JOURNAL CLUB

From animals to humans: evidence linking oxidative stress as a causative factor in muscle atrophy

Tyler Barker[1,2] and Maret G. Traber[1,3]
[1]Department of Nutrition and Exercise Sciences, [3]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA
[2]Sport Science Department, The Orthopedic Specialty Hospital, Murray, UT 84107, USA

Email: tyler.barker@intermountainmail.org

In a recent issue of *The Journal of Physiology*, Urso *et al.* (2007) demonstrated that there was a significant increase in the gene expression of various components of a major proteolytic pathway (i.e. ubiquitin proteasome pathway) in human skeletal muscle following spinal cord injury, and thus limb disuse. This finding is important because extended limb disuse causes a disruption in the muscle homeostatic balance between protein synthesis and protein degradation. This imbalance in favour of protein degradation is characterized by both a decrease in protein synthesis and an increase in protein degradation, which subsequently, manifests as muscle atrophy. The mechanisms of protein degradation and the specific proteolytic pathways are relatively well known compared with those of protein synthesis during limb disuse. Of these proteolytic pathways, Ca^{2+} activated proteases (calpains) and the ubiquitin proteasome pathway have received considerable attention as significant contributors to the catabolic state leading to skeletal muscle atrophy. Additionally, these pathways appear to be markedly influenced by oxidative stress. While various studies in experimental animals and cell culture demonstrate that oxidative stress is an important cell regulator of these proteolytic pathways, the prevalence and physiological significance of oxidative stress on limb disuse muscle atrophy in humans remains unknown. It is clear that proteolytic pathways are stimulated during limb disuse in humans (Urso *et al.* 2006, 2007; Jones *et al.* 2004), which raises the questions, is there also an increase in oxidative stress during limb disuse in humans? and is this oxidative stress a causative factor that potentiates the degree of muscle atrophy?

Hypothetically, calpain activation precedes or functions 'up-stream' from the ubiquitin proteasome pathway. It is well-known that calpains are calcium-activated proteases; however, various calpain isoforms (i.e. μ- and m-calpains) are also sensitive to oxidative stress. Moreover, calpain activation in skeletal muscle may promote the infiltration of inflammatory cells (i.e. neutrophils) that also produce reactive oxygen species (i.e. hypochlorous acid from myeloperoxidase), and thus potentiate oxidative stress. Smith & Dodd (2007) found that calpain activation increased total protein degradation, as well as specifically proteasome-dependent proteolysis. Importantly, when proteasomes were inhibited, the increase in proteolysis following calpain activation was ameliorated. Thus, calpain activation appears to be associated with increased levels of oxidative stress and increased ubiquitin proteasome protein degradation.

In attempts to identify the genes that regulate muscle atrophy in humans, Urso *et al.* (2006) and Jones *et al.* (2004) examined alterations in mRNA and protein expression following limb immobilization. These studies compliment existing experimental data because they identify various proteolytic enzymes in humans that regulate muscle atrophy and are in agreement with previous experimental animal studies (Bodine *et al.* 2001; Gomes *et al.* 2001). Urso *et al.* (2006) found increased gene expression of various components of the ubiquitin proteasome pathway following only 48 h of limb immobilization; however, they did not find changes in protein expression of these components. Furthermore, gene expression of major regulatory enzymes of the ubiquitin proteasome pathway, especially various E3 ligases (MuRF1 and MAFbx/Atrogin-1), was unchanged. In contrast to these findings, an earlier investigation in healthy, young men reported increased gene expression of MAFbx ($P < 0.01$) and a trend toward increased MuRF1 ($P = 0.1$) following 2 weeks of limb immobilization (Jones *et al.* 2004). These studies collectively suggest that there is an increase in various components of the ubiquitin proteasome pathway following limb immobilization in humans. However, the up-regulation of some of the key regulatory components associated with the ubiquitin proteasome pathway may be time dependent since 48 h of limb immobilization (Urso *et al.* 2006) did not display the observed increase in E3 ligases that was observed after 2 weeks of limb immobilization (Jones *et al.* 2004). However, it should be noted that very rarely does an individual experience limb immobilization without a preceding injury or illness, and therefore, this observation raises the question whether the aforementioned findings translate to humans following injury or illness.

Skeletal muscle atrophy occurs rapidly following a variety of injuries, which include but are not limited to spinal cord and anterior cruciate ligament injuries. Following their limb immobilization study reported in 2006 (Urso *et al.* 2006), Urso *et al.* (2007) examined the influence of limb disuse as a result of a spinal cord injury on various components of the ubiquitin proteasome pathway. Interestingly, increased gene expression was observed at 2 and 5 days post-spinal cord injury; specifically various E3 ligases (UBE3C, atrogin-1 (MAFbx/FBXO32), and MuRF1) were up-regulated. Furthermore, at 5 days but not at 2 days post-spinal cord injury, atrogin-1 protein expression increased (Urso *et al.* 2007). Thus, the temporal up-regulation of key genes involved in the regulation of protein degradation by the ubiquitin proteasome pathway was faster following an initiating event (e.g. spinal cord injury). The data provided by Urso *et al.* (2006, 2007) and Jones *et al.* (2004) are in agreement with experimental animal studies that demonstrate that the ubiquitin proteasome pathway is up-regulated during various forms of muscular disuse in humans. However, the role of oxidative stress in triggering these mechanisms has yet to be clearly defined in human skeletal muscle.

