Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
The International Journal of Biochemistry & Cell Biology 37 (2005) 2156–2168
IJBCB
www.elsevier.com/locate/biocel

Review

Novel aspects on the regulation of muscle wasting in sepsis

Per-Olof Hasselgren*, Michael J. Menconi, Moin U. Fareed, Hongmei Yang, Wei Wei, Amy Evenson

Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA

Received 8 October 2004; received in revised form 17 November 2004; accepted 7 January 2005

Abstract

Muscle wasting in sepsis is associated with increased expression of messenger RNA for several genes in the ubiquitin-proteasome proteolytic pathway, indicating that increased gene transcription is involved in the development of muscle atrophy. Here we review the influence of sepsis on the expression and activity of the transcription factors activator protein-1, nuclear factor-kappaB (NF-κB), and CCAAT/enhancer binding protein, as well as the nuclear cofactor p300. These transcription factors may be important for sepsis-induced muscle wasting because several of the genes in the ubiquitin-proteasome proteolytic pathway have multiple binding sites for activating protein-1, nuclear factor-kappaB, and CCAAT/enhancer binding protein in their promoter regions. In addition, the potential role of increased muscle calcium levels for sepsis-induced muscle atrophy is reviewed. Calcium may regulate several mechanisms and factors involved in muscle wasting, including the expression and activity of the calpain–calpastatin system, proteasome activity, CCAAT/enhancer binding protein transcription factors, apoptosis and glucocorticoid-mediated muscle protein breakdown. Because muscle wasting is commonly seen in patients with sepsis and has severe clinical consequences, a better understanding of mechanisms regulating sepsis-induced muscle wasting may help improve the care of patients with sepsis and other muscle-wasting conditions as well.

Keywords: Muscle wasting; Atrophy; Calcium; Transcription factors; Nuclear cofactors; Calpain; Calpastatin

Contents

* Corresponding author. Tel.: +1 617 667 1810; fax: +1 617 667 2978.
E-mail address: phasselg@bidmc.harvard.edu (P.-O. Hasselgren).

1357-2725/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2005.01.017

1. Introduction

A number of catabolic disease states, including sepsis, severe injury, cancer, AIDS, diabetes, and renal failure, are characterized by muscle wasting, mainly reflecting increased breakdown of myofibrillar proteins (Hasselgren & Fischer, 2001; Lecker et al., 2004; Mitch & Goldberg, 1996). One of the consequences of muscle wasting is increased peripheral release of amino acids, especially glutamine (Mittendorfer, Gore, Herndon, & Wolfe, 1999; Rennie et al., 2001; Rosenblatt, Clowes, George, Hirsch, & Lindberg, 1983). A large proportion of amino acids released from muscle is utilized in various organs and tissues and this increased amino acid utilization may be important for survival. For example, amino acids are taken up by the liver at an increased rate in conditions characterized by muscle wasting, supporting gluconeogenesis and acute phase protein synthesis (Vary & Kimball, 1992). Glutamine is utilized by cells in the immune system (Newsholme & Parry-Billings, 1990) and is an important energy source in enterocytes (Windmueller & Spaeth, 1980). There is evidence that glutamine can protect the intestinal mucosa in patients with critical illness, possibly reducing the risk for multiple organ failure (Tremel, Kienle, Weilemann, Stehle, & Furst, 1994). Thus, it can be argued that the catabolic response in skeletal muscle may be a beneficial consequence of various catabolic conditions, providing the liver, gut mucosa, and cells in the immune system with an increased amount of amino acids important for maintaining cell function and integrity.

2. Muscle wasting in sepsis has severe clinical consequences

While it may be true that amino acids released from muscle can support the function of certain vital tissues, at least during the early phase of sepsis or immediately after trauma, it is generally believed that during sustained muscle wasting, such as seen in patients with severe and protracted sepsis, burn injury, and cancer, the negative effects of muscle atrophy will outweigh any beneficial effects of muscle breakdown. Continued loss of muscle proteins, in particular myofibrillar proteins, results in muscle atrophy and weakness that may have significant clinical consequences. For example, muscle atrophy prevents or delays ambulation in patients with sepsis or severe injury, such as burn injury. Prolonged bed rest, in turn, increases the risk for thromboembolic complications and inactivity further aggravates muscle protein degradation, thus creating a vicious cycle. When respiratory muscles are affected (Reid & MacGowan, 1998), there is increased risk for pulmonary complications and patients in intensive care units may require prolonged ventilatory support.

It should be noted that although increased breakdown of proteins, in particular the contractile proteins actin and myosin, is probably the major factor behind muscle wasting and weakness in critically ill patients, other mechanisms may also be involved. Recent studies suggest that muscle weakness in critically ill patients, in particular patients in the intensive care unit on ventilatory support and treated with large doses of steroids, may reflect neuropathy (critical illness polyneuropathy, CIP) or different types of myopathy [critical illness myopathy, thick filament (myosin) myopathy or necrotizing myopathy], rather than, or in addition to, loss of muscle mass (Hund, 2001). Indeed, this type of muscle weakness may be severe and may even result in quadriplegia (Larsson et al., 2000). It is interesting to note, however, that also in these conditions, muscle atrophy is commonly reported. In a recent review of CIP and myopathy, it was pointed out that even if muscle weakness caused by CIP can explain why some of these patients are difficult to wean from ventilatory support, these patients typically also have a marked generalized

muscle atrophy (Hund, 2001). Indeed, CIP results in functional muscle denervation, a condition that is well known to result in increased protein degradation and muscle atrophy (Medina, Wing, & Goldberg, 1995; Tawa, Odessey, & Goldberg, 1997). Other reports as well suggest that even if muscle membrane dysfunction and denervation caused by muscle relaxants are important mechanisms of muscle weakness in critically ill patients, these changes result in increased muscle proteolysis and loss of muscle mass (Wagenmakers, 2001). Thus, although increased muscle proteolysis and atrophy may not be the only factors responsible for muscle weakness in patients with critical illness, muscle wasting certainly seems to be an important component of muscle weakness even in patients with prominent neuropathy and myopathy.

Mediators and mechanisms of sepsis-induced muscle cachexia were discussed in detail previously (Hasselgren & Fischer, 2001). In particular, the role of glucocorticoids and some of the proinflammatory cytokines in the regulation of muscle protein breakdown during sepsis was discussed (Hasselgren, 1999; Hasselgren & Fischer, 2001). In addition, the involvement of the ubiquitin-proteasome system in sepsis-induced muscle wasting, including changes in the expression of the ubiquitin ligases atrogin-1 and MuRF1, was reviewed in detail (Lecker, 2003; Wray, Mammen, Hershko, & Hasselgren, 2003). In other reviews, signaling pathways involved in the development of muscle atrophy were described, in particular the PI3K/Akt signaling pathway and the downstream activation of the kinase GSK3-β and the FOXO transcription factors (Glass, 2003; Sandri et al., 2004; Stitt et al., 2004). In the present report, we will discuss other aspects of muscle wasting during sepsis. In particular, we will review novel aspects on changes in the expression and activity of certain transcription factors as well as nuclear cofactors in septic muscle. Evidence for a role of calcium and calcium-regulated calpain activity as an important early, and perhaps rate-limiting, mechanism of sepsis-induced muscle proteolysis and the potential role of calcium in proteasome activation and apoptosis in skeletal muscle will also be discussed.

