

Hormones and Cytokines in the Regulation of Muscle Proteolysis During Sepsis

Per-Olof Hasselgren

Department of Surgery, University of Cincinnati, Cincinnati, Ohio

Abstract

Sepsis is associated with muscle catabolism, mainly reflecting increased protein breakdown, in particular myofibrillar protein breakdown, although reduced protein synthesis and inhibited amino acid uptake contribute to the metabolic response. There is evidence to suggest that both hormones and cytokines participate in the regulation of muscle proteolysis during sepsis. Among the various hormones, glucocorticoids are probably the most important group of regulators that stimulate muscle proteolysis. There is evidence that thyroid hormones may regulate both increased protein breakdown and reduced protein synthesis in skeletal muscle during sepsis. Insulin resistance of protein breakdown demonstrated in muscle from septic rats may contribute to the catabolic response during sepsis. Cytokines, in particular interleukin-1 (IL-1) and tumor necrosis factor (TNF) are also important for the regulation of muscle proteolysis during sepsis. Whereas the effect of TNF on muscle proteolysis is mediated by glucocorticoids, the IL-1 induced muscle proteolysis is independent of glucocorticoids. The two groups of substances, i.e., hormones and cytokines, interact in different ways in their regulation of muscle proteolysis. Cytokines stimulate the release of glucocorticoids, whereas glucocorticoids block the release of cytokines in a negative feedback manner. In addition, there is evidence for an interaction at the cellular level between cytokines and hormones, in particular glucocorticoids, to induce changes in protein metabolism.

Key words: muscle, proteolysis, sepsis, hormones, cytokines.

BAM 3 (2): 143-156, 1993

Severe injury and sepsis are associated with pronounced changes in protein metabolism in various organs and tissues, including liver [43], lungs [82], intestine [97] and skeletal muscle [12, 44]. Whereas protein synthesis is stimulated during sepsis in liver and intestine [43, 97], the metabolic response in lungs and skeletal muscle is that of catabolism [12, 44, 82].

In previous studies, evidence was found that muscle catabolism during sepsis is mainly caused by increased protein breakdown, in particular myofibrillar protein breakdown [44], but reduced protein synthesis [37, 54] and inhibited amino acid uptake [46, 47, 56] contribute to the metabolic response in skeletal muscle. It may be argued from a teleological standpoint that the catabolic response in muscle (and lungs as well) is beneficial to the organism since it results in an increased efflux of amino acids from the periphery; a large portion of these amino acids is taken up by the liver for the synthesis of acute phase proteins [87]; other amino acids, glutamine in particular, are

utilized by the gut [51, 102] and cells in the immune system [77]. Thus, muscle catabolism is probably an important part of the overall metabolic response to sepsis, providing substrates for acute phase proteins and glutamine for the support of intestinal mucosa and cells in the immune system. It should be noted, however, that during prolonged and severe sepsis, continued and uncontrolled muscle catabolism may become deleterious to the patient, resulting in muscle weakness and fatigue, delayed recovery and life-threatening pulmonary complications if respiratory muscles are affected.

Because of the clinical significance of the metabolic response to sepsis in skeletal muscle, much research has been devoted over the last few years to better understand regulatory mechanisms of muscle proteolysis. There is evidence to suggest that muscle proteolysis during sepsis is regulated by both hormones and cytokines, both individually and by an interaction between these substances.

Hormones

Glucocorticoids

There are several lines of evidence to support a role of glucocorticoids in the regulation of muscle proteolysis during sepsis and severe injury. Increased plasma concentrations of cortisol and corticotrophin in severely burned patients [96] and elevated corticosterone levels in septic rats [36] support the concept of a stimulated hypothalamic-pituitary-adrenal axis in these conditions. Stimulated muscle protein breakdown following the administration of glucocorticoids in man [13] or rats [61] are also consistent with a regulatory role of these hormones in the metabolic response to sepsis.

In previous studies, the administration of a combination of the catabolic hormones glucagon, epinephrine and glucocorticoids resulted in several metabolic changes, characteristic of severe injury and sepsis [6]. When a similar "triple hormone infusion" was performed in rats, muscle protein breakdown increased [100]. Because in that report, only the combined effects of the catabolic hormones were tested it is not known which of the three hormones was most important for the increased muscle proteolysis. Considering the strong indications that glucocorticoids regulate muscle protein degradation [13, 61], however, it is likely that corticosterone was the most important component in the "triple hormone infusion" with respect to muscle proteolysis.

In other experiments, we further tested the role of corticosterone (the predominant glucocorticoid in rats) in the regulation of muscle proteolysis [36]. Sepsis induced by cecal ligation and puncture (CLP) in rats resulted in an almost three-fold increase in plasma corticosterone levels. When administration of the hormone to normal rats resulted in similar plasma corticosterone levels as seen in septic rats, muscle protein turnover rates were not affected. However, when plasma corticosterone levels were further increased, muscle protein breakdown, in particular myofibrillar protein breakdown, was increased [36]. This was an important observation since sepsis as well mainly stimulates degradation of myofibrillar protein in skeletal muscle [44]. Other studies as well, have found evidence that myofibrillar proteins are particularly sensitive to the effects of glucocorticoids [61]. The results in our report [36], directly comparing plasma corticosterone levels and muscle protein breakdown rates in septic and hormone-treated rats, suggest that although corticosterone can induce muscle proteolysis in normal rats, increased muscle protein breakdown during sepsis is probably not caused by glucocorticoids alone.

The glucocorticoid receptor blocker RU 38486 offers another tool to test the role of glucocorticoids in sepsis-induced metabolic changes. RU 38486 is the first known antiglucocorticoid that can fully block the hormonal effects *in vivo* and *in vitro* in different tissues without agonist activity even at high concentrations [81]. In recent experiments [39], we examined the role of glucocorticoids in the regulation of muscle proteolysis by treating septic rats with

RU 38486 (5 mg/kg administered by gavage 2 h before CLP). This treatment resulted in a 28% reduction of total protein breakdown, measured as release of tyrosine from incubated extensor digitorum longus muscles, and a 44% inhibition of myofibrillar protein breakdown, measured as release of 3-methylhistidine (3-MH) in muscles from septic rats but did not affect protein breakdown in muscles from sham-operated control rats (Fig. 1). These results strongly support a role of glucocorticoids in the regulation of muscle proteolysis during sepsis. The finding that the increase in muscle protein breakdown observed in septic rats was blunted, but not normalized, following treatment with RU 38486 is consistent with the concept that sepsis-induced muscle proteolysis is not regulated by glucocorti-

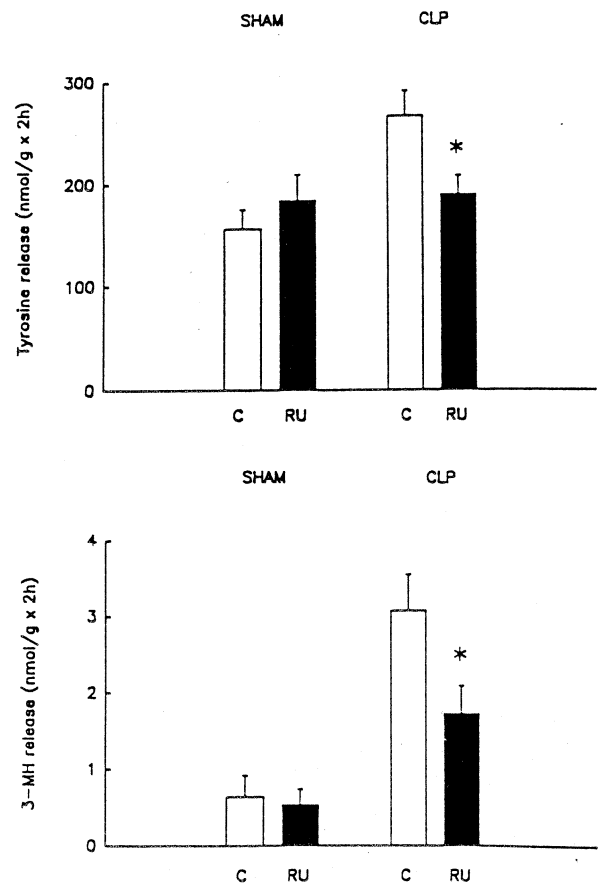


Figure 1. Total (upper panel) and myofibrillar (lower panel) protein breakdown rates in incubated extensor digitorum longus muscles from sham-operated and septic rats (16 h after CLP). Groups of rats were treated with 5 mg/kg of the glucocorticoid receptor antagonist RU 38486 (RU, filled bars) by gavage 2 h before sham-operation or CLP; control rats received corresponding volumes of solvent by gavage at the same time point (C, open bars). * $p < 0.05$ vs CLP/C. The number of rats in each groups was 7. From Ref. #39.

coids alone. In the same experiments [39], the reduced muscle protein synthesis in septic rats, measured as incorporation of ^{14}C -phenylalanine into protein in incubated extensor digitorum longus muscles, was not affected by RU 38486 (Fig. 2), suggesting that muscle protein synthesis and breakdown are regulated by different mechanisms during sepsis.

