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# The Role of Cytokines in Regulating Protein Metabolism and Muscle Function

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*Multiple lines of evidence suggest that cytokines influence different physiologic functions of skeletal muscle cells, including anabolic and catabolic processes and programmed cell death. Cytokines play an important role not only in muscle homeostasis, therefore, but also in the pathogenesis of different relevant clinical conditions characterized by alterations in protein metabolism. Recently discovered cytokines, such as ciliary neurotrophic factor and growth/differentiation factor-8, as well as the more studied tumor necrosis factor-α, interleukin-1, interleukin-6, and the interferons, have been implicated in the regulation of muscle protein turnover. Their postreceptor signaling pathways, proteolytic systems, and the mechanisms of protein synthesis inhibition involved in different catabolic conditions have been partially clarified. Moreover, recent studies have shown that cytokines can directly influence skeletal muscle contractility independent of changes in muscle protein content. Even though several gaps remain in our understanding, these observations may be useful in the development of strategies to control protein metabolism and muscle function in different clinical conditions.*

Key Words: cytokines, protein metabolism, muscle function



## Introduction

Cytokines are small nonstructural proteins, or glycoproteins, that serve as chemical messengers between cells.[1] Cytokines are mainly involved in regulation of immune response; however, recent evidence suggests they are involved in different physiologic processes such as cell growth and differentiation, tissue repair and remodeling, and aging.[2] Cytokines influence different functions of skeletal muscle cells (anabolic and catabolic processes, and contractility) and are part of the normal adaptive response of the tissue to physical stress or damage; cytokines play an important role not only in muscle homeostasis but also in disease pathogenesis. Because muscle is the major store of body protein, factors that drive muscle protein metabolism have profound effects on protein nutritional status.

## Cytokines Sources and Stimuli

Current understanding of the broad spectrum of cytokine functions in muscle homeostasis is not complete, but suggests a bidirectional approach. Although it is conventional to discuss the effects of the immune system on skeletal muscle tissue during inflammation, myocytes are also capable of producing cytokines, expressing cytokine receptors, adhesion molecules, and co-stimulatory molecules, and influencing the course of inflammation itself.[3]

Cytokines that affect muscle cell function can be produced in the muscle intrinsically or produced by non-muscle cells, either locally in the muscle (extrinsic-local) or elsewhere (extrinsic-distant) (Figure 1). Cytokines can act in an endocrine, paracrine, or autocrine manner, depending on the cytokine and the situation.[4] For example, interleukin-1β (IL-1β) has a signal peptide and thus can be secreted to act as an endocrine or paracrine signal, whereas interleukin-1α (IL-1α), which does not have a signal peptide, acts primarily in an autocrine fashion.[4]

The main extrinsic local sources of cytokines during inflammation are neutrophils and macrophages that infiltrate muscular tissue.[5] However, resident cells such as macrophages,[6] fibroblasts,[7,8] vascular smooth muscle cells,[9] and vascular endothelium,[10] can also produce cytokines in response to different stimuli such as muscle contractile activity, cytokines, lipopolysaccharide or shear stress, and possibly limiting protein intake (Yamada T et al., unpublished observations, 2001).

