**Muscle protein metabolism during sepsis**

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**Introduction**

One of the most pronounced metabolic changes after injury, sepsis and other critical illness is increased urinary excretion of nitrogen, resulting in negative nitrogen balance. There is evidence that skeletal muscle is the major source of nitrogen in these conditions. Muscle catabolism during sepsis is mainly caused by increased protein degradation, in particular myofibrillar protein breakdown, although reduced protein synthesis and inhibited amino acid uptake contribute to the catabolic response [1] (Figure 1). One consequence of muscle breakdown is release of amino acids with redistribution from the periphery to central organs and tissues, such as liver and intestine [2]. A large amount of amino acids released from skeletal muscle during catabolic conditions is taken up by the liver for gluconeogenesis and acute phase protein synthesis. Glutamine released from catabolic muscle is taken up by cells of the immune system [3] and by intestinal epithelial cells [4] and is an important energy source in these cell types. Thus, muscle catabolism is part of an integrated response to sepsis and may be beneficial to the organism, at least during the early phase of sepsis, providing essential tissues with increased amounts of amino acids. If allowed to continue uncontrolled, however, muscle breakdown will result in muscle wasting, fatigue and delayed recovery. The term 'autocannibalism' has been used to illustrate the serious consequences of muscle proteolysis during severe and protracted sepsis [5].

Considering the important clinical implications of muscle breakdown, it is not surprising that much research has been performed to define mediators and mechanisms of muscle proteolysis during sepsis. In this report, recent knowledge about the regulation of muscle protein breakdown during sepsis is reviewed together with mechanisms and molecular regulation of intracellular protein degradation.

**Abbreviations used:** EDL, extensor digitorum longus; IGF-1, insulin-like growth factor-1; IL-1, interleukin-1; IL-6, interleukin-6; IL-1ra, interleukin-1 receptor antagonist; TNF, tumour necrosis factor.

**Mediators of muscle catabolism during sepsis**

The catabolic response in muscle is mediated by multiple regulators interacting with each other. Two groups of substances are particularly important for the regulation of muscle proteolysis during sepsis, i.e. glucocorticoids and the proinflammatory cytokines tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6). It is likely, however, that other mediators are also involved since blockade of glucocorticoids or cytokines reduces, but does not normalize, protein breakdown rates during sepsis. Furthermore, neither cytokines nor glucocorticoids seem to be responsible for reduced protein synthesis in muscle during sepsis. It should also be noted that, in addition to the appearance of a factor or factors that induce muscle catabolism, it is possible that the catabolic response to sepsis may, at least in part, be caused by the lack of factors that under normal conditions promote muscle anabolism, for example, insulin-like growth factor-1 (IGF-1) [6].

**Glucocorticoids**

There are several lines of evidence to suggest that glucocorticoids regulate muscle proteolysis during sepsis. Plasma levels of cortisol are...
increased in septic and injured patients and plasma levels of corticosterone (the predominant glucocorticoid in rats) were more than doubled after caecal ligation and puncture in rats [7]. Administration of glucocorticoids in man [8] or rats [9] resulted in increased muscle protein degradation. Of special interest is the finding that myofibrillar proteins are particularly sensitive to the effects of glucocorticoids [9]. Thus, in this respect, the effect of glucocorticoids resembles the effects of sepsis [10].

More direct evidence for a role of glucocorticoids in sepsis-induced muscle proteolysis was found in recent studies in our laboratory in which septic rats were treated with the glucocorticoid receptor antagonist RU 38486 [11]. Total protein breakdown, measured as release of tyrosine by incubated extensor digitorum longus (EDL) muscles, was reduced by 28%, and myofibrillar protein breakdown, measured as release of 3-methylhistidine, was inhibited by 44% after treatment of septic rats with the glucocorticoid receptor antagonist. These results strongly support a role of glucocorticoids in sepsis-induced muscle proteolysis. In the same experiments, the reduced muscle protein synthesis observed in septic rats was not influenced by RU 38486, suggesting that sepsis-induced changes in muscle protein synthesis and breakdown are controlled by different regulators. The finding that protein breakdown rates were reduced but not normalized after treatment with RU 38486 suggests that multiple regulators, in addition to glucocorticoids, may be involved in the stimulation of muscle proteolysis during sepsis.

