrophy. This is particularly well documented for the cysteine-aspartic protease family, i.e. the caspases. The original discovery that unloading increases caspase-3 mRNA in the human diaphragm (Levine *et al.* 2008) led to animal studies confirming that caspase-3 enzyme activity is increased in unloaded diaphragm and limb muscles (Ferreira *et al.* 2009; Nelson *et al.* 2012; Talbert *et al.* 2013). Other members of the caspase family that are upregulated by unloading include caspase-6, -8, -9 and -12 (Taillandier *et al.* 1996; Andrianjafiniony *et al.* 2010). Calpain, a calcium-dependent protease, exhibits a similar response. Mechanical unloading of either diaphragm or limb muscle causes an increase in calpain mRNA and calpain activity (Taillandier *et al.* 1996; Shanely *et al.* 2002; DeRuisseau *et al.* 2005; Ferreira *et al.* 2009; Andrianjafiniony *et al.* 2010; Nelson *et al.* 2012; Talbert *et al.* 2013). Finally, emerging evidence indicates that autophagy via the lysosomal system is also increased during disuse atrophy of human and rodent muscles (Taillandier *et al.* 1996; Ikemoto *et al.* 2001; Andrianjafiniony *et al.* 2010; Hussain *et al.* 2010; Brocca *et al.* 2012). In aggregate, unloading activates all major proteolytic mechanisms – the ubiquitin–proteasome pathway, protease activity, and autophagy – thereby promoting the degradation of large molecular weight myofibrillar proteins, cytosolic proteins and smaller polypeptide residues.

Upregulation of proteolytic mechanisms (above) increases protein breakdown in unloaded skeletal muscle. This fact has been demonstrated in rodents using limb immobilization, hindlimb unloading, and mechanical ventilation (Goldspink, 1977; Loughna *et al.* 1987; Shanely *et al.* 2002) and in humans using unilateral lower limb suspension (Tesch *et al.* 2008). The latter study employed an innovative microdialysis technique to measure interstitial levels of 3-methylhistidine, an endogenous biomarker of myofibrillar degradation. Unloading for 3 days increased 3-methylhistidine levels by over 40%: evidence of increased breakdown of myofibrillar protein. This finding is consistent with recent evidence that human muscle undergoes significant atrophy within 2–5 days of being unloaded (Grosu *et al.* 2012; Suetta *et al.* 2012; Wall *et al.* 2014) and shows that greater protein breakdown is fundamental to the disuse atrophy process across species.

Conclusion

It is clear that proteolysis plays an important role in disuse atrophy, perhaps *the most* important role. This conclusion is firmly rooted in modern interdisciplinary biology (Fig. 1*A*). It incorporates what we know about muscle cell signalling, gene expression at the mRNA and protein levels, protein biochemistry, proteasome and protease activities, and regulation of protein breakdown. Plus it is inclusive, applying equally to postural and respiratory muscles of rodents and humans.

How can our learned opponent and a handful of other skeptics reject the preponderance of evidence on this issue? Historically, the position that proteolysis is unimportant has derived from measurements of fractional synthesis rate (FSR) in human vastus lateralis muscle during disuse. FSR data have been used to estimate the importance of synthesis based on first principles and assumptions that are either unstated or untested. This narrow approach (Fig. 1*B*), based on one end-point from one muscle of one species, has been justified by arguments that rodent models of disuse are 'heuristically misleading', that 'dynamic' FSR data are inherently more valid than 'snapshot' (i.e. any other) measurements, and that approaches which yield different outcomes are flawed and unreliable (Rennie *et al.* 2010). Such a position leaves little room for open-minded inquiry or free exchange of ideas. We must move beyond an either/or approach in order to broaden our understanding and advance the field. The time has come to acknowledge the fact that proteolysis is an essential component of disuse atrophy.

Figure 1. Word clouds illustrating the experimental approaches used to assess proteolysis (*A*) and protein synthesis (*B*) during disuse atrophy
3-MH, 3-methylhistidine; ULLS, unilateral lower limb suspension.

Call for comments

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Additional information

Competing interests

None declared.

Funding

This work was supported by the U.S. National Institute of Arthritis, Musculoskeletal, and Skin Diseases grants R01AR060209 (to A.R.J.), R01AR062083 (to M.B.R.), U.S. National Institute of Diabetes and Digestive and Kidney Diseases grant DK75801 (to S.C.B.), and U.S. Veterans Administration Rehabilitation Research and Development grant E7766R (to S.C.B.).