3. Transcription factors and nuclear cofactors

Previous studies provided evidence that the expression of several genes associated with proteolytic mechanisms is increased in skeletal muscle during sepsis. In particular, mRNA levels for ubiquitin, multiple 20S proteasome subunits and the 14 kDa ubiquitin conjugating enzyme E2 14k were increased in muscle from septic rats (Hobler, Wang, et al., 1999; Tiao et al., 1994) and patients (Tiao, Hobler, et al., 1997). In other experiments, evidence was found that the increased mRNA levels did not reflect increased stability of the transcripts but reflected increased transcription of the various genes (Tiao, Lieberman, Fischer, & Hasselgren, 1997). In more recent studies we found that the gene expression of the ubiquitin ligases E3α, atrogin-1 and MuRF1 was increased in muscle of septic rats (Fischer, Sun, Gang, Pritts, & Hasselgren, 2000; Wray et al., 2003). These were important observations because the expression and activity of ubiquitin ligases may be rate-limiting for the development of muscle atrophy (Lecker, 2003). A recent study suggests that calcineurin may be a target protein for atrogin-1 in heart muscle (Li et al., 2004) but the substrate for atrogin-1 in skeletal muscle, in particular in septic muscle, remains to be determined.

Because sepsis-induced muscle wasting is associated with increased transcription of various genes regulating protein degradation, it is likely that changes in the expression and activity of certain transcription factors are important for the development of muscle wasting during sepsis. In recent studies, we examined the influence of sepsis in rats on the DNA binding activity and expression of the "inflammatory" transcription factors activator protein-1 (AP-1), nuclear factor-kappaB (NF-κB), and CCAAT/enhancer binding protein (C/EBP) (Penner, Gang, Sun, Wray, & Hasselgren, 2002; Penner, Gang, Wray, Fischer, & Hasselgren, 2001). These transcription factors are of particular interest because the promoters of various genes in the ubiquitin-proteasome pathway contain multiple potential binding sites for AP-1, NF-κB, and C/EBP (Penner, Gang, Sun, et al., 2002). Sepsis, induced by cecal ligation and puncture in rats, resulted in a biphasic response in skeletal muscle of NF-κB characterized by an early increase and a late decrease in NF-κB DNA binding activity (Penner, Gang, Wray, et al., 2001). In a recent study by Cai et al. (2004), additional support for a role of NF-κB in muscle wasting was reported. In that study, activation of NF-κB through muscle-specific transgenic expression of activated IκB kinase in mice resulted in pronounced

muscle atrophy. The muscle wasting was caused by ubiquitin-proteasome-dependent protein breakdown and muscle levels of MuRF1 mRNA were increased. In the same study, muscle-specific inhibition of NF-κB through transgenic expression of an IκBα superrepressor reduced denervation- and tumor-induced muscle loss, providing additional strong evidence for a role of NF-κB in muscle wasting. Studies in cultured myotubes further support the concept that NF-κB is important for the regulation of muscle mass (Du, Mitch, Wang, & Price, 2000; Guttridge, Mayo, Madrid, Wang, & Baldwin, 2000; Ladner, Caligiuri, & Guttridge, 2003; Langen, Schols, Kelders, Wouters, & Jansen-Heininger, 2001; Li & Reid, 2000).

Although there is strong evidence that NF-κB regulates muscle mass, other transcription factors probably play an important role as well. In recent studies in our laboratory, sepsis resulted in a sustained increase in the expression and activity of AP-1 and C/EBP (Penner, Gang, Sun, et al., 2002; Penner, Gang, Wray, et al., 2001). Among these transcription factors, we have been particularly interested in the C/EBP transcription factor family because binding sites for these transcription factors are prevalent in the promoters of various genes in the ubiqitin-proteasome pathway (Penner, Gang, Sun, et al., 2002). In recent experiments we found that sepsis in rats resulted in increased expression and DNA binding activity of C/EBPβ and δ in skeletal muscle (Penner, Gang, Sun, et al., 2002). The sepsis-induced increase in the expression and activity of C/EBPβ and δ was prevented by the glucocorticoid receptor antagonist RU38486, suggesting that the transcription factors are at least in part regulated by glucocorticoids. We observed further evidence for a role of glucocorticoids in the regulation of C/EBPβ and δ in skeletal muscle in recent experiments in which treatment of rats in vivo or of cultured L6 myotubes in vitro with dexamethasone resulted in increased expression and activity of C/EBPβ and δ (Yang, Mammen, et al., 2005).

It should be noted that although a parallel regulation of various ubiquitin-proteasome-related genes and C/EBPβ and δ by glucocorticoids and sepsis is consistent with the concept that the genes are regulated by these transcription factors in atrophying muscles, additional experiments are needed to test if there is a causal relationship between C/EBP and ubiquitin-proteasome genes in septic muscle. We are presently in the process of testing the role of C/EBP in sepsis-induced ubiquitin-proteasome-dependent muscle proteolysis by examining the effect of sepsis in C/EBPβ-deficient mice.

In recent studies, evidence was found that Foxo transcription factors regulate the atrogin-1 gene in muscle cells (Sandri et al., 2004; Stitt et al., 2004) but the expression and activity of Foxo transcription factors in skeletal muscle during sepsis are not known.

It is well known that gene transcription is regulated not only by transcription factors but by other mechanisms as well. Among such mechanisms, the expression and activity of various nuclear cofactors are particularly important. Nuclear cofactors influence gene transcription by different mechanisms, including histone acetyl transferase activity and protein–protein interaction with various transcription factors (Janknecht, 2002).

In recent experiments, we tested the influence of glucocorticoids (an important mediator of sepsis-induced muscle proteolysis) on the expression and activity of the nuclear cofactor p300 in skeletal muscle. In those experiments, treatment of cultured L6 myotubes with dexamethasone resulted in time- and dose-dependent increases in protein and gene expression of p300 (Yang, Menconi, Wei, Petkova, & Hasselgren, 2005). Interestingly, treatment of the myotubes with dexamethasone also resulted in increased protein–protein interaction between p300 and C/EBPβ as determined by co-immunoprecipitation. Thus, glucocorticoid-induced muscle proteolysis may be associated with, and perhaps at least in part caused by, increased expression and activity of various transcription factors and nuclear cofactors and an interaction between these factors. These observations further support the concept that muscle wasting is regulated at the gene level.

4. Calcium and muscle wasting

Calcium is a ubiquitous second messenger regulating numerous cell functions, including gene expression, some forms of programmed cell death, motility, secretion, proliferation, and cell survival (Carafoli & Klee, 1999; Rosen, Ginty, & Greenberg, 1995). Research during the last 10–15 years has

provided evidence that increased calcium levels and redistribution of calcium between different intracellular compartments are important signaling mechanisms by which a number of hormones, growth factors, and cytokines regulate cell metabolism. Binding of calcium to calmodulin and subsequent activation of calcium-calmodulin dependent kinases (CaMK), in particular CaMK II, are important upstream events in many calcium-induced biological effects. Interestingly, calcium does not only regulate phosphorylation (by activating CaMK II and other kinases, including protein kinase A and C), but can also regulate dephosphorylation by activating phosphatases, such as calcineurin (Ferrand-Drake et al., 2003).