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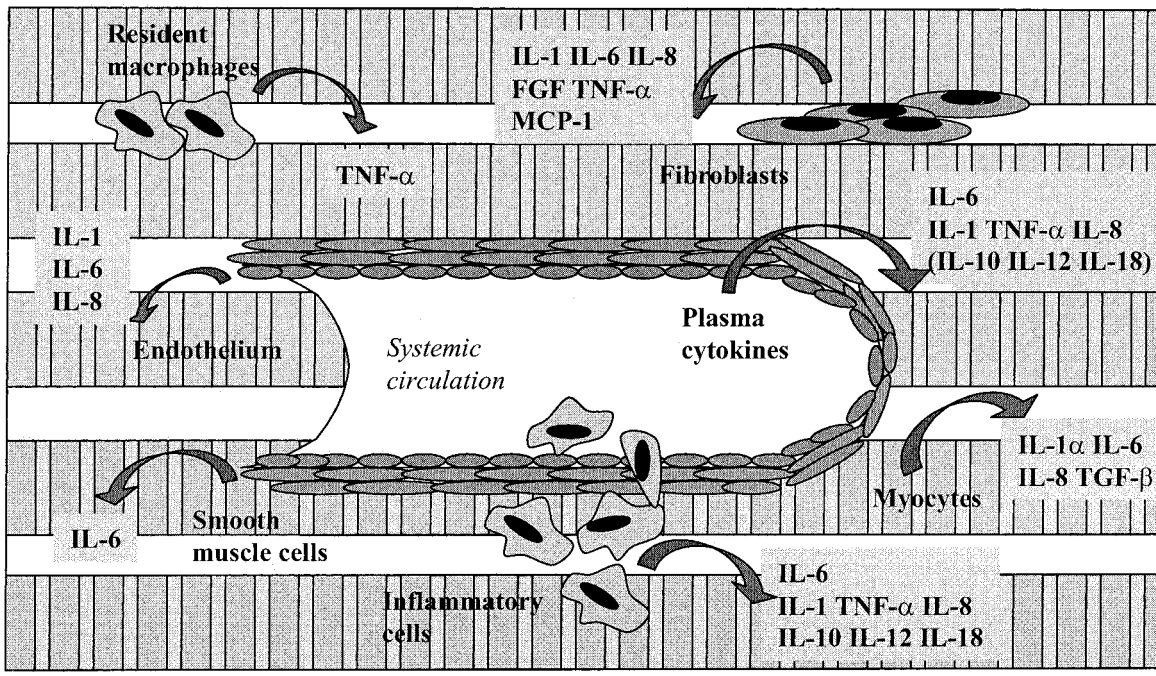


**Figure 1.** Source of cytokines in muscle tissue. IL = interleukin, FGF = fibroblast growth factor, MCP =monocyte cytokine protein, TNF = tumor necrosis factor, TGF = transforming growth factor.

There are limited studies of the intrinsic production of cytokines by muscle cells, but all of these data point to the capacity of muscle tissue to produce cytokines constitutively and in response to a variety of inflammatory and noninflammatory stimuli. Human skeletal muscle cells secrete IL-1$\beta$, IL-6, IL-8, and transforming growth factor beta constitutively and under the stimulation of a variety of proinflammatory cytokines.[8,11,12] Myoblasts do not synthesize granulocyte-macrophage colony-stimulating factor (GM-CSF) in unstimulated conditions, but IL-1$\alpha$, IL-1$\beta$, and tumor necrosis factor alpha (TNF-$\alpha$) induce production of GM-CSF in a dose-dependent manner.[12] Other data suggest that stimulation with TNF-$\alpha$ and interferon-$\gamma$ (IFN-$\gamma$) alone or in combination is capable of inducing the production of other cytokines and chemokines like IL-8-$\alpha$ and RANTES (regulated on activation, normal T-expressed and secreted).[8] The synthesis of mRNAs for IFN-$\gamma$, TNF-$\alpha$, macrophage inflammatory protein-1$\alpha$, and IL-1$\beta$ has also been shown, but the meaning of this finding is unclear because secreted proteins were not always detected.[12]

## Muscle Catabolism

### *Relevance of Cytokines*

Different pathologic and physiologic conditions such as cancer cachexia, sepsis, HIV wasting, burns and trauma, inflammatory disorders, chronic heart and renal failure, muscle denervation or immobilization, fasting, and aging are characterized by skeletal muscle catabolism. Extensive investigations have been carried out to identify the mediators directly involved in muscle protein breakdown. Since the publication of the article by Clowes et al. in 1983,[13] inflammatory cytokines have been thought to play an important role in muscle protein degradation. Clowes et al. first presented data supporting the existence (in patients with sepsis and trauma) of a circulating peptide with the ability to increase protein degradation rates in rat muscle strips incubated ex vivo.[13] However, this circulating factor, released into the plasma by leukocytes, has yet to be molecularly defined.[14] More recently, a 24-K proteoglycan isolated from mice with cancer cachexia with no homology with other known proteins, was shown to accelerate skeletal muscle breakdown in vitro and in vivo and was detected in urine of patients with cancer cahexia.[15] Which cytokines or circulating factors are involved in the regulation of muscle protein degradation, however, is a question that has not yet been completely answered.