Thyroid Hormone

There is evidence that thyroid hormones regulate muscle protein turnover, both *in vitro* and *in vivo* [64]. Interestingly, thyroid hormones have opposite effects at low and high concentrations; physiological concentrations of thyroid hormone are necessary for normal growth of muscle and other tissues whereas high hormone concentrations result in muscle catabolism. During hyperthyroidism, muscle catabolism is mainly caused by increased protein breakdown [1] whereas protein synthesis in the hyperthyroid state has been reported to be unchanged [1, 40], increased [9], or reduced [25]. Previous studies suggest that thyroid hormones regulate both the lysosomal and nonlysosomal, ATP-dependent proteolytic pathways in muscle [63].

During sepsis and other critical illness, circulating levels of triiodothyronine (T_3) are reduced, a condition usually referred to as the "low T_3 syndrome" [101]. Although altered peripheral metabolism of thyroxine (T_4) is probably the principal mechanism of low T_3 levels in critical illness, increased uptake by different tissues, including skeletal muscle, may contribute to reduced serum levels [16, 34]. There is evidence that intracellular uptake of T_3 by different tissues is regulated by specific and concentrative transport systems [78].

In a recent study, we tested the role of T_3 in sepsis-induced changes in muscle protein turnover by inducing sepsis in thyroidectomized rats and by measuring T_3 levels in muscle tissue [41]. The increase in muscle protein breakdown observed in septic, thyroidectomized rats was

less pronounced than that in septic, sham-thyroidectomized rats (Fig. 3). This effect of thyroidectomy on the response to sepsis was more pronounced in white, fast-twitch than in red, slow-twitch muscle. The reduction in muscle protein synthesis observed in septic rats was completely abolished in thyroidectomized animals (Fig. 3). These results support the concept that thyroid hormone may participate in the regulation of muscle protein metabolism during sepsis.

In the same study [41], we measured T_3 concentration in muscle tissue during sepsis. Despite reduced serum levels of the hormone, consistent with the "low T_3 syndrome", muscle levels of T_3 were increased (although not significantly so) during sepsis. The results demonstrated that reduced serum levels of T_3 during sepsis do not necessarily reflect tissue levels. From a metabolic standpoint it is, of course, the tissue levels that are important. Thus, it is possible that uptake of T_3 by skeletal muscle is increased during sepsis and that this phenomenon may contribute to both reduced circulating levels of the hormone and reduced synthesis and stimulated breakdown of muscle protein. It will be important in future studies to measure actual uptake rates of T_3 by muscle to test that hypothesis.

Insulin

Insulin is an important regulator of muscle protein metabolism and exerts its anabolic effect by stimulating protein synthesis and inhibiting protein breakdown [58]. The roles of these components in the *in vivo* effects of the hormone are not completely understood. Systemic administration of insulin in humans increased whole body protein synthesis and reduced protein breakdown [10, 30, 93] but results from such experiments must be interpreted with caution since whole body protein turnover rates are influenced by protein metabolism in tissues other than skeletal muscle. When insulin was infused directly into the brachial artery and forearm amino acid flux studies were performed, evidence was found that in healthy humans, physiological elevations of insulin promote net muscle protein anabolism primarily by inhibiting protein breakdown, rather than by stimulating protein synthesis [32]. The importance of maintaining normal plasma amino acid concentrations during hormone infusion was demonstrated in several studies [10, 31, 93]. When hypoaminoacidemia was allowed to develop, the anabolic effect of insulin was prevented and protein synthesis could even be reduced.

The mechanisms by which insulin regulates muscle protein turnover were reviewed previously [57, 58, 64, 65, 91]. The hormone stimulates protein synthesis mainly at the translational level by enhancing peptide-chain initiation but may also increase the capacity for protein synthesis by increasing the concentration of ribosomes [2, 57]. Previous studies suggest that the effect of insulin on protein synthesis is not dependent on the influence of the hormone on amino acid and glucose transport [24, 84]. The mechanism(s) by which insulin blocks protein breakdown are less well understood but inhibition of the lysosomal proteolytic

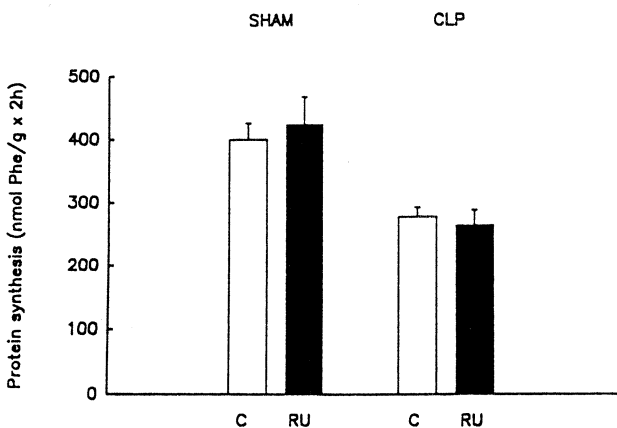


Figure 2. Protein synthesis, measured as incorporation of radiolabeled phenylalanine into protein, in incubated extensor digitorum longus muscles from sham-operated and septic rats, treated with RU 38486 or control gavage as described in Fig. 1.

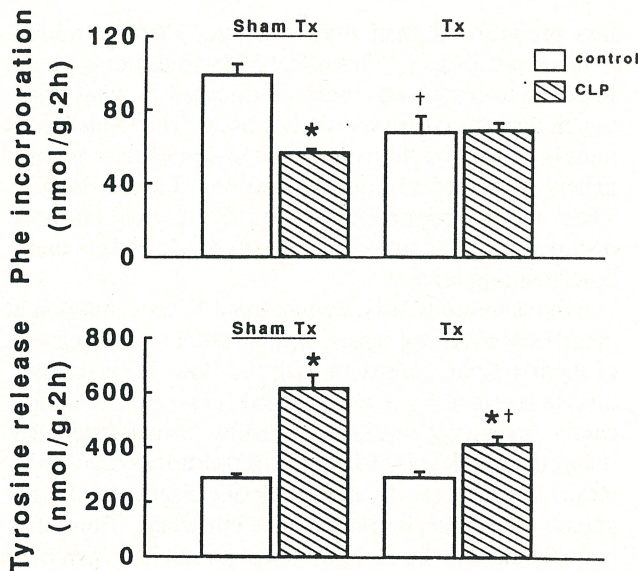


Figure 3. Protein synthesis (upper panel) and breakdown (lower panel) in incubated muscles from control and septic rats that had undergone either sham thyroidectomy (sham Tx) or thyroidectomy (Tx) 5 days before induction of sepsis by CLP. * $p < 0.05$ vs control; + $p < 0.05$ vs corresponding sham Tx group. Based on data in Ref. #41.

pathway and reduced formation of autophagosomes have been reported [58, 64, 68].

In previous reports there was evidence for reduced insulin sensitivity in skeletal muscle during various catabolic conditions. Thus, regulation of muscle protein synthesis and/or degradation became resistant to the hormone after burn injury [76], blunt trauma [94] and denervation [14].

Recent experiments in our laboratory suggest that the response to insulin of muscle protein breakdown is reduced during sepsis [42, 44, 48]. When muscles from non septic rats were incubated in the presence of different concentrations of insulin, total protein breakdown, measured as release of tyrosine, was inhibited by up to 20%. The lowest concentration at which insulin inhibited protein breakdown in these muscles was 100 $\mu\text{U/ml}$ (Fig. 4). In contrast, protein breakdown in muscle from septic rats (16 h after CLP) was not significantly affected by insulin until the hormone concentration was increased to $10^4 \mu\text{U/ml}$ and even at this high concentration, the reduction of proteolysis was less than half of that observed in non-septic muscles (Fig. 4).

Previous studies suggest that the breakdown of myofibrillar proteins is not regulated by insulin in normal muscle [68, 91], although this is somewhat controversial [88]. We recently tested the effect of insulin on myofibrillar protein breakdown, measured as release of 3-MH, in muscle from septic rats [44] and found that the hormone significantly inhibited 3-MH release (Fig. 5). In line with previous reports [68], insulin did not regulate myofibrillar protein

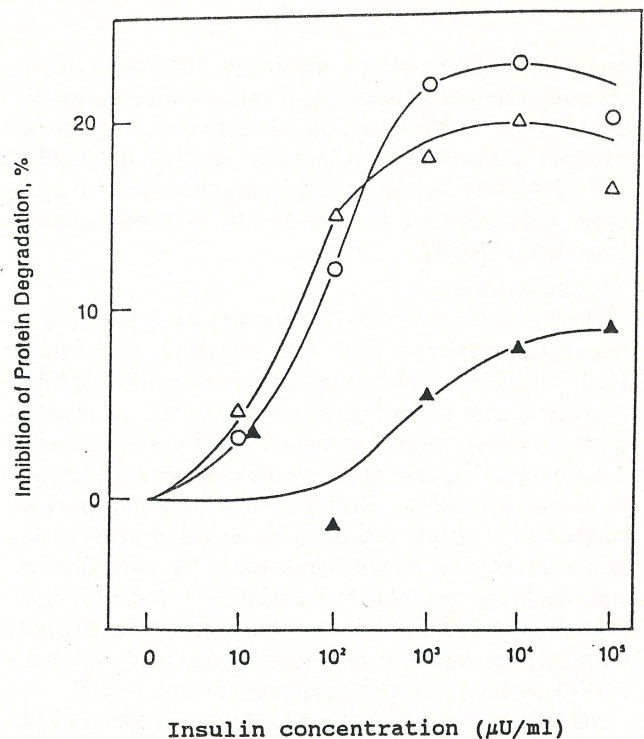


Figure 4. Relationship between insulin concentration in incubation medium and inhibition of protein degradation, measured as release of tyrosine, in extensor digitorum longus muscles from untreated (O), sham-operated (Δ), and septic ($\blacktriangle$) rats. From Ref. #48.