Several lines of evidence support a role of calcium in muscle wasting. First, calcium levels are increased in skeletal muscle in various conditions characterized by muscle atrophy, including sepsis (Fischer et al., 2001; Wray, Sun, Gang, & Hasselgren, 2002), burn injury (Sayeed, 2000), denervation (Joffe, Savage, & Isaacs, 1981) and muscular dystrophy (Engel, Mokri, Jerusalem, Sakakibarda, & Paulson, 1997). Second, treatment of muscles in vitro with calcium or the calcium ionophore A23187 increased protein degradation (Baracos, Greenberg, & Goldberg, 1986; Furuno & Goldberg, 1986; Kameyama & Etlinger, 1979; Lewis, Anderson, & Goldspink, 1982). Third, treatment of experimental animals with "calcium antagonists" inhibited muscle proteolysis in various catabolic conditions. For example, in previous studies in this and other laboratories, treatment of rats with dantrolene, a substance that blocks the net release of calcium from the sarcoplasmic reticulum into the sarcoplasm (Krause, Gerbershagen, Fiege, Weisskorn, & Wappler, 2004), prevented the sepsis-induced increase in muscle calcium levels and protein breakdown rates (Fischer et al., 2001; Hotchkiss & Karl, 1994; Wray et al., 2002). In other experiments, treatment of mice with the L-type calcium channel blocker nifedipine prevented muscle protein loss caused by hindlimb immobilization (Wagatsuma, Fujimoto, & Yamada, 2002).

In addition to stimulating protein degradation, there is evidence to suggest that calcium may regulate multiple other mechanisms involved in the development of muscle wasting. These mechanisms include activation of the calpain–calpastatin system, the ubiquitin-proteasome pathway, various transcription factors, and apoptosis (Fig. 1).

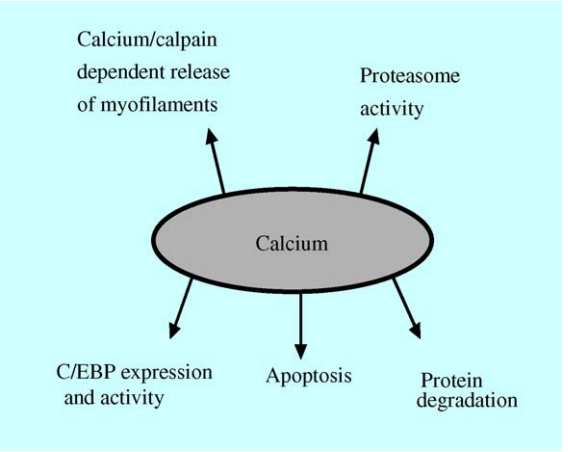

Fig. 1. Increased muscle calcium levels may regulate multiple processes that participate in the development of muscle wasting during sepsis. Note that some of the potential effects of calcium in skeletal muscle proposed here are speculative presently. For example, although increased calcium levels in cultured myotubes are associated with activation of caspase-3, consistent with increased apoptosis (Menconi et al., 2004), recent studies in our laboratory suggest that caspase-3 is not activated in skeletal muscle during sepsis (Wei et al., 2005). The influence of calcium on C/EBP and other transcription factors involved in muscle wasting also remains to be determined.

5. Muscle wasting and the calpain–calpastatin system

Although calcium may influence muscle protein metabolism through multiple mechanisms, the most important mechanism is probably regulation of the calpain–calpastatin system. The calpains constitute a family consisting of at least 14 members that may be ubiquitous enzymes, such as μ- and m-calpain, or tissue specific proteins, such as the muscle specific calpain 3, also called p94 (Goll, Thompson, Li, Wei, & Cong, 2003). The regulation of calpain activity is complex. The most important activator of calpain is calcium but calpain activity is also regulated by the endogenous inhibitor calpastatin (Goll et al., 2003). Interestingly, calcium does not only activate calpains but also regulates the binding of calpastatin to calpain, resulting in inhibited calpain activity. In addition, calpain is autocatalyzed so that activated calpain degrades itself. Finally, activated calpain can degrade calpastatin, adding further complexity to the regulation of the calpain–calpastatin system.

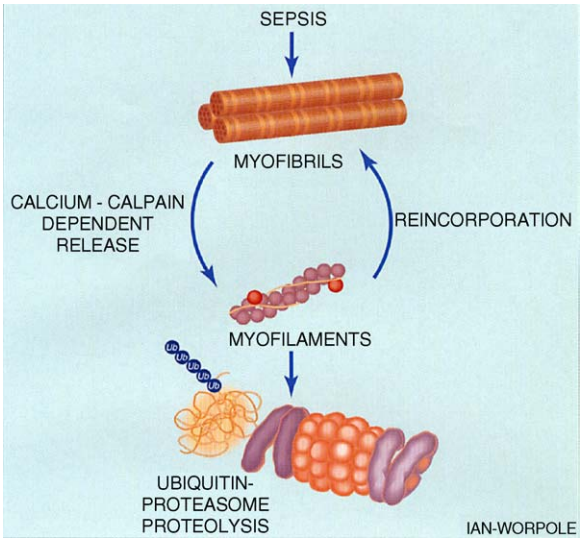

Fig. 2. Calcium-calpain dependent release of myofilaments from the myofibrils may be an early, and perhaps rate-limiting, component of sepsis-induced muscle wasting. In this model, myofilaments (mainly actin and myosin) released from the myofibrils are ubiquitinated and subsequently degraded by the 26S proteasome. Reprinted from Hasselgren, P.O. (2002). Molecular regulation of muscle wasting. *Science* & *Medicine, 8*, 230–239 (with permission).

Support for a role of calpains in muscle wasting caused by sepsis was provided in previous studies in which we found that the gene expression of μ- and m-calpain and p94 was increased in muscle of septic rats (Williams et al., 1999). In reports from other laboratories, muscle calpain activity in rats was increased during sepsis (Bhattacharyya, Thompson, & Sayeed, 1991). In more recent experiments, we found evidence that the sepsis-induced increase in calpain activity in skeletal muscle was secondary to inhibited calpastatin activity (Wei et al., 2005). Based on those and similar observations, we and others have proposed a model in which increased calpain activity provides an early, and perhaps rate-limiting, step in muscle wasting, accounting for degradation of Z-band-associated proteins, in particular titin and alpha-actinin and release of myosin and actin from the myofibrils (Goll et al., 2003; Hasselgren & Fischer, 2001; Huang & Forsberg, 1998; Solomon, Baracos, Sarraf, & Goldberg, 1998; Solomon, Lecker, & Goldberg, 1998; Williams et al., 1999). In this model, the released actin and myosin are subsequently ubiquitinated and degraded by the 26S proteasome (Fig. 2).

It should be noted that in addition to sepsis, other catabolic conditions as well have been associated with increased calpain expression and activity. For example, a role of calpain-mediated proteolysis was reported in patients with acute quadriplegic myopathy (Showalter & Engel, 1997) and in various muscular dystrophies (Tidball & Spencer, 2000). Activation of calcium-dependent proteolysis (Costelli, Tullio, Baccino, & Melloni, 2001) and increased gene expression of m-calpain (Busquets et al., 2000) were observed in tumor-bearing rats. Transfection of cultured myotubes with a dominant negative m-calpain resulted in a 30% inhibition of protein degradation and when calpastatin was overexpressed, protein degradation was reduced by 63% (Huang & Forsberg, 1998). In the same study, inhibition of calpain activity also stabilized nebulin and it was concluded that calpains play a key role in the disassembly of sarcomeric proteins.