*TNF-$\alpha$.* TNF-$\alpha$ was first identified as a possible cachectic factor in studies in chronically infected rabbits;[16] it was originally designated "cachectin" in recognition of its catabolic action. Increased concentrations of TNF-$\alpha$ have been found in cachectic subjects with sepsis,[17] AIDS,[18] and chronic heart failure.[19] Although many studies have failed to document elevated circulating levels of TNF in cachectic cancer patients,[20] human recombinant TNF-$\alpha$ given intravenously as part of an antineoplastic trial caused an increase in whole-body protein turnover;[21] in experimental rat models of cancer cachexia or inflammatory weight loss, moreover, en-

hanced fractional rates of muscle protein degradation were associated with increased levels of TNF-$\alpha$.[22–24] Further confirmation of the catabolic role of TNF-$\alpha$ comes from studies with Chinese hamster ovary (CHO) cells transfected with the human TNF-$\alpha$ gene: 87% of mice inoculated intramuscularly with this cell line developed, in combination with high serum levels of TNF-$\alpha$, severe cachexia and weight loss.[25] Administration of TNF-$\alpha$ to laboratory animals induces a state of net nitrogen loss[26] and muscle tissue catabolism,[27] especially in red-type muscles.[28] In addition, anti-TNF antibody treatment in septic[29] and neoplastic rats,[23] as well as pretreatment of infected rats with pentoxifylline (an inhibitor of TNF synthesis),[30] reduced or prevented the muscle protein loss characteristic of these experimental models of animal cachexia.

Although the presence of TNF-$\alpha$ receptors in rat skeletal muscle cells has been proved,[31] and TNF-$\alpha$ receptor–deficient mice have been shown to be resistant to the development of cancer cachexia,[32] for some time it was not possible to demonstrate a direct effect of TNF-$\alpha$ in vitro,[33–35] suggesting an indirect mechanism of action mediated by either another cytokine or a conventional hormone. Methodological problems such as the validity of the techniques used to assess rates of protein synthesis and degradation in vitro, the composition of the incubation medium, and the time and conditions of incubation itself, could be at least partially responsible for the discrepancy between in vivo and in vitro studies. Two recent reports support a direct effect of TNF-$\alpha$ on muscle catabolism.[36,37] Incubation of isolated rat soleus muscle in the presence of human recombinant TNF-$\alpha$ resulted in increased expression of genes related to the ubiquitin-dependent proteolytic system.[36] Moreover Li et al. found a reduction in total protein content and myosin heavy chains after TNF-$\alpha$ incubation of differentiated myotubes in mice.[37]

*IL-1$\beta$.* IL-1$\beta$ has many effects similar to those of TNF-$\alpha$. A role for this cytokine in muscle proteolysis has been suggested since 1983, when Clowes et al. proposed that the proteolysis-inducing factor isolated from septic plasma was a cleavage product of IL-1$\beta$, and Baracos et al.[38] showed that the addition of partially purified human IL-1$\beta$ to incubated rat skeletal muscle induced proteolysis and prostaglandin $E_2$ production. After the availability of pure recombinant IL-1$\beta$, however, several studies failed to demonstrate an effect of this cytokine on muscle protein breakdown in vitro.[33,34,39] More contrasting are the results from in vivo studies: in different conditions, treatment of rats with IL-1$\beta$ had no effect,[35] only a synergistic effect with TNF-$\alpha$,[26] or a positive effect in enhancing muscle proteolysis.[40] The administration of interleukin-1 receptor antagonist (IL-1ra) to rats bearing the Yoshida ascites hepatoma was ineffective in preventing tissue depletion and protein hypercatabolism,[41] whereas septic rats treated with high doses of IL-1ra showed a normalization of muscle proteolysis rate.[42] These conflicting results, cast doubt on a definitive and direct role of IL-1$\beta$ in the induction of skeletal muscle breakdown.

*IL-6.* IL-6 is a proinflammatory cytokine of the gp130 class of signal molecules that is capable of causing a variety of metabolic responses. Because TNF-$\alpha$ induces the production of IL-6,[1] the cachectic changes mediated by TNF-$\alpha$ may overlap and complement the effects of IL-6. In the report by Strassmann et al.,[43] increased levels of IL-6 in mice bearing murine colon-26 adenocarcinoma correlated with the development of cachexia; in the same study, the treatment with anti-IL-6 antibody was able to reverse significantly the key parameters of cachexia.[43] Mice inoculated with CHO cells transfected with the murine IL-6 gene showed a decrease in body weight.[44] Moreover, IL-6-transgenic mice that overexpress IL-6 developed muscle atrophy,[45] whereas the administration of IL-6 receptor antibody completely blocked the catabolic response observed in this experimental model.[46] In vitro, treatment of C2C12 myotubes with IL-6 was found to shorten the half-life of long-lived muscle proteins and to increase the activity of both non-lysosomal and lysosomal proteolytic pathways.[47] This evidence may suggest an important role for IL-6 in the regulation of muscle proteolysis.