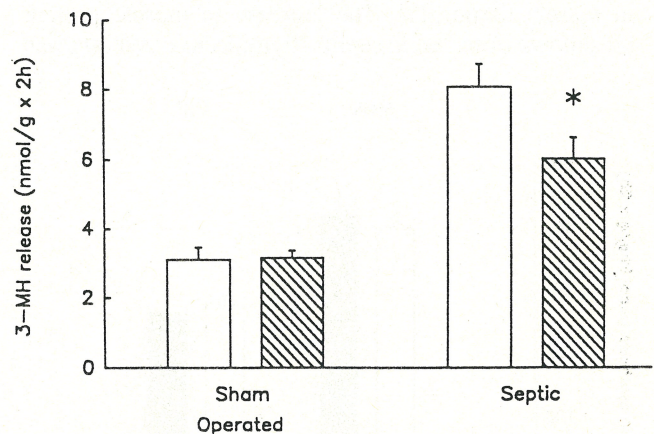


Figure 5. Effect of insulin on myofibrillar protein breakdown (3-MH release) in incubated extensor digitorum longus muscles from sham-operated and septic (16 h after CLP) rats. Muscles were incubated in the absence (open bars) or presence (hatched bars) of insulin ($10^4 \mu\text{U/ml}$). Results are from 7 paired muscles from each group of animals. * $p < 0.05$ vs no insulin. Based on data in Ref. #44.

breakdown in muscle from non-septic rats. The mechanism of insulin inhibition of myofibrillar proteolysis in septic muscle is unclear. Interestingly, a previous study suggests that the hormone inhibits myofibrillar protein breakdown in muscle from rats with diabetes, another catabolic condition associated with stimulated myofibrillar protein breakdown [88].

In the same muscles from septic rats in which total protein breakdown was resistant to insulin, protein synthesis and amino acid uptake were stimulated by the hormone [48]. This finding of a selective impairment of insulin responsiveness in different metabolic pathways suggests that insulin resistance of total protein breakdown in septic muscle is at the postreceptor level [60].

Previous studies suggest that insulin resistance of muscle glucose disposal observed after injury and sepsis is mediated by β -adrenoreceptor activity [7, 11]. We recently found evidence that the same mechanism may be responsible for insulin resistance of total protein breakdown in septic muscle [42]. When muscles from non-septic rats were incubated in the presence of 1 μ M isoproterenol, the dose-response curve for insulin concentration and inhibition of protein degradation was shifted downward and to the right, thus mimicking the situation in septic muscle. Conversely, when septic muscles were incubated in the presence of 1 μ M propranolol, the dose-response curve was shifted upward and to the left, suggesting that the insulin resistance was reversed by β -adrenoreceptor blockade [42].

It should be noted that although there is evidence that muscle protein breakdown becomes refractory to insulin during sepsis, it is not known if this phenomenon is a mechanism of sepsis-induced muscle proteolysis. In previous studies, whole-body or forearm protein catabolism was inhibited by the administration of insulin to injured patients [103] or animals [92], and the use of insulin to reduce the catabolic response to trauma was suggested. Improved nitrogen balance following treatment with insulin in patients with trauma, however, has been short-lasting [69, 103], possibly reflecting insulin resistance of protein metabolism. The effect of *in vivo* administration of insulin on muscle catabolism during sepsis remains to be determined.

Growth Hormone and Insulin-like Growth Factor I

The anabolic effects of growth hormone are well recognized. Previous studies suggest that the hormone promotes muscle protein synthesis but does not prevent muscle protein breakdown [19, 50, 59, 70, 99]. In several reports, administration of recombinant growth hormone improved nitrogen balance in patients with catabolic conditions, such as burn injury [50], surgical trauma [59, 99], critical illness [19], and in patients receiving parenteral nutrition [83] or healthy volunteers treated with glucocorticoids [53].

There is evidence that growth hormone exerts its anabolic effects via the production in the liver of insulin-like growth factor I (IGF-I) [18]. Abundant receptors for IGF-I are present in muscle [27]. IGF-I promotes protein balance

in cultured myoblasts [3] and stimulates protein synthesis and inhibits protein breakdown in incubated rat muscles [64]. A protein sparing effect following the *in vivo* administration of IGF-I has also been demonstrated [18].

The effect of IGF-I on muscle protein balance is of particular interest since during sepsis and other catabolic conditions, plasma levels of the hormone are reduced, concomitantly with increased concentrations of growth hormone [85]. Thus, it is possible that reduced IGF-I levels contribute to muscle catabolism during sepsis. Recent studies in animals suggest that IGF-I prevents weight loss after burn injury [90], inhibits protein degradation during starvation [79] and reduces protein loss during infusion of tumor necrosis factor (TNF) [18]. These observations suggest that IGF-I may have a role in the treatment of patients with sepsis and other catabolic conditions.

Cytokines

Cytokines are regulatory proteins released from a variety of cells although in trauma and sepsis, macrophages are the most important source. The role of cytokines as mediators of metabolic changes during inflammation, severe injury and sepsis was reviewed recently [15, 22, 29]. Cytokines primarily act locally in a paracrine fashion but increased plasma levels of the cytokines have been observed in septic patients [98], suggesting that they may also have a hormone-like function.

Among the cytokines, interleukin-1 (IL-1) and TNF have been most extensively studied with respect to regulation of muscle protein breakdown. Other cytokines are more important for the regulation of protein metabolism in other organs and tissues. For example, it is well established that IL-6 is the principle regulator of acute-phase protein synthesis in the liver during sepsis [49]. Although previous reports on the influence of cytokines on muscle protein breakdown were contradictory [62, 73], recent studies in our laboratory suggest that both IL-1 and TNF regulate muscle protein degradation [104-108]. In other experiments we have found evidence that these substances influence muscle proteolysis through different mechanisms (see below).

Interleukin-1 (IL-1)

In experiments reported approximately 10 years ago, Clowes et al [12] found evidence that muscle proteolysis during sepsis is mediated by a circulating factor. Addition of plasma from septic or injured patients to incubated human muscle or rat soleus muscles stimulated protein breakdown, measured as release of tyrosine. Isolation and purification of the factor in septic plasma that caused increased muscle protein degradation provided evidence that the "Proteolysis Inducing Factor", or PIF, was a peptide with a molecular weight of approximately 4,200 daltons and it was suggested that PIF was a cleavage product of IL-1. In similar experiments in our laboratory, plasma from septic rats stimulated protein breakdown when added to incubated muscles from nonseptic rats but this phenomenon was observed only under very specific *in vitro*

conditions and was not characterized by the typical septic response of a predominant increase in myofibrillar protein breakdown [45]. Thus, the role of PIF in sepsis-induced muscle proteolysis remains controversial.

Although the role of PIF in the regulation of muscle protein breakdown is unclear, there are several lines of evidence to suggest that IL-1 is a mediator of sepsis-induced muscle proteolysis. In recent studies in our laboratory, administration of recombinant IL-1 α (rIL-1 α) to rats (300 μ g/kg divided into three i.p. doses over 16 h) resulted in increased muscle protein breakdown, in particular an increase in myofibrillar proteolysis [104, 107, 108], similar to the metabolic response to sepsis (Fig. 6). Other studies as well have found evidence that administration of IL-1 results in muscle catabolism. Fong et al [28] reported decreased muscle protein content and mRNA levels for myofibrillar proteins following chronic administration of rIL-1 α in rats. In another report, 6 h infusion of rIL-1 β in rats augmented the catabolic effect of rTNF α , assessed as changes in the specific radioactivity in the precursor pool following infusion of 14 C-leucine [26].

To further test the role of IL-1 in the regulation of muscle proteolysis, we recently treated septic or endotoxemic rats with recombinant IL-1 receptor antagonist (rIL-1ra) [106]. When endotoxemic rats were treated with a total dose of 105 mg/kg of IL-1ra, the increase in muscle total protein breakdown was blocked (Fig. 7). The increase in myofibrillar proteolysis during endotoxemia was inhibited by an even smaller dose of rIL-1ra, i.e., 45 mg/kg [106]. Similar results were observed when rats with sepsis, induced by CLP, were treated with IL-1ra (unpublished observations). These results support the concept that IL-1 participates in the regulation of muscle proteolysis during sepsis and endotoxemia. They do not, however, clarify whether IL-1 is the only mediator of muscle proteolysis in these conditions (in fact, other studies discussed in this article suggest that IL-1 is not the only mediator of muscle proteolysis during sepsis). It is also not known from the experiments described above whether IL-1 has a direct effect on muscle or acts through other substances. In vitro experiments, in which various concentrations of rIL-1 α were added to incubated muscles, failed to demonstrate a direct effect of this cytokine on muscle protein breakdown [45, 55, 73].