The mechanism by which glucocorticoids stimulate muscle proteolysis is not fully known. Recent studies suggest, however, that glucocorticoids may exert their control of muscle proteolysis at the transcriptional level. During starvation in rats, the ubiquitin-dependent proteolytic pathway was stimulated, as evidenced by increased levels in muscle of ubiquitin-conjugated proteins and ubiquitin mRNA [12]. This response to starvation was blocked in adrenalectomized rats but was restituted by the administration of dexamethasone. Thus, it is possible that the activated glucocorticoid receptor complex acts as a transcription factor on the ubiquitin gene in skeletal muscle. Recent studies in our laboratory (G. Tiao, J. Fagan, V. Roegner, M. Lieberman, J. J. Wang, J. E. Fischer and P. O. Hasselgren, unpublished work) suggest that glucocorticoids act through a similar mechanism during sepsis (see below).

Cytokines

In addition to glucocorticoids, the proinflammatory cytokines TNF, IL-1 and IL-6 participate in the regulation of protein metabolism during sepsis. It is generally believed that cytokines act primarily in an autocrine or paracrine fashion and that circulating levels of the substances represent an ‘overflow’ of cytokines locally produced in various tissues [13,14]. Because injection of cytokines in healthy humans or animals can induce several sepsis-like metabolic changes, it is possible that cytokines can act in an endocrine fashion as well.

Several studies have provided evidence that IL-1 participates in the regulation of muscle protein breakdown. After chronic administration of recombinant IL-1α in rats, muscle protein content and mRNA levels for myofibrillar proteins decreased [15]. In other studies, infusion of IL-1β in rats augmented the catabolic effect of TNF-α [16]. In experiments in our laboratory, treatment of rats with recombinant IL-1α (300 μg/kg divided into three equal intraperitoneal doses over 16 h) resulted in stimulated muscle protein breakdown, in particular myofibrillar protein breakdown [17,18]. Muscle protein synthesis was not affected by the recombinant IL-1α treatment.

To further test the role of IL-1 in sepsis-induced muscle proteolysis, we treated septic or endotoxaemic rats with IL-1 receptor antagonist (IL-1ra). Administration of recombinant IL-1ra blunted the increase in muscle proteolysis induced by caecal ligation and puncture [19] or 1 mg/kg of endotoxin in rats [20]. These results suggest that IL-1 participates in the regulation of muscle protein breakdown during sepsis.

In addition to IL-1, TNF and IL-6 also regulate muscle protein breakdown. Administration of recombinant TNF-α in rats (300 μg/kg divided into three intraperitoneal doses over 16 h) resulted in a sepsis-like increase in muscle protein breakdown, in particular myofibrillar protein breakdown [18]. When septic rats were pre-treated with TNF-antiserum, the increase in muscle protein breakdown was significantly blunted, although the increase in myofibrillar protein breakdown was not completely prevented [21]. Administration of TNF to normal rats or TNF-antiserum to septic rats did not significantly influence muscle protein synthesis, supporting the concept that protein synthesis and breakdown are differentially regulated during sepsis.

In a recent report, administration of IL-6 (two doses of 125 μg/kg intraperitoneally) in rats...
resulted in increased myofibrillar protein breakdown, measured as release of 3-methylhistidine by incubated EDL muscles [22]. In the same study, incubation of muscles in the presence of IL-6 in vitro (750 ng/ml) for 6 h did not influence protein breakdown rate, suggesting that IL-6 does not have a direct effect on skeletal muscle.