The negative results of other studies, however, raise doubts about a direct regulatory role of IL-6 in muscle protein degradation.[48–50] IL-6 did not affect the rate of protein breakdown in vitro in rat muscle preparations,[48] and in vivo, when repeatedly administered to mice, IL-6 induced an acute-phase response without producing cachexia.[49] Moreover, sepsis increased muscle proteolysis in the absence of detectable plasma IL-6 levels in IL-6 knockout mice, and the treatment of normal mice or myotubes with IL-6 did not induce muscle protein breakdown.[50] In the interpretation of these contradictory results, one must consider the anti-inflammatory properties of IL-6 and the ability of this cytokine to down-regulate TNF-$\alpha$ and IL-1 levels.[1] Further research seems necessary to understand the complex network of interactions of different signals involved in the regulation of muscle protein turnover.

*Other gp130 cytokines.* Recent studies indicate that another member of the IL-6 superfamily, ciliary neurotrophic factor (CNTF), can affect muscle protein turnover.[49,51,52] Mice implanted with C6 glioma cells genetically modified to secrete CNTF developed a severe and lethal form of cachexia that was independent of the induction of other catabolic cytokines.[51] CNTF administration resulted in a 218% increase in carcass protein breakdown rates compared with controls that had only a

slight rise in protein synthesis.[49] In the same study, however, no significant effect on protein turnover was detected in vitro, suggesting an indirect mechanism of action.[49] Wang and Forsberg[52] recently reported different effects of CNTF on muscle: CNTF seems capable of regulating both protein synthesis and degradation in myogenic cell cultures in a dose-dependant manner, and catabolic effects were found only at high doses.[51,52] Much remains to be learned about CNTF actions on skeletal muscle tissue; it seems to exert both anabolic and catabolic effects in vivo, playing a role in muscle adaptation to denervation and injury.

*Interferons.* Interest in the role of interferons in muscle proteolysis developed following observations that severe cachexia occurred in nude mice inoculated with CHO cells producing IFN-$\gamma$;[53] passive immunization against IFN-$\gamma$ prior to tumor cell inoculation prevented the development of the syndrome.[53] In this study, however, both IFN-$\gamma$ release and the presence of tumor cells were required to induce cachexia.[53] In mice bearing the Lewis lung tumor, weight loss was associated with the production of IFN-$\gamma$.[54] Anti-IFN-$\gamma$ antibody treatment attenuated the loss of body fat, however, but had no effect on total body protein.[54] Serum IFN-$\alpha$ levels were significantly elevated in patients with AIDS compared with seronegative controls, but there was no relationship between circulating levels of this cytokine and the presence of wasting as measured by total body potassium.[55]

*Growth/differentiation factor-8.* A recently discovered member of the TGF-$\beta$ superfamily, growth/differentiation factor-8 (GDF-8) or myostatin, expressed specifically in developing and adult skeletal muscle tissue has been identified.[56] It appears that GDF-8 is produced by type 2 fibers at higher levels than in type 1 fibers (Vannier E, Hamada K, Yamada T, Roubenoff R, unpublished observations, 2001). This factor seems to represent an important negative regulator of skeletal muscle growth because it was shown that GDF-8-null animals develop muscle hyperplasia and hypertrophy.[56] The serum and intramuscular concentrations of myostatin–immunoreactive protein were increased in HIV-infected men with wasting and were inversely correlated with fat-free mass.[57] In hindlimb muscle atrophy in the rat, moreover, a 37% increase in myostatin expression was observed in type 2 fibers.[58] Investigators have also found an inverse correlation between myostatin mRNA levels and type 2 fibers area in patients with chronic disuse atrophy.[59]

In conclusion, the evidence presented in the literature indicates the existence of a complex network of mediators involved in the regulation of muscle protein turnover. It is still difficult to distinguish single contributions, but globally cytokines seem to play an important role.