Evidence of inhibited calpastatin activity as a mechanism of increased calpain activity, as seen in recent experiments in our laboratory in muscle from septic rats (Wei et al., 2005), has been reported by others as well. For example, Tidball and Spencer, (2002) found that muscle-specific overexpression of calpastatin in mice reduced muscle atrophy by approximately 30% in a model of muscle wasting consisting of hindlimb unloading for 10 days. Costelli et al. (2001) reported that calcium-dependent muscle proteolysis in tumor-bearing rats reflected reduced calpastatin activity. In other studies, calpain-dependent protein degradation in differentiating myoblasts reflected reduced calpastatin expression and increased calpain/calpastatin ratio (Barnoy, Supino-Rosin, & Kosower, 2000).

It should be noted that although we and others have found evidence that increased calpain activity plays an important role in muscle wasting conditions, contradictory results have also been reported. Thus, in a recent study, Du et al. (2004) provided data suggesting that increased caspase-3 activity, rather than calpain activity, may be the “upstream” mechanism by which myofilaments are released from the myofibrils followed by subsequent degradation by the ubiquitin-proteasome system in skeletal muscle from rats with acute diabetes or chronic uremia. It is possible that the roles of calpains and caspases differ in different conditions because we found that in septic muscle (in which calpain activity was increased) caspase-3 activity was not altered (Wei et al., 2005). The question whether calpains or cas-

pases are most important for the development of muscle wasting is further complicated by the fact that there is evidence for a complex interaction (cross-talk) between the two systems (Neumar, Xu, Gada, Guttmann, & Siman, 2003).

6. Calcium and proteasome activity

Although there may be some controversy with regards to the role of calpains versus caspases in muscle wasting, there is more universal agreement that upregulated expression and activity of the ubiquitin-proteasome proteolytic pathway is essential for the development of muscle atrophy (Hasselgren & Fischer, 2001; Lecker, 2003; Lecker et al., 2004; Mitch & Goldberg, 1996). In this mechanism, proteins are targeted for degradation by conjugation of multiple ubiquitin molecules to the protein. Ubiquitinated proteins are recognized by the 26S proteasome and degraded in the central channel of the 20S proteasome.

The mechanisms by which the 26S proteasome activity is increased in atrophying muscle are not fully understood. One important mechanism regulating the rate by which proteins are degraded by the ubiquitin-proteasome pathway is the ubiquitination of proteins, a process that in turn is regulated by a number of different enzymes, including the ubiquitin activating enzyme, ubiquitin conjugating enzymes and ubiquitin ligases. The mechanisms regulating the ubiquitination of proteins in muscle wasting conditions were reviewed previously (Hasselgren & Fischer, 2001; Lecker, 2003; Lecker et al., 2004; Mitch & Goldberg, 1996). In addition to an increased supply of ubiquitinated proteins, the "intrinsic" activity of the proteasome is probably increased as well as suggested by increased activity in proteasomes isolated from septic muscle (Hobler, Williams, et al., 1999). Although multiple reports from our and other laboratories suggest that the gene expression of several proteasome subunits is upregulated in catabolic muscle, the protein levels of the subunits are not increased (Bailey et al., 1996; Hobler, Williams, et al., 1999). Thus, the stimulated proteasome proteolytic activity is probably caused by mechanisms other than increased abundance of the proteasome. One such mechanism may be increased calcium levels.

The first evidence for a potential role of calcium in the regulation of proteasome activity was reported by Kawahara and Yokosawa (1994). In that study, treatment of unfertilized ascidian eggs with the calcium ionophore A23187 resulted in increased proteasome activity, determined by measuring the degradation of proteasome-specific substrates. This effect of A23187 was abolished by the cell-permeable calcium chelating agent BAPTA-AM, suggesting that the proteasome activity was regulated by increased intracellular calcium levels. In subsequent work from the same group (Aizawa, Kawahara, Tanaka, & Yokosawa, 1996), treatment of Xenopus eggs with A23187 resulted in increased proteasome activity, suggesting that regulation of the proteasome by calcium is not cell specific.

In recent studies in our laboratory, the role of calcium in the regulation of proteasome activity in muscle cells was tested (Menconi, Wei, Yang, Wray, & Hasselgren, 2004). In those experiments, treatment of cultured L6 myotubes with A23187 resulted in a time- and dose-dependent increase in proteasome activity. When different metabolic inhibitors were used, results suggested that activation of CaMK II is an important upstream mechanism of calcium-mediated proteasome activation. In addition, we found evidence that both caspases and calpains are involved in activation of the proteasome by calcium. Furthermore, treatment of the myotubes with thapsigargin, a substance that increases cytoplasmic calcium levels by blocking uptake of calcium into the sarcoplasmic reticulum, stimulated proteasome activity. This was an important observation because it suggests that increasing cytoplasmic calcium levels through different mechanisms may have a similar effect on the proteasome.

The results from our experiments with A23187-treated myotubes are in line with previous reports in which we and others found that treatment of rats with dantrolene prevented the sepsis-induced increase in muscle calcium levels, protein breakdown rates and proteasome activity (Fischer et al., 2001; Hotchkiss & Karl, 1994; Wray et al., 2002). Although those results provided indirect evidence that calcium may be important for the regulation of proteasome activity in skeletal muscle, the experiments in which myotubes were treated with A23187 or thapsigargin were important because they suggest that calcium has a direct effect on proteasome activity in muscle.

7. Calcium and C/EBP transcription factors

In addition to regulating calpain and proteasome activities, there may be other mechanisms by which calcium influences muscle wasting. One such mechanism is the regulation of the expression and activity of various transcription factors. Considering our previous observations that C/EBPβ and δ may be involved in sepsis- and glucocorticoid-induced muscle atrophy (Penner, Gang, Sun, et al., 2002), previous studies indicating that calcium may regulate the activity of different members of the C/EBP transcription factor family are particularly interesting. Wegner, Cao, and Rosenfeld (1992) reported that treatment of cultured pituitary cells with A23187 resulted in increased C/EBP DNA binding determined by electrophoretic mobility shift assay. Supershift analysis provided evidence that C/EBPβ, but not C/EBPα or δ, was activated by the calcium ionophore. Additional experiments, using specific inhibitors, showed that the activation of C/EBPβ was mediated by CaMK II and at least in part reflected phosphorylation of Ser 276.

In other studies, Bartlett, Luethy, Carlson, Sollott, and Holbrook (1992) found that treatment of Hela cells with A23187 upregulated the expression of an additional member of the C/EBP family, i.e., gadd 153. Gadd 153 can form heterodimers with other C/EBP proteins and prevent their binding to DNA, suggesting that gadd 153 may act as an inhibitor of those transcriptional activators. Thus, the effect of calcium on C/EBP-regulated gene activation is probably complex and may reflect a balance between transcriptional stimulation and inhibition. The regulation of C/EBP by calcium in muscle is not known but experiments are presently being performed in our laboratory testing the hypothesis that calcium regulates C/EBP expression and activity in skeletal muscle as well.