Tumor Necrosis Factor (TNF)

TNF plays a central role in the development of the sepsis syndrome [95]. Several studies have shown improved survival in sepsis and endotoxemia following treatment with anti-TNF antibodies [8, 52, 71] but conflicting results have also been reported [21]. TNF is usually considered to be a proximal mediator in the cascade of proinflammatory cytokines released during sepsis, and there is evidence of TNF-induced production of IL-1, IL-6, IL-8 and TNF itself [95].

Although earlier studies indicated that TNF does not regulate muscle protein metabolism [62, 73], more recent experiments, both in our [104, 105] and other laboratories [26, 33] suggest that TNF stimulates muscle proteolysis

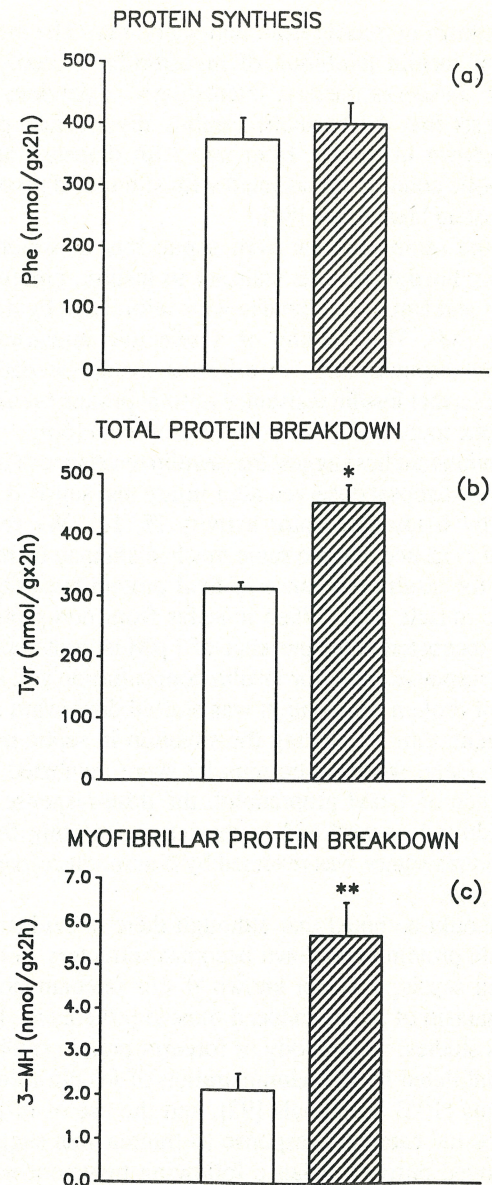


Figure 6. Effect of rIL-1 α injected intraperitoneally in rats at a dose of 100 μ g/kg three times over 16 h on protein synthesis (a), total protein breakdown (b), and myofibrillar protein breakdown (c) in incubated extensor digitorum longus muscles. Control rats, open bars; rIL-1 α treated rats, hatched bars. * $p < 0.05$; ** $p < 0.01$. From Ref. # 108.

and may participate in the regulation of the catabolic response in muscle during sepsis. Following administration of rTNF α to rats (100 μ g/kg intraperitoneally, repeated three times over 16 h), protein breakdown in incubated EDL muscles, dissected 2 h after the last cytokine injection, was increased with a marked stimulation of myofibrillar protein breakdown [105] (Fig. 8), similar to the metabolic pattern seen in sepsis. In the same study, muscle protein synthesis was not affected by the cytokine.

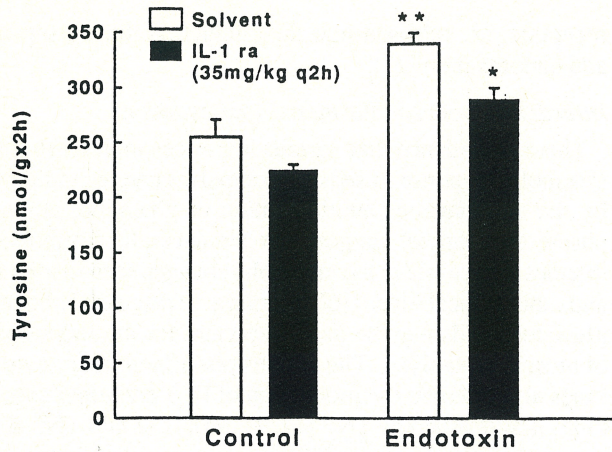


Figure 7. Total protein breakdown rates in incubated extensor digitorum longus muscles from control and endotoxin (1 mg/kg) injected rats. Muscles were incubated 4 h after control or endotoxin injection. Groups of rats were treated with recombinant IL-1 receptor antagonist (rIL-1ra; 35 mg/kg every 2 h) or corresponding volumes of solvent. The number of rats in each group was 7. * $p < 0.05$ vs endotoxin/solvent; ** $p < 0.05$ vs all other groups. From Ref. #106.

The interval between cytokine administration and muscle incubation in these experiments was probably critical since in another study from our laboratory no changes in protein turnover rates were noted in muscles harvested 8 h after TNF injection [38]. The timing between TNF administration and measurement of protein breakdown may also explain why previous studies from other laboratories were negative. In a study by Kettelhut and Goldberg [62], in which no changes in protein turn over rates were noted, muscles were harvested up to 6 h after injection of 4 mg/kg of TNF.

To test the role of TNF in sepsis-induced muscle proteolysis, we treated septic rats with TNF-antiserum [105]. Administration of TNF-antiserum (1 ml/100 g b.w. intraperitoneally 2 h before CLP) significantly blunted, but did not normalize, the increased muscle protein breakdown rates seen in septic rats (Fig. 9). These results suggest that TNF is a mediator of muscle proteolysis during sepsis but do not answer the question whether TNF has a direct effect on muscle or acts through other mediators. In the same experiments, the reduced muscle protein synthesis, observed in septic rats, was not significantly affected by TNF-antiserum (Fig. 9). This finding supports the concept that increased protein breakdown and reduced protein synthesis during sepsis are mediated by different regulators.

It should be noted that although muscle protein breakdown was inhibited in septic rats treated with TNF-antiserum, proteolytic rates were not normalized (Fig. 9). Thus, TNF is probably only one of several metabolic mediators

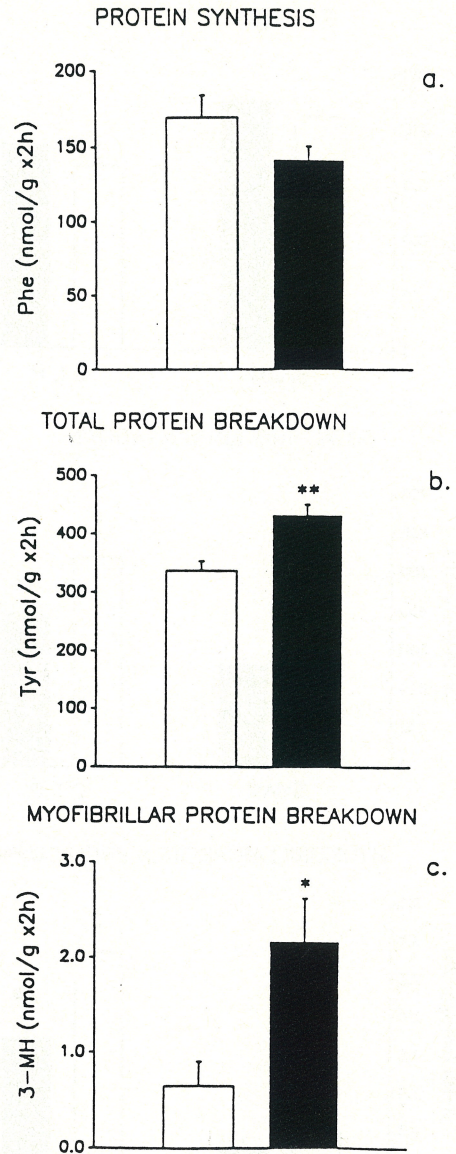


Figure 8. Protein synthesis (a), total protein breakdown (b), and myofibrillar protein breakdown (c) in incubated extensor digitorum longus muscles from rats that had been treated with three i.p. doses of 100 μ g/kg of rTNF α over 16 h (filled bars) or corresponding volumes of saline (open bars). * $p < 0.05$; ** $p < 0.01$. From Ref. #105.

that work in concert during sepsis. Evidence of a synergistic effect between TNF and IL-1 [26] and between TNF and glucocorticoids [38] in the regulation of muscle protein turnover supports that concept.