### Mechanisms of Protein Degradation

Skeletal muscle cells contain distinct proteolytic systems that can be distinguished as lysosomal and non-lysosomal (Table 1). The lysosomal pathway, including proteases such as cathepsins B, H, L, and D, and other hydrolases, is mainly involved in the proteolysis of extracellular proteins and cell surface receptors.[60] Among the non-lysosomal mechanisms, the ATP-ubiquitin-dependent pathway and the calcium-activated system of calpains are important in the regulation of intracellular muscle protein degradation.[60]

Several studies have provided evidence that the ATP-ubiquitin-dependent pathway is up-regulated in skeletal muscle under different catabolic conditions such as sepsis, cancer cachexia, AIDS wasting, burn or traumatic injury, fasting, denervation, inactivity, and metabolic acidosis.[60,61] The same pattern of enzymatic activation was found in different animal experimental models of muscle catabolism, which further supports the relevance of this mechanism in muscle protein breakdown.[60,61] In this process, proteins are targeted for degradation by conjugation to ubiquitin.[60,61] Ubiquitin is first activated by the ubiquitin-activating enzyme, E1, in an energy-requiring step; it is then transferred by a family of carrier proteins, E2, to the ubiquitin-protein ligase, E3, that finally catalyzes the binding of long ubiquitin chains to different substrate proteins.[60,61] The conjugation, which is mediated by the 14-kDa E2, has been regarded as the rate-limiting step in the regulation of this pathway.[60,61] The ubiquitinated proteins are recognized by the proteolytic complex, the 26S proteasome; after unfolding, the proteins are transferred to the catalytic core of the complex, the 20S proteasome, where they are hydrolyzed in an energy-dependent process.[60,61] Finally, the proteasome releases short oligopeptides that are rapidly degraded by cytosolic peptidases into amino acids.[60,61]

Some studies suggest that TNF-$\alpha$ can stimulate muscle proteolysis by a direct activation of the ATP-ubiquitin-dependent pathway. In 1994, Garcia-Martinez reported that in vivo administration of TNF-$\alpha$ resulted in an increase in both gene expression and levels of free and conjugated ubiquitin in rat skeletal muscle.[62] Moreover, anti-TNF-$\alpha$ treatment abolished the increase in muscle ubiquitin gene expression that was observed in tumor-bearing rats,[63] whereas TNF-$\alpha$ receptor 1–deficient mice did not show any increase in ubiquitin gene expression after implantation of the Lewis lung carcinoma.[32] A direct effect in vitro on the induction of genes related to this proteolytic pathway was subsequently shown for TNF-$\alpha$ in isolated rat skeletal muscle tissue[36] and in C2C12 rat muscle cell cultures.[37] Finally, the TNF-$\alpha$ inhibitor pentoxifylline administered to Yoshida sarcoma–bearing rats suppressed the enhanced expression

**Table 1.** Role of Different Proteolytic Systems in the Degradation of Various Classes of Muscle Cell Proteins

| Pathway | Enzymes | Target |
|---|---|---|
| ***Lysosomal System*** | Cathepsins B, D, H, L<br>Hydrolases | Extracellular proteins<br>Membrane proteins |
| ***Nonlysosomal Systems*** | | |
| *Energy-dependent* | | |
| Ubiquitin-dependent | 26S Proteasome | Cytosolic short- and long-lived normal protein, abnormal proteins |
| Ubiquitin-independent | Caspases or ICE- (IL-1$\beta$ converting enzyme) related proteases | Cytosolic proteins (during apoptosis) |
| *Energy-independent* | | |
| Calcium-dependent | Calpains<br>ubiquitous: $\mu$-calpain, m-calpain<br>skeletal muscle tissue-specific: p94, CL-1,calpain3 | Cytosolic proteins (during normal protein turnover, tissue injury, necrosis, and apoptosis) |
| ***Mitocondrial System*** | | |
| *Energy-dependent* | Different proteases | Mitochondrial proteins |

IL = interleukin.