Over the past 15 years oxidative stress has received increasing recognition as an intracellular mediator of the signalling pathways that regulate muscle atrophy, especially in the experimental animal literature. Servais *et al.* (2007) examined the influence of vitamin E supplementation on oxidative stress and skeletal muscle proteolysis in rats exposed to 14 days of muscular disuse (e.g. hindlimb suspension). Vitamin E

 DOI: 10.1113/jphysiol.2007.139378

supplementation to rats for 21 days prior to and during the 14 days of hindlimb suspension reduced soleus muscle atrophy by ∼17%, ameliorated the increase in muscle thiobarbituric acid-reactive substance (TBARS) content and MuRF1 mRNA, and showed a tendency to prevent the up-regulation of MAFbx and μ-calpain mRNA (Servais *et al.* 2007). Thus, antioxidant supplementation prior to experimental manipulations appears to minimize muscle atrophy in rats, and therefore suggests in humans that long-term antioxidant supplementation or routine consumption of high antioxidant-containing diets may play an important role in the protection against muscle atrophy and oxidative stress during periods of skeletal muscle disuse. Currently, it remains unknown if dietary antioxidant status or supplementation in humans provides protection against muscle atrophy during periods of limb disuse or reduced activity.

A novel class of oxidative stress-related factors has been identified in muscle atrophy studies. Metallothioneins are low molecular weight metal-binding (i.e. zinc and copper) proteins that contain cysteine residues and are induced by oxidative stress, among other stressors (i.e. glucocorticoids, catecholamines, etc.). Metallothioneins have also been suggested to display antioxidant properties because they can scavenge reactive oxygen species, and therefore may provide the protection against the elevation in oxidative stress that was responsible for their increased expression. Both metallothionein gene (Lecker *et al.* 2004) and protein (Kondo *et al.* 1992) expression increase in atrophying muscle in experimental animals. Importantly, metallothionein gene expression also increases in human muscle following limb immobilization (Urso *et al.* 2006) and spinal cord injury (Urso *et al.* 2007). Metallothioneins have been implicated in the atrophy programme gene (Lecker *et al.* 2004) and protein (Kondo *et al.* 1992) response as a result of oxidative stress in experimental animal studies. However, it is currently unknown if the observed increase in metallothioneins in atrophying muscle seen in humans is due to an increase in oxidative stress. Therefore, regulation of metallothioneins and their role in muscle atrophy requires further investigation.

Oxidative stress may be the link tying together various aspects of muscle atrophy. The gene expression of ubiquitin proteasome pathway components as well as various metallothioneins, increase during limb disuse as a result of a spinal cord injury in human skeletal muscle (Urso *et al.* 2007). These findings are important because they identify a specific proteolytic pathway and a component involved in antioxidant protection in human skeletal muscle following an initiating event where both the former and latter have been demonstrated to be induced by oxidative stress in experimental animal studies. These studies also emphasize that data obtained in various experimental animal limb disuse and cell culture studies potentially apply to human disuse muscle atrophy. However, it is currently unknown if there is an increase in oxidative stress in atrophying muscle in humans. Applying the acquired scientific knowledge gained from experimental animal studies pertaining to the physiological interaction between the proteolytic pathways and oxidative stress to humans could have a significant impact on the rehabilitation from various muscular disuse atrophy conditions.

References

Bodine SC, Latres E, Baumhueter S, Lai VKM, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan Z-Q, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD & Glass DJ (2001). Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* **294**, 1704–1708.

Gomes MD, Lecker SH, Jogaoae RT, Navon A & Goldberg AF (2001). Atrogin 1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A* **98**, 14440–14445.

Jones SW, Hill RJ, Krasney PA, O'Conner B, Peirce N & Greenhaff PL (2004). Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass. *FASEB J* **18**, 1025–1027.

Kondo H, Miura M, Nakagaki I, Sasaki S & Itokawa Y (1992). Trace element movement and oxidative stress in skeletal muscle atrophied by immobilization. *Am J Physiol Endocrinol Metab* **262**, E583–E590.

Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, Price SR, Mitch WE & Goldberg AL (2004). Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J* **18**, 39–51.

Servais S, Letexier D, Favier R, Duchamp C & Desplanches D (2007). Prevention of unloading-induced atrophy by vitamin E supplementation: links between oxidative stress and soleus muscle proteolysis? *Free Rad Biol Med* **42**, 627–635.

Smith IJ & Dodd SL (2007). Calpain activation causes a proteasome dependent increase in protein degradation and inhibits the Akt signaling pathway in rat diaphragm muscle. *Exp Physiol* **92**, 561–573.

Urso ML, Chen YW, Scrimgeour AG, Lee PC, Lee KF & Clarkson PM (2007). Alterations in mRNA expression and protein products following spinal cord injury in humans. *J Physiol* **579**, 877–892.

Urso ML, Scrimgeour AG, Chen YW, Thompson PD & Clarkson PM (2006). Analysis of human skeletal muscle after 48 h immobilization reveals alterations in mRNA and protein for extracellular matrix components. *J Appl Physiol* **101**, 1136–1148.