Whereas the reports described suggest that TNF, IL-1 and IL-6 may regulate muscle protein breakdown in vivo, several studies have failed to demonstrate a direct effect of any of these cytokines on skeletal muscle [22-25]. Thus, it is likely that the cytokines induce muscle proteolysis through some other mediator released by the cytokines (e.g. glucocorticoids) or by an interaction among themselves. Another mechanism through which the cytokines may induce muscle proteolysis could be by inhibiting an anabolic agent, for example, IGF-1. In a recent study, cytokines (in particular TNF) blocked the anabolic effects of IGF-1 in chondrocytes [26]. It remains to be determined whether a similar relationship exists between the proinflammatory cytokines and IGF-1 (or any anabolic substance) in skeletal muscle.

**Cellular mechanisms of muscle proteolysis during sepsis**

Intracellular protein degradation is regulated by different proteolytic pathways. Proteolysis in the different pathways is regulated individually during various pathophysiological conditions. Much knowledge has been gained during recent years about the mechanisms of intracellular protein degradation in skeletal muscle and extensive reviews have appeared elsewhere [e.g. 1,27].

Proteins can be degraded by lysosomal or non-lysosomal mechanisms. Among the non-lysosomal pathways, there is evidence for both energy-dependent and energy-independent mechanisms. Protein breakdown is regulated by specific proteolytic enzymes in the different proteolytic pathways. The lysosomal and the energy (ATP)—ubiquitin-dependent pathways are the most important mechanisms for regulation of protein degradation during various physiological and pathophysiological conditions. It is generally believed that the lysosomal pathway accounts for the breakdown of long-lived proteins and membrane proteins and that abnormal proteins, short-lived normal proteins and myofibrillar proteins are degraded in the ATP—ubiquitin-dependent pathway. A recent study from our laboratory suggests that sepsis more or less selectively stimulates the energy—ubiquitin-dependent protein breakdown in skeletal muscle without affecting other proteolytic pathways [28]. Other studies have shown that the ubiquitin system is also involved in muscle breakdown in other catabolic conditions, including starvation [12], cancer [29], burn injury [30], muscle denervation [31] and metabolic acidosis [32].

**Energy—ubiquitin-dependent protein breakdown**

The unique features of the ubiquitin system are that it requires energy and that proteins are targeted for breakdown by conjugation to multi-ubiquitin chains (Figure 2). Ubiquitin is a 76 amino acid polypeptide, present in all eukaryotic cells. Proteins conjugated to ubiquitin are recognized and degraded by the high molecular mass 26S protease complex. The 26S protease complex consists of three subunits, the most important being the 20S proteasome which is the 'catalytic core' of the 26S protease complex. The three-dimensional structure of the 20S proteasome was published only recently [33]. The complex consists of four stacked rings, each consisting of seven subunits, forming a 'tunnel' through which the protein is directed for proteolysis. Before the protein is directed through the 20S proteasome, it is dissociated from ubiquitin and then unfolded. Unfolding of the substrate is one of the reasons why ATP is required in this proteolytic pathway. Various aspects of the ubiquitin system, including molecular genetics, different biological roles, biochemistry, enzymology and involvement in pathological conditions, have been reviewed extensively in several recent reports [34-36]. Genes encoding ubiquitin and several enzymes involved in the ubiquitin pathway have been cloned and this has opened up the possibility of studying the regulation of protein degradation at the molecular level. More than 40 gene products are involved in trafficking ubiquitin.

It should be noted that classification of intracellular proteolysis into lysosomal and non-lysosomal mechanisms may be an oversimplification. Thus, there is evidence that different proteolytic pathways interact with each other and that the different mechanisms are not completely distinct. For example, it was reported recently that the ubiquitin-activating enzyme E3 is required for stress-induced activation of lysosomal breakdown of cellular proteins [37]. Other studies have provided evidence that ubiquitin—
protein conjugates are enriched in lysosomes of fibroblasts [38]. Free ubiquitin has also been found in lysosomes [39]. It is also possible that the formation and function of autophagic vacuoles and/or the uptake of proteins in lysosomes are regulated by the ubiquitin system. In addition, certain proteins may be degraded by different pathways, perhaps involving initial cleavage of the protein outside lysosomes followed by final hydrolysis within lysosomes. In fact, there is evidence that the final breakdown of myofibrillar proteins may involve lysosomes [40].