of ubiquitin, the 14-kDa ubiquitin-conjugating enzyme E2, and different subunits of the 26S proteasome.[64] Recently, investigators elucidated the postreceptor mechanism leading to the activation of the ATP-ubiquitin-dependent system by TNF-$\alpha$ (Figure 2).[37] TNF-$\alpha$ bound to surface receptors activates the transcriptional factor nuclear factor-$\kappa$B (NF-$\kappa$B) through a cascade of events that result in the degradation of I-$\kappa$B$\alpha$, the protein that inhibits NF-$\kappa$B.[37] This process depends on mitochondrial production (induced by TNF-$\alpha$) of reactive oxygen species (ROS) that leads to rapid activation of the ubiquitin pathway and proteasomal degradation of I-$\kappa$B$\alpha$.[37] These events result in NF-$\kappa$B translocation to the nucleus and expression of genes involved in muscle proteolysis induction.[37] The specific genes that respond to NF-$\kappa$B have not been determined yet, but most likely represent regulatory components of the ubiquitin-proteasome system. The role of NF-$\kappa$B in mediating the catabolic response of muscle cells to TNF-$\alpha$ recently received further support from the study by Li and Reid,[65] in which the protein content of muscular cells overexpressing the inhibitor protein I-$\kappa$B$\alpha$ was unaltered by treatment with TNF-$\alpha$.

Although many other cytokines, such as IL-1, IL-6, and IFN-$\gamma$, have been proposed as mediators of muscle protein degradation in different catabolic conditions, few studies have considered the proteolytic mechanism involved. In skeletal muscle from septic rats the administration of IL-1$\beta$ did not cause an increase in the levels of ubiquitin mRNA.[66] IL-1 and IFN-$\gamma$ were able to induce the expression of the ubiquitin mRNA in rats in another study, however, whereas administration of IL-6 and leukemia inhibitory factor resulted in no significant change.[67] Nevertheless, increased mRNA levels of ubiquitin, together with enhanced expression of mRNA of cathepsins, was found in IL-6 transgenic mice.[46] In a cell culture system of C2C12 myotubes, IL-6 increased the activities and enhanced the transcription of both cathepsins and proteasomes.[47] The scarcity of reports and the contradictory results of different studies prevents advancing any hypothesis about the mechanisms involved in the activation of the ubiquitin-dependent pathway by cytokines other than TNF-$\alpha$.

Although several studies point at the involvement of the ubiquitin-proteasome pathway in myofibrillar protein degradation, it is not clear if the proteasome system is the key rate-limiting protease in muscle catabolism. If so, it may in turn regulate dietary protein requirement. However, dividing proteolytic mechanisms into distinct pathways independent of each other may be an oversimplification: the various proteolytic pathways probably do not regulate protein degradation independently and most likely neither do cytokines.

The calcium-activated pathway of calpains is also up-regulated in conditions of muscle protein breakdown.[68] Sepsis in rats results in an early calcium-dependent disruption of Z disks and release of myofilaments in the cytoplasm.[69] Although calpains are unable to degrade myofibrillar proteins, they could play a role in the initiation of protein degradation by the cleavage of a limited number of specific sites.[68] Because the proteasome does not degrade intact myofibrils,[70] Hasselgren has recently suggested that the ubiquitin-proteasome pathway may not regulate muscle breakdown.[71] Rather, proteasome-mediated myofibrillar protein degradation may follow from the initial Z-band disintegration by calpains, and the rate-limiting step in muscle protein degradation may be this initial protein dissociation from the myofibrils.[71] No study has shown the existence of a mechanism linking the increased level of cytokines in different catabolic con-