8. Glucocorticoids and calcium

Multiple previous observations support the concept that glucocorticoids are important mediators of muscle wasting in sepsis as well as in other catabolic conditions (Hasselgren, 1999). For example, the increase in ubiquitin-proteasome-dependent proteolysis observed in skeletal muscle during sepsis and after severe injury was blocked by the glucocorticoid receptor antagonist RU38486 (Fang, James, Ogle, Fischer, & Hasselgren, 1995; Tiao et al., 1996). Treatment of rats or humans with glucocorticoids resulted in increased muscle protein breakdown (Darmaun, Matthews, & Bier, 1988; Hall-Angeras, Angeras, Hasselgren, & Fischer, 1990; Tiao et al., 1996). When cultured myotubes were exposed to glucocorticoids, protein degradation was increased (Wang, Luo, Wang, & Hasselgren, 1998). In fact, dexamethasone-treated myotubes have been used as an in vitro model of muscle wasting in several reports from our and other laboratories (Du et al., 2000; Sandri et al., 2004; Stitt et al., 2004; Wang et al., 1998).

The precise mechanisms by which glucocorticoids induce muscle proteolysis are not known, but it is possible that calcium may be involved in the regulation of glucocorticoid-induced muscle proteolysis. Thus, in previous reports, treatment with glucocorticoids of different cell types, including cultured human muscle cells, resulted in increased basal cytoplasmic calcium levels (Bian, Hughes, Huang, Cidlowski, & Putney, 1997; Lam, Dubyak, & Distelhorst, 1993; McConkey et al., 1989; Vandebrouck, Imbert, Duport, Cognard, & Raymond, 1999). Although several mechanisms may be involved, stimulated store operated calcium entry (SOCE) is an important mechanism by which glucocorticoids may increase cellular calcium levels (Gardner & Zhang, 1999). The role of calcium in glucocorticoid-induced muscle proteolysis has not been reported, but recent experiments in our laboratory (unpublished) seem to support a role of calcium in this response to glucocorticoids. Thus, treatment of cultured L6 myotubes with the calcium chelator BAPTA or the CaMK II inhibitor KN-62 significantly reduced the dexamethasone-induced increase in protein degradation. In addition, the calpain inhibitor calpeptin blocked the dexamethasone-induced proteolysis (Wei et al., 2005). Taken together, these observations support a role of calcium in glucocorticoid-induced muscle proteolsysis.

It should be noted that although several previous reports suggest that glucocorticoids may increase cellular calcium concentrations (Bian et al., 1997; Lam et al., 1993; McConkey et al., 1989; Simard et al., 1999; Vandebrouck et al., 1999), apparently conflicting results have also been reported (Leijendekker, Passaquin, Metzinger, & Ruegg, 1996; Metzinger, Passaquin, Leijendekker, Poindron, & Ruegg, 1995; Passaquin, Lhote, & Ruegg, 1998). For example, when cultured C2C12

myotubes were treated with glucocorticoids, calcium uptake (determined by measuring uptake of extracellular 45Ca) was inhibited (Passaquin et al., 1998). Those results do not necessarily contradict previous reports, however, because it is possible that glucocorticoids increase cytoplasmic calcium levels through other mechanisms than uptake of extracellular calcium, such as by influencing calcium uptake and release from the sarcoplasmic reticulum (Gardner and Zhang, 1999). More studies are needed to better define the role of glucocorticoids in the regulation of muscle calcium levels and protein degradation.

9. Muscle wasting, apoptosis, and calcium

Although the role of apoptosis in sepsis-induced muscle wasting is not known at present, previous studies suggest that apoptosis may contribute to muscle atrophy in various conditions characterized by muscle metabolism. Those conditions include cancer cachexia (Belizario, Lorite, & Tisdale, 2001; van Royen et al., 2000), spinal muscular atrophy (Tews & Goebel, 1997), chronic heart failure (Libera, Zennaro, Sandri, Ambrosio, & Vescovo, 1999), hindlimb unweighing (Allen et al., 1997), muscle denervation (Tews et al., 1997), muscle dystrophy (Sandri et al., 1995), and burn injury (Yasuhara et al., 2000). Other reports provided evidence that TNFα may induce apoptosis in different cell types, including cardiac myocytes (Carbo et al., 2002), further supporting the concept that apoptosis may contribute to, or at least be associated with, muscle wasting. Interestingly, the role of TNFα in the induction of apoptosis may reflect ubiquitin-proteasome activity. Thus, in a recent study, treatment of cultured endothelial cells with TNFα induced dephosphorylation of bcl-2, targeting this antiapoptotic protein for degradation by the ubiquitin system (Dimmeler, Breitschopf, Haendeler, & Zeiher, 1999).

Previous studies suggest that calcium may, at least in part, regulate apoptosis (Nicotera, Zhivotovsky, & Orrenius, 1994). The role of calcium in the induction of apoptosis is probably multifactorial and may reflect activation of endonucleases, protein kinases, phosphatases, and proteases. In addition, there is evidence that calcium is actively transported into the nucleus where it may regulate chromatin unfolding and gene transcription, providing additional apoptotic mechanisms. In recent experiments in our laboratory, treatment of cultured L6 myotubes with A23187 resulted in increased caspase-3 activity, suggesting that calcium may regulate apoptosis in muscle cells (Menconi et al., 2004). More studies are needed, however, to further establish a role of calcium in muscle apoptosis and its potential role in sepsis-induced muscle wasting.

10. Concluding remarks

Sepsis-induced muscle wasting mainly reflects ubiquitin-proteasome-dependent breakdown of myofibrillar proteins. Mechanisms regulating ubiquitin-dependent muscle proteolysis in various muscle wasting conditions, as well as the important role of glucocorticoids, were reviewed in detail elsewhere. In this review we have emphasized some additional and novel aspects of sepsis-induced muscle wasting, in particular the potential role of the transcription factors C/EBPβ and δ and the influence of calcium. A continued elaboration of mechanisms regulating muscle wasting may help future attempts to prevent or treat this debilitating complication of sepsis.

Acknowledgments

Supported in part by NIH grants R01 DK37908 and R01 NR008545. AE was supported by a Ruth L. Kirchstein National Research Service Award from the NIH.

References

Aizawa, H., Kawahara, H., Tanaka, K., & Yokosawa, H. (1996). Activation of the proteasome during Xenopus egg activation implies a link between proteasome activation and intracellular calcium release. *Biochem. Biophys. Res. Commun., 218*, 224–228.

Allen, D. L., Linderman, J. K., Roy, R. R., Bigbee, A. J., Grindeland, R. E., Mukku, V., et al. (1997). Apoptosis: a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting. *Am. J. Physiol., 273*, C579–C587.

Bailey, J. L., Wang, X., England, B. K., Price, S. R., Ding, X., & Mitch, W. E. (1996). The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent ubiquitin-proteasome pathway. *J. Clin. Invest., 97*, 1447–1453.

Baracos, V., Greenberg, R. E., & Goldberg, A. L. (1986). Influence of calcium and other divalent cations on protein turnover in rat skeletal muscle. *Am. J. Physiol., 250*, E702–E710.

Barnoy, S., Supino-Rosin, L., & Kosower, N. S. (2000). Regulation of calpain and calpastatin in differentiating myoblasts: mRNA levels, protein synthesis and stability. *Biochem. J.*, *351*, 413–420.

Bartlett, J. D., Luethy, J. D., Carlson, S. G., Sollott, S. J., & Holbrook, N. J. (1992). Calcium ionophore A23187 induces expression of the growth arrest and DNA damage inducible CCAAT/enhancer-binding protein (C/EBP)-related gene, gadd153. Ca2+ increases transcriptional activity and mRNA stability. *J. Biol. Chem.*, *267*, 20465–20470.