Thus, our studies provide evidence that both TNF and IL-1 participate in the regulation of muscle proteolysis during sepsis. Because TNF induces the release of IL-1 [17, 74] it is possible that the effects caused by injection of TNF in normal rats or by TNF-antiserum in septic rats reflect the regulatory effect of IL-1. It is also possible that

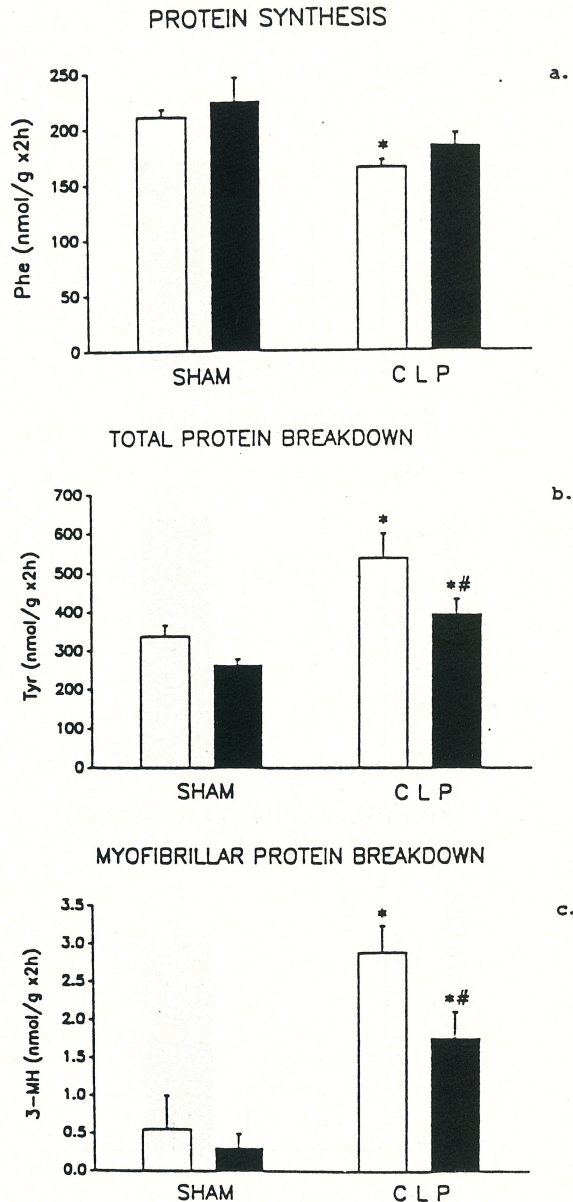


Figure 9. Protein synthesis (a), total protein breakdown (b) and myofibrillar protein breakdown (c) in incubated extensor digitorum longus muscles from sham-operated (non-septic) and septic (16 h after CLP) rats. Groups of rats were treated with anti-TNF antiserum, administered i.p. 2 h before sham-operation or CLP (filled bars); other rats received corresponding injections of control serum (open bars). * $p < 0.05$ vs corresponding sham group; ** $p < 0.05$ vs CLP/control serum. Based on results in Ref. #105.

both cytokines exert their effects through other mediators, for example cytokines such as IL-6 [75], or hormones, such as glucocorticoids. The interaction between the different cytokines is further complicated by the fact that endogenous inhibitors of the cytokines, such as IL-1ra and TNF

receptors, are released into the circulation during sepsis and endotoxemia [23].

Interaction between hormones and cytokines

There is evidence that various hormones interact in the metabolic response to sepsis. Metabolic changes induced by the simultaneous administration of glucagon, epinephrine and cortisol suggest that the catabolic hormones interact to give rise to the metabolic changes seen in sepsis and other critical illness [6], although, as discussed above, glucocorticoids may be most important for the induction of muscle proteolysis. The various proinflammatory cytokines also interact; the production of IL-1 and other cytokines is stimulated by TNF [17, 74, 95]; IL-1 and TNF act synergistically on muscle [26] and the interaction between various cytokines is important for metabolic alterations in other tissues as well, such as liver [4].

Perhaps even more interesting is the interaction between the two types of substances, i.e. hormones and cytokines. Both TNF and IL-1 stimulate the hypothalamic-pituitary-adrenal axis with release of corticotropin and glucocorticoids [35, 86]. Glucocorticoids, in turn, inhibit the release of cytokines in a negative feed back like fashion [5]. There is also evidence of an interaction between thyroid hormones and cytokines; administration of TNF or IL-1 results in low serum levels of thyroid hormone [20, 80], similar to the "low T_3 syndrome" in sepsis [101].

Also with respect to metabolic changes, there is evidence for an interaction between hormones and cytokines. In the hepatocyte, dexamethasone is an important cofactor to IL-6 in the induction of acute phase protein synthesis [4, 66]. A similar interaction between glucocorticoids and TNF stimulates muscle proteolysis. In a recent study from our laboratory [38], administration of 200 $\mu\text{g/kg}$ of rTNF α to rats stimulated muscle proteolysis only when the cytokine was given together with corticosterone and when the treatment resulted in increased plasma corticosterone levels (Fig. 10). When the same protocol was used in adrenalectomized rats, plasma corticosterone was not increased and muscle protein breakdown rates were unaffected by the cytokine, further supporting the concept of an interaction between glucocorticoids and TNF in the stimulation of muscle protein breakdown.

A recent study by Mealy et al [72] supports the concept that glucocorticoids are necessary for the metabolic effects of TNF. Administration of TNF in normal rats resulted in increased adrenal weight and plasma corticotropin levels, concomitant with reduced carcass nitrogen content and nitrogen balance. In contrast, when TNF was infused in adrenalectomized rats, the urinary nitrogen loss was less pronounced than seen in intact, TNF-infused rats. Although protein turnover rates were not measured in that study, the results were interpreted as indicating that TNF-stimulated muscle breakdown was mediated by glucocorticoids [72].

In a recent study in our laboratory, muscle protein turnover rates were measured in TNF-treated rats that had also been treated with the glucocorticoid receptor antagonist

Muscle proteolysis in sepsis

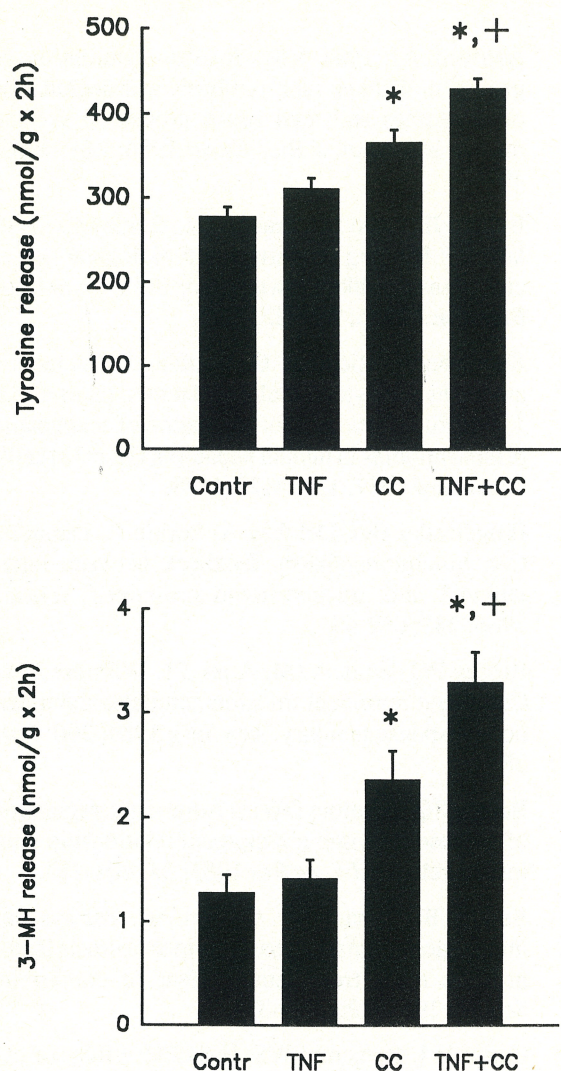


Figure 10. Total (upper panel) and myofibrillar (lower panel) protein breakdown rates in incubated extensor digitorum longus muscles from rats that had been treated with rTNF α , corticosterone (CC) or a combination of rTNF α and corticosterone. * $p < 0.05$ vs control rats; + $p < 0.05$ vs cc. From Ref. #38.

RU 38486 [104]. The increase in both total and myofibrillar protein breakdown induced by TNF was almost completely abolished by RU 38438 (Fig. 11), supporting the concept that TNF-induced muscle proteolysis is mediated by glucocorticoids.