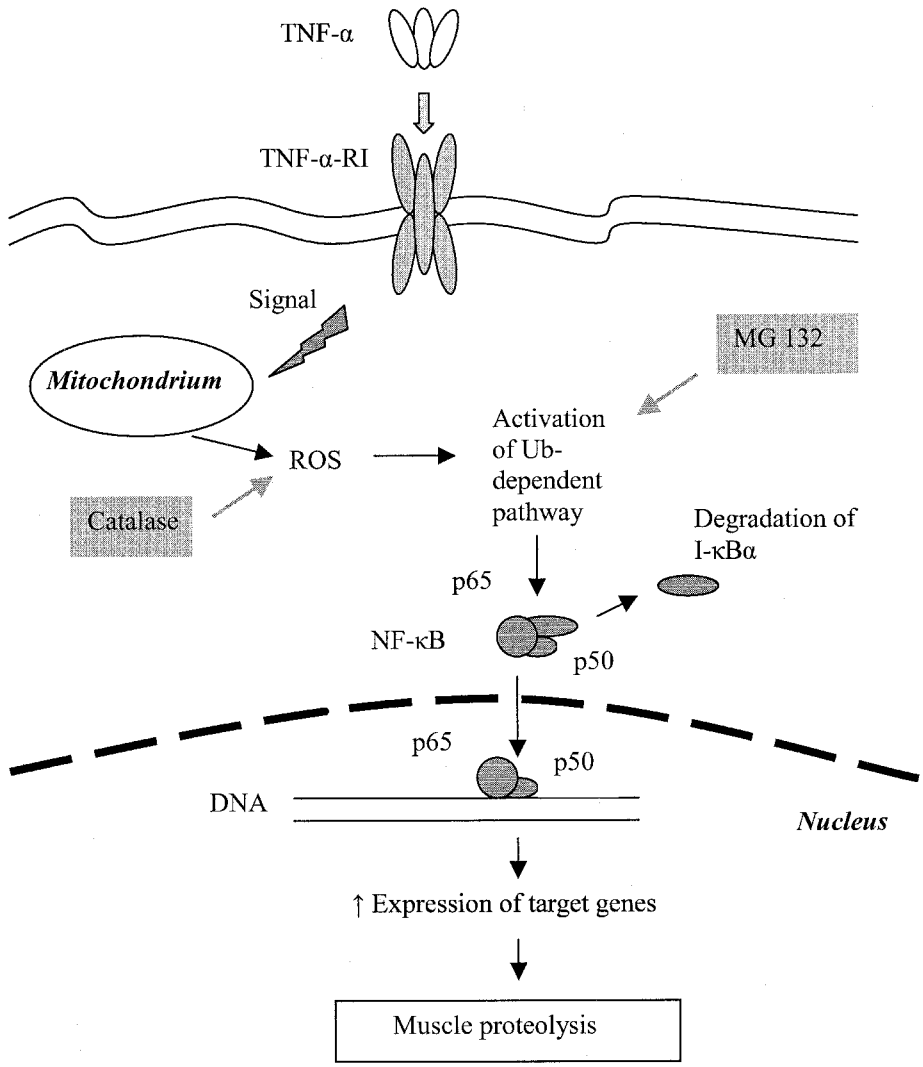


**Figure 2.** Postreceptor mechanism leading to the activation of the ATP-ubiquitin-dependent system by TNF-α. Grey arrows = inhibitory effect, black arrows = stimulatory effect. TNF-α = tumor necrosis factor-α, TNF-α-RI = TNF-α receptor type 1, ROS = reactive oxygen species, Ub = ubiquitin, Ub-dependent pathways = ATP-ubiquitin-dependent pathway, NF-κB = nuclear factor κB, I-κBα = inhibitor κBα

ditions to the activation of the calcium-dependent pathway of calpains in skeletal muscular tissue.

The functional importance of lysosomal proteolysis in muscle degradation has been questioned, and the role of cathepsins in muscle proteolysis is uncertain.[60] However, enzymatic activities and mRNA levels of lysosomal cathepsins were observed to increase in vitro in C2C12 myotubes treated with IL-6,[47] and in vivo in muscle of IL-6-transgenic mice with muscle atrophy.[45] The administration of anti-IL-6 receptor antibody in the same experimental model,[46] as well as in colon-26 adenocarcinoma–bearing mice,[72] prevented the activation of the lysosomal pathway. Moreover, the intramuscular injection of turpentine oil in mice was followed by the development of muscle atrophy, and by an increase in serum IL-6 level and enzymatic activity of cathepsins.[73] It therefore seems that the signaling processes induced by IL-6 in certain conditions of muscle atrophy may be closely linked to the production of cathepsins, which may enhance the degradation of endogenous or alternatively endocytosed proteins. However, inhibition of cathepsins does not affect overall protein breakdown or myofibrillar proteolysis in other conditions of muscle protein degradation, such as fasting, denervation, atrophy, and sepsis.[46] Further research seems necessary to clarify the role of this proteolytic pathway in IL-6-induced muscle atrophy.

In conclusion, even though future research must address several unanswered questions, cytokines, through different proteolytic pathways (Figure 3), seem to represent important regulatory molecules in the complex network of signals that control muscle protein breakdown.