Several experimental approaches can be used to test the role of the energy–ubiquitin-dependent proteolytic pathway in various catabolic conditions. First, the role of energy for the stimulated proteolytic rates can be determined by incubating muscles in the presence of 2,4-dinitrophenol, which blocks energy production in the tissue and almost completely depletes the incubated muscles of ATP. Second, the involvement of ubiquitin itself can be tested by measuring levels of free and conjugated ubiquitin and by measuring ubiquitin mRNA levels. Using these experimental approaches, we have found evidence that both sepsis-induced [28] and burn-induced [30] muscle proteolysis mainly reflect increased energy-dependent protein breakdown, in particular energy-dependent myofibrillar protein breakdown. Tissue levels of ubiquitin mRNA were increased in muscle from septic rats (Figure 3). These results are consistent with stimulated activity of the energy–ubiquitin-dependent proteolytic pathway. In the same studies we found evidence that the lysosomal and calcium-dependent mechanisms do not contribute substantially to muscle breakdown in sepsis, supporting the concept that different proteolytic pathways are individually regulated in various pathophysiological conditions.

The mechanism of upregulated ubiquitin gene activity during sepsis and other catabolic conditions remains to be determined. Preliminary results in our laboratory (G. Tiao, J. Fagan, V. Roegner, M. Lieberman, J. J. Wang, J. E. Fischer and P. O. Hasselgren, unpublished work) suggest that glucocorticoids are important for the regulation of the ubiquitin pathway during sepsis. Thus, treatment of septic rats with the glucocorticoid receptor antagonist RU 38486 blocked the energy-dependent component of muscle proteolysis and abolished the increase in ubiquitin mRNA levels seen in muscle from septic rats. These results suggest that the activated glucocorticoid–receptor complex may act as a transcription factor, stimulating the activity of the ubiquitin gene.

**Clinical implications**

The catabolic response in skeletal muscle during sepsis and after trauma is important from a clinical standpoint for several reasons. During the early phase of sepsis, muscle catabolism may be beneficial to the organism since it results in release of amino acids which are taken up by the liver for gluconeogenesis and acute phase protein
and continued muscle breakdown will result in significant whole-body protein loss. The recent finding of energy-dependent proteolysis during sepsis [28] also suggests that the catabolic response in skeletal muscle may be a mechanism of increased energy expenditure in this condition.

Treatment of patients with muscle catabolism should, of course, primarily be directed towards eliminating the source of catabolism; for example, antibiotics, adequate drainage of abscesses and removal of devitalized tissue remain the cornerstones of treatment of septic patients. Despite treatment of the underlying source of muscle catabolism, however, muscle breakdown may still continue and pose a threat to the patient. Additional pharmacological and/or nutritional therapy has therefore been tested although results have been marginal at best.

Because early studies indicated that the branched-chain amino acids, in particular leucine and its keto-acid α-ketoisocaproic acid, inhibited protein breakdown in normal skeletal muscle [41], treatment of injured and septic patients with amino acid solutions enriched with branched-chain amino acids has been tested to reduce muscle catabolism. One reason why results from such studies have not been impressive may be that muscle becomes refractory to the anabolic effects of leucine during sepsis [42].