In contrast to TNF, IL-1 seems to induce muscle protein breakdown through a glucocorticoid-independent pathway. The IL-1 induced muscle proteolysis was not affected by RU 38486 [104] (Fig. 12) and IL-1 stimulated muscle proteolysis both in sham-adrenalectomized and adrenalectomized rats [108]. The finding that IL-1 stimulates muscle proteolysis independently of glucocorticoids does not necessarily mean that IL-1 has a direct effect on muscle. Previous studies in which IL-1 was added to incubated

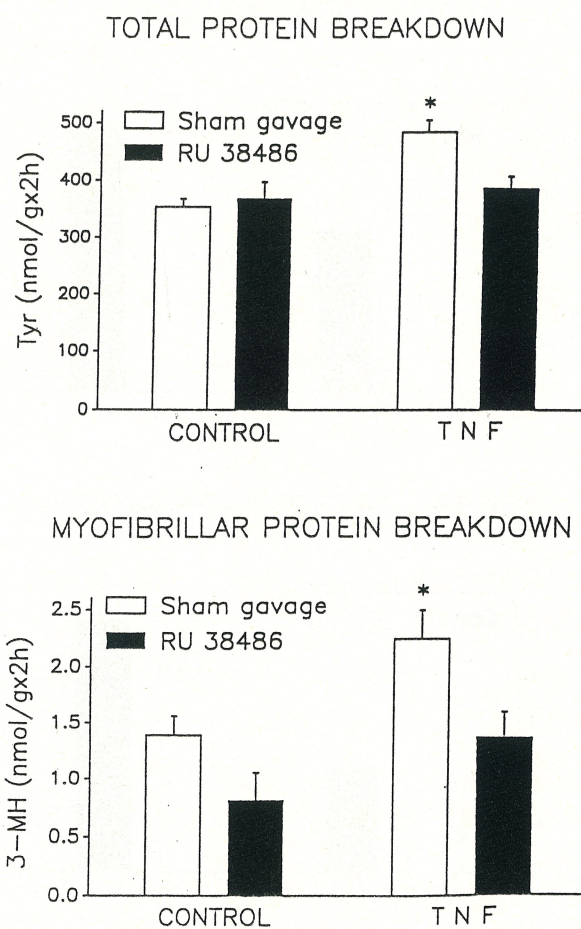


Figure 11. Total (upper panel) and myofibrillar (lower panel) protein breakdown rates in incubated extensor digitorum longus muscles from rats that had been treated with three i.p. injections of 100 μ g/kg of rTNF α over 16 h or corresponding injections of saline (control). Groups of rats were treated with 5 mg/kg of RU 38486 by gavage 2 h before each injection of TNF or saline or received corresponding volumes of solvent by gavage (sham gavage). * $p < 0.05$ vs all other groups. From Ref. #104.

muscles at different concentrations failed to demonstrate a direct effect of the cytokine on muscle protein turnover [45, 55, 73]. It is possible that IL-1 induces muscle proteolysis through some other substance than corticosterone, perhaps another cytokine. TNF and/or IL-1 may stimulate the release of IL-6 and previous studies suggest that several metabolic effects of IL-1 are mediated by IL-6 [75, 89]. The effect of IL-6 on muscle protein turnover remains to be determined.

Another possible mechanism by which IL-1 may increase muscle proteolysis could be inhibited release of a factor that normally promotes muscle anabolism. In a recent study, acute treatment of mice with IL-1 α , but not TNF α , resulted in a significant decline in free and total

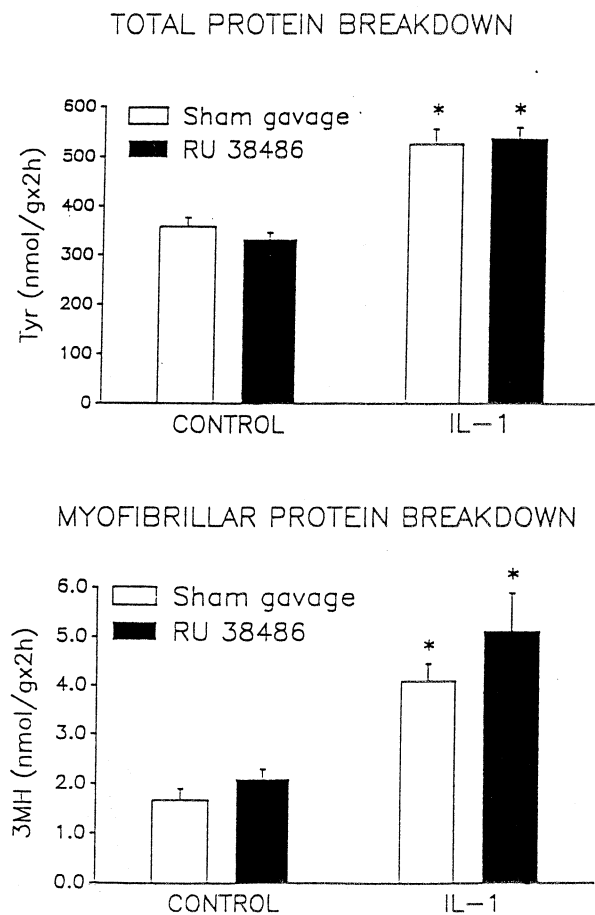


Figure 12. Total and myofibrillar protein breakdown rates in incubated extensor digitorum longus muscles. Experimental conditions were identical to those in Fig 11 except that rIL-1 α was injected instead of rTNF α . * $p < 0.05$ vs corresponding control group.

circulating IGF-I levels [67], offering another example of interaction between cytokines and hormones. The anabolic effects of IGF-I were discussed above. Thus, it may be speculated that during sepsis, TNF contributes to muscle proteolysis through an interaction with glucocorticoids whereas IL-1 promotes muscle catabolism by inhibited release of IGF-I.

Address correspondence to:

Dr. Per-Olof Hasselgren, Department of Surgery, University of Cincinnati Medical Center, 231 Bethesda Avenue, Cincinnati, Ohio 45267-0558

References

- [1] Angerås U, Hasselgren PO: Protein turnover in different types of skeletal muscle during experimental hyperthyroidism in rats. *Acta Endocrinol* 1985; 109: 90-95.
- [2] Ashford AJ, Pain VM: Insulin stimulation of growth in diabetic rats: synthesis and degradation of ribosomes and total tissue protein in skeletal muscle and heart. *J Biol Chem* 1986; 261: 4066-4070.
- [3] Ballard JF, Read LC, Francis GL, Bagley CJ, Wallace JC: Binding properties and biological potencies of insulin-like growth factor in L6 myoblasts. *Biochem J* 1986; 233: 223-230.
- [4] Baumann H, Richards C, Gaudie J: Interaction among hepatocyte-stimulating factors, interleukin-1, and glucocorticoids for regulation of acute phase plasma proteins in human hepatoma (HepG2) cells. *J Immunol* 1987; 139: 4122-4128.
- [5] Besedovsky HO, Del Rey A, Sorkin E, Dinarello CA: Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* 1986; 233: 652-654.
- [6] Bessey PQ, Watters JM, Aoki TT, Wilmore DW: Combined hormonal infusion simulates the metabolic response to injury. *Ann Surg* 1984; 200: 264-281.
- [7] Bessey PQ, Wilmore DW: β -Adrenergic regulation of glucose disposal: a reciprocal relationship with insulin release. *J Surg Res* 1983; 34: 404-414.
- [8] Beutler B, Milsark IW, Cerami AC: Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* 1985; 229: 869-871.
- [9] Carter WJ, Benjamin WS, Faas FH: Effect of experimental hyperthyroidism on protein turnover in skeletal and cardiac muscle as measured by [14 C]-tyrosine infusion. *Biochem J* 1982; 204: 69-74.
- [10] Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of insulin and plasma amino acid concentrations on leucine metabolism in man: role of substrate availability on estimates of whole body protein synthesis. *J Clin Invest* 1987; 80: 1784-1793.
- [11] Chiasson JL, Shikama H, Chu DTW, Exton JH: Inhibitory effect of epinephrine on insulin-stimulated glucose uptake by rat skeletal muscle. *J Clin Invest* 1981; 68: 706-713.
- [12] Clowes GHA, George BC, Villee CA, Saravis CA: Muscle proteolysis induced by a circulating peptide in patients with sepsis or trauma. *N Engl J Med* 1983; 308: 545-552.
- [13] Darman D, Matthews ED, Bier DM: Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am J Physiol* 1988; 255: E366-E373.