Belizario, J. E., Lorite, M. J., & Tisdale, M. J. (2001). Cleavage of caspases-1, -3, -6, -8 and -9 substrates by proteases in skeletal muscles from mice undergoing cancer cachexia. *Br. J. Cancer*, *84*, 1135–1140.

Bhattacharyya, J., Thompson, K., & Sayeed, M. M. (1991). Calcium-dependent and calcium-independent protease activities in skeletal muscle during sepsis. *Circ. Shock*, *35*, 117–122.

Bian, X., Hughes, F. M., Jr., Huang, Y., Cidlowski, J. A., & Putney, J. W., Jr. (1997). Roles of cytoplasmic Ca2+ and intracellular Ca2+ stores in induction and suppression of apoptosis in S49 cells. *Am. J. Physiol.*, *272*, C1241–C1249.

Busquets, S., Garcia-Martinez, C., Alvarez, B., Carbo, N., Lopez-Soriano, F. J., & Argiles, J. M. (2000). Calpain-3 gene expression is decreased during experimental cancer cachexia. *Biochim. Biophys. Acta*, *1475*, 5–9.

Cai, D., Frantz, J. D., Tawa, N. E., Melendez, P. A., Oh, B. C., Lidov, H. G. W., et al. (2004). IKKβ/NF-κB activation causes severe muscle wasting in mice. *Cell*, *119*, 285–298.

Carafoli, E., & Klee, C. (Eds.). (1999). *Calcium as a cellular regulator*. Oxford, New York: Oxford University Press.

Carbo, N., Busquets, S., van Royen, M., Alvarez, B., Lopez-Soriano, F. J., & Argiles, J. M. (2002). TNF-alpha is involved in activating DNA fragmentation in skeletal muscle. *Br. J. Cancer*, *86*, 1012–1016.

Costelli, P., Tullio, R. D., Baccino, F. M., & Melloni, E. (2001). Activation of Ca(2+)-dependent proteolysis in skeletal muscle and heart in cancer cachexia. *Br. J. Cancer*, *84*, 946–950.

Darmaun, D., Matthews, D. E., & Bier, D. M. (1988). Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am. J. Physiol.*, *255*, E366–E373.

Dimmeler, S., Breitschopf, K., Haendeler, J., & Zeiher, A. M. (1999). Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. *J. Exp. Med.*, *189*, 1815–1822.

Du, J., Mitch, W. E., Wang, X., & Price, S. R. (2000). Glucocorticoids induce proteasome C3 subunit expression in L6 muscle cells by opposing the suppression of its transcription by NF-kappa B. *J. Biol. Chem.*, *275*, 19661–19666.

Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., et al. (2004). Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J. Clin. Invest.*, *113*, 115–123.

Engel, A. G., Mokri, B., Jerusalem, F., Sakakibarda, N., & Paulson, O. B. (1997). In L. P. Rowland (Ed.), *Pathogenesis of human muscle dystrophies* (pp. 310–324). Amsterdam: Excerpta Medica.

Fang, C. H., James, H. J., Ogle, C., Fischer, J. E., & Hasselgren, P. O. (1995). Influence of burn injury on protein metabolism in different types of skeletal muscle and the role of glucocorticoids. *J. Am. Coll. Surg.*, *180*, 33–42.

Ferrand-Drake, M., Zhu, C., Gido, G., Hansen, A. J., Karlsson, J. O., Bahr, B. A., et al. (2003). Cyclosporin A prevents calpain activation despite increased intracellular calcium concentrations, as well as translocation of apoptosis-inducing factor, cytochrome c and caspase-3 activation in neurons exposed to transient hypoglycemia. *J. Neurochem.*, *85*, 1431–1442.

Fischer, D., Sun, X., Gang, G., Pritts, T., & Hasselgren, P. O. (2000). The gene expression of ubiquitin ligase E3alpha is up-regulated in skeletal muscle during sepsis in rats-potential role of glucocorticoids. *Biochem. Biophys. Res. Commun.*, *267*, 504–508.

Fischer, D. R., Sun, X., Williams, A. B., Gang, G., Pritts, T. A., James, J. H., et al. (2001). Dantrolene reduces serum TNFalpha and corticosterone levels and muscle calcium, calpain gene expression, and protein breakdown in septic rats. *Shock*, *15*, 200–207.

Furuno, K., & Goldberg, A. L. (1986). The activation of protein degradation in muscle by Ca2+ or muscle injury does not involve a lysosomal mechanism. *Biochem. J.*, *237*, 859–864.

Gamrin, L., Andersson, K., Hultman, E., Nilsson, E., Essen, P., & Wernerman, J. (1997). Longitudinal changes of biochemical parameters in muscle during critical illness. *Metabolism*, *46*, 756–762.

Gardner, J. P., & Zhang, L. (1999). Glucocorticoid modulation of Ca2+ homeostasis in human B lymphoblasts. *J. Physiol.*, *514*, 385–396.

Glass, D. J. (2003). Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat. Cell Biol.*, *5*, 87–90.

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiol. Rev.*, *83*, 731–801.

Guttridge, D. C., Mayo, M. W., Madrid, L. V., Wang, C. Y., & Baldwin, A. S. (2000). NF-κB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science*, *289*, 2363–2366.

Hall-Angeras, M., Angeras, U., Hasselgren, P. O., & Fischer, J. E. (1990). Corticosterone alone does not explain increased muscle proteolysis in septic rats. *J. Surg. Res.*, *48*, 368–372.

Hasselgren, P. O. (1999). Glucocorticoids and muscle catabolism. *Curr. Opin. Clin. Nutr. Metab. Care*, *2*, 201–215.

Hasselgren, P. O. (2002). Molecular regulation of muscle wasting. *Sci. Med.*, *8*, 230–239.

Hasselgren, P. O., & Fischer, J. E. (2001). Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. *Ann. Surg.*, *233*, 9–17.

Hobler, S. C., Wang, J. J., Williams, A. B., Melandri, F., Sun, X., Fischer, J. E., et al. (1999). Sepsis is associated with increased ubiquitin conjugating enzyme E214k mRNA in skeletal muscle. *Am. J. Physiol.*, *276*, R468–R473.

Hobler, S. C., Williams, A., Fischer, D., Wang, J. J., Sun, X., Fischer, J. E., et al. (1999). Activity and expression of the 20S proteasome are increased in skeletal muscle during sepsis. *Am. J. Physiol.*, *277*, R434–R440.

Hotchkiss, R. S., & Karl, I. E. (1994). Dantrolene ameliorates the metabolic hallmarks of sepsis in rats and improves survival in a mouse model of endotoxemia. *Proc. Natl. Acad. Sci. U.S.A.*, *91*, 3039–3043.

Huang, J., & Forsberg, N. E. (1998). Role of calpain in skeletal-muscle protein degradation. *Proc. Natl. Acad. Sci. U.S.A.*, *95*, 12100–12105.

Hund, E. (2001). Neurological complications of sepsis: critical illness polyneuropathy and myopathy. *J. Neurol.*, *248*, 929–934.

Janknecht, R. (2002). The versatile functions of the transcriptional coactivators p300 and CBP and their roles in disease. *Histol. Histopathol.*, *17*, 657–668.