Reduced muscle glutamine levels after injury and during sepsis have been suggested to be a mechanism of increased muscle protein breakdown and inhibited protein synthesis in these conditions [43]. It has therefore been hypothesized that administration of glutamine can reverse the catabolic response in skeletal muscle, and results observed in patients after elective surgery seem to support that hypothesis [44]. During sepsis, when muscle catabolism is a more significant clinical problem than after most uncomplicated operations, prevention of muscle catabolism with glutamine has not been successful [45]. In a recent study from our laboratory [46], the catabolic state in muscle from septic rats was not influenced by elevating intracellular levels of glutamine. The universal use of glutamine to treat patients with sepsis or other significant catabolic conditions needs to await the results from controlled clinical studies. This is particularly important considering the high cost of specific amino acid solutions.

The recent detailed knowledge of mediators and mechanisms of muscle proteolysis during
sepsis may open up new treatment modalities; for example, treatment with cytokine antibodies and/or receptor blockers may become part of the future armamentarium in the treatment of septic and other catabolic patients. Treatment of septic rats with anti-TNF antibodies [21] or IL-1ra [19,20] significantly blunted the catabolic response in muscle. Although speculative at this point, manipulation of the genetic control of muscle proteolysis may prove beneficial in the future. The recent knowledge about the molecular regulation of the ubiquitin-dependent proteolytic pathway during sepsis may generate new treatment modalities for septic and other critically ill patients.

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Septic shock in man is characterized by microbial invasion of the bloodstream, hypotension, hyporeactivity to vasopressor agents, vascular leak and circulatory failure leading to multiple organ dysfunction and death [1]. A common cause of septic shock is Gram-negative bacterial infection, and administration of Gram-negative bacterial endotoxin in animals and man produces a shock-like syndrome [2,3]. A variety of host mediators have been implicated in the pathogenesis of shock, in particular cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interferon-γ (IFN-γ). Indeed, administration of TNF-α and IL-1 in animals and man produces the systemic and pathological features of endotoxin-induced shock.

Emerging evidence suggests that overproduction of NO may be the common mechanism by which endotoxin and cytokines bring about their deleterious action [4].

The L-arginine: NO pathway [5] is involved in a variety of physiological processes in the cardiovascular and nervous system where NO is generated via constitutive Ca²⁺-dependent NO synthases [5,6]. In the vasculature, the NO synthase releases relatively small amounts of NO in response to receptor stimulation and shear stress and maintains a vasodilator tone [5,7]. An inducible Ca²⁺-independent NO synthase is expressed in phagocytic and other cells after activation by endotoxin and/or cytokines. Much larger amounts of NO are generated by this enzyme, which is responsible for the cytotoxicity of macrophages towards certain micro-organisms and tumour cells and may account for the phenomenon of non-specific immunity [5].

Endotoxin and/or cytokines also induce the expression of a Ca²⁺-independent NO synthase in vascular endothelial and smooth muscle cells and cardiac myocytes [4,8–10]. Isolated blood vessels treated with endotoxin show a time-dependent expression of the inducible NO synthase over a period of 8 h, which begins after a lag period of 2 h. This is accompanied by an increase in the levels of cyclic GMP in the tissue over the same time course. These observations, together with the finding that precontracted vessels exhibit a time-dependent relaxation which also begins after a lag period of approximately 2 h, indicate that endotoxin increases the synthesis of NO in these tissues ([4] and Figure 1). The relaxation over the 8 h period is attenuated by polymyxin B which binds and neutralizes endotoxin [11], confirming that endotoxin provides the stimulus for induction of the Ca²⁺-independent NO synthase. The expression of this enzyme is abolished by preincubation with cycloheximide, an inhibitor of protein synthesis, indicating that the increased amount of NO is dependent upon the synthesis of new enzyme. The Ca²⁺-dependent (constitutive) NO synthase activity is unchanged over this time course and is unaffected by preincubation with cycloheximide, indicating that the synthesis of NO by this enzyme does not contribute to the loss of tone [4].

The activation of the inducible NO synthase by endotoxin results in much larger quantities of NO being produced than that of the constitutive enzyme. The inducible NO synthase is expressed...