- [14] Davis TA, Karl IE: Resistance of protein and glucose metabolism to insulin in denervated rat muscle. *Biochem J* 1988; 254: 667-675.
- [15] Del Rey A, Besedovsky HO: Metabolic and neuroendocrine effects of proinflammatory cytokines. *Europ J Clin Invest* 1992; 22: 10-15.
- [16] De Rubertis FR, Woeber KA: Accelerated cellular uptake and metabolism of L-thyroxine during acute *Salmonella typhimurium* sepsis. *J Clin Invest* 1973; 52: 78-87.
- [17] Dinarello CA, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MA, O'Connor J: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin-1. *J Exp Med* 1986; 163: 1433-1450.
- [18] Douglas RG, Gluckman PD, Breier BH, McCall JL, Parry B, Shaw JHF: Effects of recombinant IGF-I on protein and glucose metabolism in rTNF-infused lambs. *Am J Physiol* 1991; 261: E606-E612.
- [19] Douglas RG, Humberstone DA, Haystead A, Shaw JHF: Metabolic effects of recombinant human growth hormone: isotropic studies in the postabsorptive state and during total parenteral nutrition. *Br J Surg* 1990; 77: 785-790.
- [20] Dubuis JM, Dayer JM, Siegrist-Kaiser CA, Burger AG: Human recombinant interleukin-1 β decreases plasma thyroid hormone and thyroid stimulating hormone levels in rats. *Endocrinology* 1988; 123: 2175-2181.
- [21] Eskandari MK, Bolgos G, Miller C, Nguyen DT, DeForge LE, Remick DG: Anti-tumor necrosis factor antibody therapy fails to prevent lethality after cecal ligation and puncture or endotoxemia. *J Immunol* 1992; 148: 2724-2730.
- [22] Fischer JE, Hasselgren PO: Cytokines and glucocorticoids in the regulation of the "hepato-skeletal muscle axis" in sepsis. *Am J Surg* 1991; 161: 266-271.
- [23] Fischer E, Van Zee KJ, Marano MA, Rock CS, Kenney JS, Pontsiaka DD, Dinarello CA, Lowry SF, Moldawer LL: Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease. *Blood* 1992; 79: 2196-2200.
- [24] Flaim KE, Kochel PJ, Kira Y, Kobayashi ET, Jefferson LS, Morgan HE: Insulin effects on protein synthesis are independent of glucose and energy metabolism. *Am J Physiol* 1983; 245: C133-C143.
- [25] Flaim KE, Li JB, Jefferson LS: Effects of thyroxine on protein turnover in rat skeletal muscle. *Am J Physiol* 1978; 235: E231-E236.
- [26] Flores EA, Bistrian BR, Pomposelli JJ, Dinarello CA, Blackburn GG, Istfan NW: Infusion of tumor necrosis factor/cachectin promotes muscle catabolism in the rat. A synergistic effect with interleukin-1. *J Clin Invest* 1989; 83: 1614-1622.
- [27] Florini JR: Hormonal control of muscle growth. *Muscle Nerve* 1987; 10: 577-598.
- [28] Fong Y, Moldawer LL, Marano M, Wei H, Barber A, Manogue K, Tracey KJ, Kuo G, Fischman DA, Cerami A, Lowry SF: Cachectin/TNF or IL-1 α induces cachexia with redistribution of body proteins. *Am J Physiol* 1989; 253: R659-R665.
- [29] Fong Y, Moldawer LL, Shires T, Lowry SF: The biological characteristics of cytokines and their implication in surgical injury. *Surg Gynecol Obstet* 1990; 170: 363-378.
- [30] Fukugawa NK, Minaker KL, Rowe JW, Goodman MN, Matthews DE, Bier DM, Young VR: Insulin-mediated reduction of whole body protein breakdown: dose-response effects on leucine metabolism in postabsorptive men. *J Clin Invest* 1985; 76: 2306-2311.
- [31] Garlick PJ, Grant I: Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin: effect of branched-chain amino acids. *Biochem J* 1988; 254: 579-584.
- [32] Gelfand RA, Barrett EJ: Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest* 1987; 80: 1-6.
- [33] Goodman MN: Tumor necrosis factor induces skeletal muscle protein breakdown in rats. *Am J Physiol* 1991; 260: E727-E730.
- [34] Goretzki PE, Cavalier RR, Wahl RA, Ellis RJ, McMahon FA, Goldfien S, Gerend P, Clark OH: Serum and tissue T₃ levels in endotoxin shock in the rat. *J Surg Res* 1983; 35: 211-217.
- [35] Gwosdow AR, Kumar ASR, Bode HH: Interleukin-1 stimulation of the hypothalamic-pituitary-adrenal axis. *Am J Physiol* 1990; 258: E65-E70.
- [36] Hall-Ångerås M, Ångerås U, Hasselgren PO, Fischer JE: Corticosterone alone does not explain increased muscle proteolysis in septic rats. *J Surg Res* 1990; 48: 368-372.
- [37] Hall-Ångerås M, Ångerås U, von Allmen D, Higashiguchi T, Zamir O, Hasselgren PO, Fischer JE: Influence of sepsis in rats on muscle protein turnover *in vivo* and in tissue incubated under different *in vitro* conditions. *Metabolism* 1991; 40: 247-251.
- [38] Hall-Ångerås M, Ångerås U, Zamir O, Hasselgren PO, Fischer JE: Interaction between corticosterone

- and tumor necrosis factor stimulated protein breakdown in rat skeletal muscle, similar to sepsis. *Surgery* 1990; 108: 460-466.
- [39] Hall-Angerås M, Angerås U, Zamir O, Hasselgren PO, Fischer JE: Effect of the glucocorticoid receptor antagonist RU 38486 on muscle protein breakdown in sepsis. *Surgery* 1991; 109: 468-473.
- [40] Hasselgren PO, Adlerberth A, Angerås U, Stenström G: Protein metabolism in skeletal muscle tissue from hyperthyroid patients following pre-operative treatment with antithyroid drug or selective beta-blocking agent: results from a prospective randomized study. *J Clin Endocrinol Metab* 1984; 59: 835-839.
- [41] Hasselgren PO, Chen IW, James JH, Sperling M, Warner BW, Fischer JE: Studies on the possible role of thyroid hormone in altered muscle protein turnover during sepsis. *Ann Surg* 1987; 206: 18-24.
- [42] Hasselgren PO, Fischer JE: Regulation by insulin of muscle protein metabolism during sepsis and other catabolic conditions. *Nutrition* 1992; 8: 434-439.
- [43] Hasselgren PO, Jagenburg R, Karlström L, Seeman T: Changes of protein metabolism in liver and skeletal muscle following trauma complicated by sepsis. *J Trauma* 1984; 24: 224-228.
- [44] Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Hiyama DT, Li S, Fischer JE: Total and myofibrillar protein breakdown in different types of rat skeletal muscle: effects of sepsis and regulation by insulin. *Metabolism* 1989; 38: 634-640.
- [45] Hasselgren PO, James JH, Benson DW, Li S, Fischer JE: Is there a circulating proteolysis-inducing factor during sepsis. *Arch Surg* 1990; 125: 510-514.
- [46] Hasselgren PO, James JH, Fischer JE: Inhibited amino acid uptake in sepsis. *Ann Surg* 1986; 203: 360-365.
- [47] Hasselgren PO, James JH, Warner BW, Ogle CK, Takehara H, Fischer JE: Reduced muscle amino acid uptake in sepsis and the effects in vitro of septic plasma and interleukin-1. *Surgery* 1986; 100: 222-228.
- [48] Hasselgren PO, Warner BW, James JH, Takehara H, Fischer JE: Effect of insulin on amino acid uptake and protein turnover in skeletal muscle from septic rats. Evidence for insulin resistance of protein breakdown. *Arch Surg* 1987; 122: 228-233.
- [49] Heinrich PC, Castell JV, Andus T: Interleukin-6 and the acute phase response. *Biochem J* 1990; 265: 621-636.
- [50] Herndon DN, Barrow RE, Kunkel KR, Broemeling L, Rutan RL: Effects of recombinant human growth hormone on donor-site healing in severely burnt children. *Ann Surg* 1990; 212: 424-429.
- [51] Higashiguchi T, Hasselgren PO, Frederick JA, Wagner K, Fischer JE: Protein synthesis in isolated rat enterocytes and the influence of glutamine. *JPEN* 1993; 17: 307-314.
- [52] Hinshaw L, Olson P, Kuo G: Efficacy of post-treatment with anti-TNF monoclonal antibody in preventing the pathophysiology and lethality of sepsis in the baboon. *Circ Shock* 1989; 27: 362-368.
- [53] Horber FF, Haymond MW: Human growth hormone prevents the protein catabolic side effects of prednisolone in humans. *J Clin Invest* 1990; 86: 265-272.
- [54] Hummel RP, Hasselgren PO, James JH, Warner BW, Fischer JE: The effects of sepsis in rats on skeletal muscle protein synthesis *in vivo* in periphery and central core of incubated muscle preparations *in vitro*. *Metabolism* 1988; 37: 1120-1127.
- [55] Hummel RP, Warner BW, Pederson P, Hasselgren PO, James JH, Ogle CK, Fischer JE: In vitro effects of TNF, IL-1 α , and other monokines on skeletal muscle amino acid uptake and protein degradation. *Surg Forum* 1987; 38: 13-15.
- [56] James JH, Hasselgren PO, Hummel RP, Warner BW, Fischer JE: Effect of sepsis on amino acid transport system A and its response to insulin in incubated rat skeletal muscle. *Metabolism* 1990; 39: 335-340.
- [57] Jefferson LS: Role of insulin in the regulation of protein synthesis. *Diabetes* 1980; 29: 487-496.
- [58] Jefferson LS, Rannels DE, Munger BL, Morgan HE: Insulin in the regulation of protein turnover in heart and skeletal muscle. *Fed Proc* 1974; 33: 1098-1104.
- [59] Jiang ZM, He GZ, Zhang SY, Wang XR, Yang NF, Zhu YU, Wilmore DW: Low-dose growth hormone and hypocaloric nutrition attenuate the protein-catabolic response after major operation. *Ann Surg* 1989; 210: 513-524.
- [60] Kahn CR: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism* 1978; 27: 1893-1902.
- [61] Kayali AG, Young VR, Goodman MN: Sensitivity of myofibrillar proteins to glucocorticoid-induced muscle proteolysis. *Am J Physiol* 1987; 252: E621-E626.
- [62] Kettelhut IC, Goldberg AL: Tumor necrosis factor can induce fever in rats without activating protein