Joffe, M., Savage, N., & Isaacs, H. (1981). Increased muscle calcium. A possible cause of mitochondrial dysfunction and cellular necrosis in denervated rat skeletal muscle. *Biochem. J.*, *196*, 663–667.

Kameyama, T., & Etlinger, J. D. (1979). Calcium-dependent regulation of protein synthesis and degradation in muscle. *Nature*, *279*, 344–346.

Kawahara, H., & Yokosawa, H. (1994). Intracellular calcium mobilization regulates the activity of 26 S proteasome during the metaphase-anaphase transition in the ascidian meiotic cell cycle. *Dev. Biol.*, *166*, 623–633.

Krause, T., Gerbershagen, M. U., Fiege, M., Weisskorn, R., & Wappler, F. (2004). Dantrolene—a review of its pharmacology, therapeutic use and new developments. *Anesthesia*, *59*, 364–373.

Ladner, K. J., Caligiuri, M. A., & Guttridge, D. C. (2003). Tumor-necrosis factor-regulated biphasic activation of NF-κB is required for cytokine-induced loss of skeletal muscle gene products. *J. Biol. Chem.*, *278*, 2294–2303.

Lam, M., Dubyak, G., & Distelhorst, C. W. (1993). Effect of glucocorticosteroid treatment on intracellular calcium homeostasis in mouse lymphoma cells. *Mol. Endocrinol.*, *7*, 686–693.

Langen, R. C., Schols, A. M., Kelders, M. C., Wouters, E. F., & Jansen-Heininger, Y. M. (2001). Inflammatory cytokines inhibit myogenic differentiation through activation of NF-κB. *FASEB J.*, *15*, 1169–1180.

Larsson, L., Li, X., Edstrom, L., Eriksson, L. I., Zackrisson, H., Argentini, C., et al. (2000). Acute quadriplegia and loss of muscle myosin in patients treated with nondepolarizing neuromuscular blocking agents and corticosteroids: mechanisms at the cellular and molecular levels. *Crit. Care Med.*, *28*, 34–45.

Lecker, S. H. (2003). Ubiquitin-protein ligases in muscle wasting: multiple parallel pathways? *Curr. Opin. Clin. Nutr. Metab. Care*, *6*, 271–275.

Lecker, S. H., Jagoe, R. T., Gilbert, A., Gomes, M., Baracos, V., Bailey, J., et al. (2004). Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J.*, *18*, 39–51.

Leijendekker, W. J., Passaquin, A. C., Metzinger, L., & Ruegg, U. T. (1996). Regulation of cytosolic calcium in skeletal muscle cells of the mdx mouse under conditions of stress. *Br. J. Pharmacol.*, *118*, 611–616.

Lewis, S. E., Anderson, P., & Goldspink, D. F. (1982). The effects of calcium on protein turnover in skeletal muscles of the rat. *Biochem. J.*, *204*, 257–264.

Li, H. H., Kedar, V., Zhang, C., McDonough, H., Arya, R., Wang, D. Z., et al. (2004). Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J. Clin. Invest.*, *114*, 1058–1071.

Li, Y. P., & Reid, M. B. (2000). NF-κB mediates the protein loss induced by TNF-α in differentiated skeletal muscle myotubes. *Am. J. Physiol.*, *279*, R1165–R1170.

Libera, L. D., Zennaro, R., Sandri, M., Ambrosio, G. B., & Vescovo, G. (1999). Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. *Am. J. Physiol.*, *277*, C982–C986.

McConkey, D. J., Nicotera, P., Hartzell, P., Bellomo, G., Wyllie, A. H., & Orrenius, S. (1989). Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca2+ concentration. *Arch. Biochem. Biophys.*, *269*, 365–370.

Medina, R., Wing, S. S., & Goldberg, A. L. (1995). Increase in levels of polyubiquitin and proteasome mRNA in skeletal muscle during starvation and denervation atrophy. *Biochem. J.*, *307*, 631–637.

Menconi, M. J., Wei, W., Yang, H., Wray, C. J., & Hasselgren, P. O. (2004). Treatment of cultured myotubes with the calcium ionophore A23187 increases proteasome activity via a CaMK II-caspase-calpain-dependent mechanism. *Surgery*, *136*, 135–142.

Metzinger, L., Passaquin, A. C., Leijendekker, W. J., Poindron, P., & Ruegg, U. T. (1995). Modulation by prednisolone of calcium handling in skeletal muscle cells. *Br. J. Pharmacol.*, *116*, 2811–2816.

Mitch, W. E., & Goldberg, A. L. (1996). Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N. Engl. J. Med.*, *335*, 1897–1905.

Mittendorfer, B., Gore, D. C., Herndon, D. N., & Wolfe, R. R. (1999). Accelerated glutamine synthesis in critically ill patients cannot maintain normal intramuscular free glutamine concentration. *J. Parenter. Enteral. Nutr.*, *23*, 243–250.

Neumar, R. W., Xu, Y. A., Gada, H., Guttmann, R. P., & Siman, R. (2003). Cross-talk between calpain and caspase proteolytic systems during neuronal apoptosis. *J. Biol. Chem.*, *278*, 14162–14167.

Newsholme, E. A., & Parry-Billings, M. (1990). Properties of glutamine release from muscle and its importance for the immune system. *J. Parenter. Enteral. Nutr.*, *14*, 63S–67S.

Nicotera, P., Zhivotovsky, B., & Orrenius, S. (1994). Nuclear calcium transport and the role of calcium in apoptosis. *Cell Calcium*, *16*, 279–288.

Passaquin, A. C., Lhote, P., & Ruegg, U. T. (1998). Calcium influx inhibition by steroids and analogs in C2C12 skeletal muscle cells. *Br. J. Pharmacol.*, *124*, 1751–1759.

Penner, G., Gang, G., Sun, X., Wray, C., & Hasselgren, P. O. (2002). C/EBP DNA-binding activity is upregulated by a glucocorticoid-dependent mechanism in septic muscle. *Am. J. Physiol.*, *282*, R439–R444.

Penner, C. G., Gang, G., Wray, C., Fischer, J. E., & Hasselgren, P. O. (2001). The transcription factors NF-kappab and AP-1 are differentially regulated in skeletal muscle during sepsis. *Biochem. Biophys. Res. Commun.*, *281*, 1331–1336.

Reid, W. D., & MacGowan, N. A. (1998). Respiratory muscle injury in animal models and humans. *Mol. Cell Biochem.*, *179*, 63–80.

Rennie, M. J., Ahmed, A., Khogali, S. E., Low, S. Y., Hundal, H. S., & Taylor, P. M. (2001). Glutamine metabolism and transport in skeletal muscle and heart and their clinical relevance. *J. Nutr.*, *126*, 1142S–1145S.

Rosen, L. B., Ginty, D. D., & Greenberg, M. E. (1995). Calcium regulation of gene expression. *Adv. Second Messenger Phosphoprotein Res.*, *30*, 225–253.

Rosenblatt, S., Clowes, G. H., Jr., George, B. C., Hirsch, E., & Lindberg, B. (1983). Exchange of amino acids by muscle and liver in sepsis. *Arch. Surg.*, *118*, 167–175.

Sandri, M., Carraro, U., Podhorska-Okolov, M., Rizzi, C., Arslan, P., Monti, D., et al. (1995). Apoptosis, DNA damage and ubiquitin expression in normal and mdx muscle fibers after exercise. *FEBS Lett.*, *373*, 291–295.

Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., et al. (2004). Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*, *117*, 399–412.

Sayeed, M. M. (2000). Signaling mechanisms of altered cellular responses in trauma, burn, and sepsis: role of Ca2+. *Arch. Surg.*, *135*, 1432–1442.

Showalter, C. J., & Engel, A. G. (1997). Acute quadriplegic myopathy: analysis of myosin isoforms and evidence for calpain-mediated proteolysis. *Muscle Nerve*, *20*, 316–322.

Simard, M., Couldwell, W. T., Zhang, W., Song, H., Liu, S., Cotrina, M. L., et al. (1999). Glucocorticoids-potent modulators of astrocytic calcium signaling. *Glia*, *28*, 1–12.

Solomon, V., Baracos, V., Sarraf, P., & Goldberg, A. L. (1998). Rates of ubiquitin conjugation increase when muscles atrophy, largely through activation of the N-end rule pathway. *Proc. Natl. Acad. Sci. U.S.A.*, *95*, 12602–12607.

Solomon, V., Lecker, S. H., & Goldberg, A. L. (1998). The N-end rule pathway catalyzes a major fraction of the protein degradation in skeletal muscle. *J. Biol. Chem.*, *273*, 25216–25222.

Stitt, T. N., Drujan, D., Clarke, B. A., Panaro, F., Timofeyva, Y., Kline, W. O., et al. (2004). The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol. Cell*, *14*, 395–403.

Tawa, N. E., Odessey, R., & Goldberg, A. L. (1997). Inhibitors of the proteasome reduce the accelerated proteolysis in atrophying rat skeletal muscles. *J. Clin. Invest.*, *100*, 197–203.

Tews, D. S., & Goebel, H. H. (1997). Apoptosis-related proteins in skeletal muscle fibers of spinal muscular atrophy. *J. Neuropathol. Exp. Neurol.*, *56*, 150–156.

Tews, D. S., Goebel, H. H., Schneider, I., Gunkel, A., Stennert, E., & Neiss, W. F. (1997). DNA-fragmentation and expression of apoptosis-related proteins in experimentally denervated and reinnervated rat facial muscle. *Neuropathol. Appl. Neurobiol.*, *23*, 141–149.

Tiao, G., Fagan, J., Roegner, V., Lieberman, M., Wang, J. J., Fischer, J. E., et al. (1996). Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. *J. Clin. Invest.*, *97*, 339–348.

Tiao, G., Fagan, J. M., Samuels, N., James, J. H., Hudson, K., Lieberman, M., et al. (1994). Sepsis stimulates nonlysosomal, energy-dependent proteolysis and increases ubiquitin mRNA levels in rat skeletal muscle. *J. Clin. Invest.*, *94*, 2255–2264.

Tiao, G., Hobler, S., Wang, J. J., Meyer, T. A., Luchette, F. A., Fischer, J. E., et al. (1997). Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. *J. Clin. Invest.*, *99*, 163–168.

Tiao, G., Lieberman, M., Fischer, J. E., & Hasselgren, P. O. (1997). Intracellular regulation of protein degradation during sepsis is different in fast- and slow-twitch muscle. *Am. J. Physiol.*, *272*, R849–R856.

Tidball, J. G., & Spencer, M. J. (2000). Calpains and muscular dystrophies. *Int. J. Biochem. Cell Biol.*, *32*, 1–5.

Tidball, J. G., & Spencer, M. J. (2002). Expression of a calpastatin transgene slows muscle wasting and obviates changes in myosin isoform expression during murine muscle disuse. *J. Physiol.*, *545*, 819–828.

Tremel, H., Kienle, B., Weilemann, L. S., Stehle, P., & Furst, P. (1994). Glutamine dipeptide-supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology*, *107*, 1595–1601.

Vandebrouck, C., Imbert, N., Duport, G., Cognard, C., & Raymond, G. (1999). The effect of methylprednisolone on intracellular calcium of normal and dystrophic human skeletal muscle cells. *Neurosci. Lett.*, *269*, 110–114.

van Royen, M., Carbo, N., Busquets, S., Alvarez, B., Quinn, L. S., Lopez-Soriano, F. J., et al. (2000). DNA fragmentation occurs in skeletal muscle during tumor growth: a link with cancer cachexia? *Biochem. Biophys. Res. Commun.*, *270*, 533–537.

Vary, T. C., & Kimball, S. R. (1992). Regulation of hepatic protein synthesis in chronic inflammation and sepsis. *Am. J. Physiol.*, *262*, C445–C452.

Wagatsuma, A., Fujimoto, K., & Yamada, S. (2002). Effect of treatment with nifedipine, an L-type calcium channel blocker, on muscular atrophy induced by hindlimb immobilization. *Scand. J. Med. Sci. Sports*, *12*, 26–30.

Wagenmakers, A. J. (2001). Muscle function in critically ill patients. *Clin. Nutr.*, *20*, 451–454.

Wang, L., Luo, G. J., Wang, J. J., & Hasselgren, P. O. (1998). Dexamethasone stimulates proteasome- and calcium-dependent proteolysis in cultured L6 myotubes. *Shock*, *10*, 298–306.

Wegner, M., Cao, Z., & Rosenfeld, M. G. (1992). Calcium-regulated phosphorylation within the leucine zipper of C/EBP beta. *Science*, *256*, 370–373.

Wei, W., Fareed, M., Evenson, A., Menconi, M., Yang, H., Petkova, V., et al. (2005). Sepsis stimulates calpain activity in skeletal muscle by decreasing calpastatin activity but does not activate caspase-3. *Am. J. Physiol.*, *288*, R580–R590.

Williams, A. B., Decourten-Myers, G. M., Fischer, J. E., Luo, G., Sun, X., & Hasselgren, P. O. (1999). Sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. *FASEB J.*, *13*, 1435–1443.

Windmueller, H. G., & Spaeth, A. E. (1980). Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. *J. Biol. Chem.*, *255*, 107–112.

Wray, C. J., Mammen, J. M., Hershko, D. D., & Hasselgren, P. O. (2003). Sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. *Int. J. Biochem. Cell Biol.*, *35*, 698–705.

Wray, C. J., Sun, X., Gang, G. I., & Hasselgren, P. O. (2002). Dantrolene downregulates the gene expression and activity of the ubiquitin-proteasome proteolytic pathway in septic skeletal muscle. *J. Surg. Res.*, *104*, 82–87.

Yang, H., Mammen, J., Wei, W., Menconi, M., Petkova, V., & Hasselgren, P. O. (2005). The expression and activity of C/EBPβ and δ are upregulated by dexamethasone in skeletal muscle. *J. Cell Physiol.*, in press.

Yang, H., Menconi, M. J., Wei, W., Petkova, V., & Hasselgren, P. O. (2005). Dexamethasone upregulates the expression of the nuclear cofactor p300 and its interaction with C/EBPβ in cultured myotubes. *J. Cell Biochem.*, in press.

Yasuhara, S., Perez, M. E., Kanakubo, E., Yasuhara, Y., Shin, Y. S., Kaneki, M., Fujita, T., & Martyn, J. A. (2000). Skeletal muscle apoptosis after burns is associated with activation of proapoptotic signals. *Am. J. Physiol.*, *279*, 1114–1121.