- breakdown in muscle or lipolysis in adipose tissue. *J Clin Invest* 1988; 81: 1384-1389.
- [63] Kettelhut IC, Leopold B, Tawa NE, Goldberg AL: Protein deficiency and lack of pituitary hormones reduce both lysosomal and ATP-dependent (non-lysosomal) proteolytic processes in skeletal muscle. *Fed Proc* 1988; 2: A564.
- [64] Kettelhut IC, Wing SS, Goldberg AL: Endocrine regulation of protein breakdown in skeletal muscle. *Diabetes Metab Rev* 1988; 4: 751-772.
- [65] Kimball SR, Jefferson LS: Cellular mechanisms involved in the action of insulin on protein synthesis. *Diabetes Metab Rev* 1988; 4: 773-787.
- [66] Koj A, Gauldie J, Regoeczi E, Sander DN, Sweeney GD: The acute phase response of cultured rat hepatocytes. *Biochem J* 1984; 224: 505-514.
- [67] Lazarus DD, Lowry SF, Moldawer LL: Cytokines acutely decrease circulating insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3). *Surg Forum* 1992; 43: 92-94.
- [68] Lowell BB, Ruderman NB, Goodman MN: Evidence that liposomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. *Biochem J* 1986; 234: 237-240.
- [69] Macfie J, Yule AG, Hill GL: Effect of added insulin on body composition of gastroenterologic patients receiving intravenous nutrition - a controlled clinical trial. *Gastroenterology* 1981; 81: 285-289.
- [70] Manson JM, Smith RJ, Wilmore DW: Growth hormone stimulates protein synthesis during hypocaloric parenteral nutrition. *Ann Surg* 1988; 208: 136-142.
- [71] Mathison JC, Wolfson E, Ulevitch RJ: Participation of tumor necrosis factor in the mediation of gram-negative bacterial lipopolysaccharide-induced injury in rabbits. *J Clin Invest* 1988; 81: 1925-1937.
- [72] Mealy K, van Lanschot JJB, Robinson BG, Ramos J, Wilmore DW: Are the catabolic effects of tumor necrosis factor mediated by glucocorticoids? *Arch Surg* 1990; 125: 42-48.
- [73] Moldawer LL, Svaninger G, Gelin J, Lundholm K: Interleukin-1 and tumor necrosis factor do not regulate protein balance in skeletal muscle. *Am J Physiol* 1987; 253: C766-C773.
- [74] Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D: Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin-1. *J Exp Med* 1986; 163: 1363-1375.
- [75] Neta R, Vogel SN, Sipe JD, Wong GG, Nordan RP: Comparison of in vivo effects of human recombinant IL-1 and human recombinant IL-6 in mice. *Lymphokine Res* 1988; 7: 403-412.
- [76] Newman JJ, Goodwin CW, Mason AD, Pruitt BA: Altered protein metabolism in diaphragms from thermally injured rats. *J Surg Res* 1984; 36: 177-183.
- [77] Newsholme EA, Parry-Billings M: Properties of glutamine release from muscle and its importance for the immune system. *JPEN* 1990; 14: 63S-67S.
- [78] Oppenheimer JH, Schwartz HL: Stero-specific transport of triiodothyronine from plasma to cytosol and from cytosol to nucleus in rat liver, kidney, brain and heart. *J Clin Invest* 1985; 75: 147-154.
- [79] O'Sullivan U, Gluckman PD, Breier BH, Woodall S, Siddiqui RA, McCutcheon SN: Insulin-like growth factor I (IGF-I) in mice reduced weight loss during starvation. *Endocrinology* 1989; 125: 2793-2794.
- [80] Ozawa M, Sato K, Han DC, Kawakawa M, Tsushima T, Shizume K: Effects of tumor necrosis factor- α /cachectin on thyroid hormone metabolism in mice. *Endocrinology* 1988; 123: 1461-1467.
- [81] Philiberg D: RU 38486: An original multifaceted antihormone in vivo. In: Agarwal MK (ed) *Adrenal Steroid Antagonism*. Walter de Gruyter & Co., Hawthorne, NY 1984; pp 77-100.
- [82] Plumley DA, Souba WW, Hautamaki RD, Martin T, Flynn T, Rout R, Copeland E: Accelerated lung amino acid release in hyperdynamic septic surgical patients. *Arch Surg* 1990; 125: 57-61.
- [83] Ponting GA, Ward HC, Halliday D, Sim AJW: Protein and energy metabolism with biosynthetic human growth hormone in patients on full intravenous nutritional support. *JPEN* 1990; 14: 437-441.
- [84] Rannels DE, Kao R, Morgan HE: Effect of insulin on protein turnover in heart muscle. *J Biol Chem* 1975; 250: 1694-1701.
- [85] Ross RJM, Miell JP, Freeman E, Jones J, Matthews D, Preece M, Buchanan C: Critical ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of insulin-like growth factor-I. *Clin Endocrinol* 1991; 35: 47-54.
- [86] Sapolsky RC, Rivier G, Yamamoto P, Plotsky P, Vale W: Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 1987; 238: 522-524.
- [87] Sax HC, Talamini MA, Hasselgren PO, Rosenblum L, Ogle CK, Fischer JE: Increased synthesis of

- secreted hepatic proteins during abdominal sepsis. *J Surg Res* 1988; 44: 109-116.
- [88] Smith OLK, Wong CY, Gelfand RA: Skeletal muscle proteolysis in rats with acute streptozocin-induced diabetes. *Diabetes* 1989; 38: 1117-1122.
- [89] Starnes HF, Pearce MK, Tewari A, Yim JH, Zou JC, Abrams JS: Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor challenge in mice. *J Immunol* 1990; 145: 4185-4191.
- [90] Strock LL, Singh H, Abdullah A, Miller JA, Herndon DN: The effect of insulin-like growth factor I on postburn hypermetabolism. *Surgery* 1990; 108: 161-164.
- [91] Sugden PH, Fuller SJ: Regulation of protein turnover in skeletal and cardiac muscle. *Biochem J* 1991; 273: 21-37.
- [92] Tannuri U, Amaral LA, Maksoud JG: Effect of insulin on protein catabolism after injury in young animals. *J Pediatr Surg* 1982; 17: 296-299.
- [93] Tessari P, Inchiostro S, Biolo G, Trevisan R, Giuseppe F, Marescotti MC, Iori E, Tiengo A, Crepaldi G: Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo: evidence for distinct mechanisms in regulation of new amino acid deposition. *J Clin Invest* 1987; 79: 1062-1069.
- [94] Tischler ME, Fagan JM: Response to trauma of protein, amino acid, and carbohydrate metabolism in injured and uninjured rat skeletal muscles. *Metabolism* 1983; 32: 853-868.
- [95] Tracey KJ: Tumor necrosis factor (cachetin) in the biology of septic shock syndrome. *Circ Shock* 1991; 35: 123-128.
- [96] Vaughan GM, Becker RA, Allen JP, Goodwin CW, Pruitt BA, Mason AD: Cortisol and corticotrophin in burned patients. *J Trauma* 1982; 22: 263-273.
- [97] Von Allmen D, Hasselgren PO, Higashiguchi T, Frederick J, Zamir O, Fischer JE: Increased intestinal protein synthesis during sepsis and following the administration of tumor necrosis factor α or interleukin-1 α . *Biochem J* 1992; 286: 585-589.
- [98] Waage A, Halstensen A, Espevik T: Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* 1987; 1: 355-357.
- [99] Ward JC, Halliday D, Sim JW: Protein and energy metabolism with biosynthetic human growth hormone after gastrointestinal surgery. *Ann Surg* 1987; 206: 56-61.
- [100] Warner BW, Hasselgren PO, Hummel RP, James JH, Pedersen P, Fischer JE: Effect of catabolic hormone infusion on protein turnover and amino acid uptake in skeletal muscle. *Am J Surg* 1990; 159: 295-300.
- [101] Wartofsky L, Burman KD: Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome." *Endocrine Rev* 1982; 3: 164-217.
- [102] Windmueller HG, Spaeth AE: Respiratory fuels and nitrogen metabolism *in vivo* in small intestinal of fed rats. *J Biol Chem* 1980; 255: 107-112.
- [103] Woolfson AMJ, Heatley RV, Allison SP: Insulin to inhibit protein catabolism after injury. *N Engl J Med* 1979; 300: 14-17.
- [104] Zamir O, Hasselgren PO, Higashiguchi T, Frederick JA, Fischer JE: Tumor necrosis factor (TNF) and interleukin-1 (IL-1) induce muscle proteolysis through different mechanisms. *Mediators Inflamm* 1992; 1: 247-250.
- [105] Zamir O, Hasselgren PO, Kunkel SL, Frederick J, Higashiguchi T, Fischer JE: Evidence that tumor necrosis factor participates in the regulation of muscle proteolysis during sepsis. *Arch Surg* 1992; 127: 170-174.
- [106] Zamir O, Hasselgren PO, O'Brien W, Thompson RC, Fischer JE: Muscle protein breakdown during endotoxemia in rats after treatment with interleukin-1 receptor antagonist (IL-1ra). *Ann Surg* 1992; 216: 381-387.
- [107] Zamir O, Hasselgren PO, von Allmen D, Fischer JE: The effect of interleukin-1 α and the glucocorticoid receptor blocker RU 38486 on total and myofibrillar protein breakdown in skeletal muscle. *J Surg Res* 1991; 50: 579-583.
- [108] Zamir O, Hasselgren PO, von Allmen D, Fischer JE: In vivo administration of interleukin-1 α induces muscle proteolysis in normal and adrenalectomized rats. *Metabolism* 1993; 42: 204-208.

Supported in part by NIH grant #DK37908 and by grant #15861 from the Shriners of North America