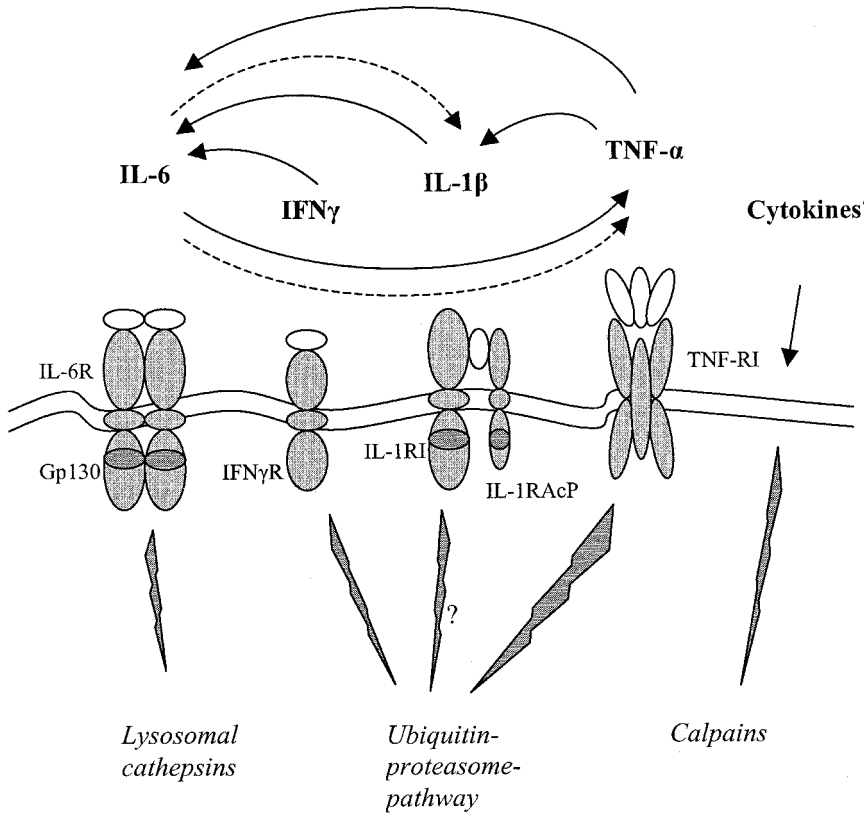


**Figure 3.** The role of cytokines in regulating control of muscle protein degradation. Full arrows = stimulatory effect, dashed arrows = inhibitory effect. IL-6 = interleukin 6, IL-6R = IL6 receptor, Gp 130 = glycoprotein 130, IFNγ = interferon γ, IFNγR = IFNγ receptor, IL-1RI = IL1 receptor type 1, IL-1RacP = IL1 receptor accessory protein, TNF-α = tumor necrosis factor-α; TNF-α-RI = TNF-α receptor type 1.

### *Inhibition of Protein Synthesis and Muscle Repair*

Depletion of skeletal muscle tissue can be the result of both an increase in muscular protein breakdown and a decrease in muscle protein synthesis. However, the respective roles of protein synthesis and degradation in muscle tissue differ from condition to condition. Reduced rates of protein synthesis have been reported in cancer patients with weight loss:[74] muscle protein synthesis in cachectic patients accounted for only 8% of total protein synthesis compared with 53% in healthy control subjects.[75] Several reports indicate that sepsis is associated with an impairment of protein synthesis;[76] muscles with a predominance of fast-twitch fibers seem to be particularly affected.[77] Although older reports suggest that aging is associated with reduced protein synthesis,[78] a more recent report disputes this.[79] In HIV infection with wasting, protein synthesis fails to rise to match the increase in protein degradation,[80] but this can be reversed with exercise.[81]

The decrease in muscular protein synthesis in these conditions appears to be not only the consequence of a failure of the normal anabolic stimuli and/or in the supply of amino acids and energy, but also the result of different signals that actively inhibit muscular protein synthesis. Infusion of IL-1ra in rodents prevented the sepsis-induced loss of muscle protein and inhibition of protein synthesis in gastrocnemius,[82,83] suggesting an active role for IL-1 in this process. Because changes in the muscular mRNA of septic rats could not account for the inhibition in protein synthesis,[82,83] an alteration of the translational efficiency, rather than of ribosome content, was proposed as the major mechanism responsible for the inhibition of protein synthesis during sepsis.

Translation is regulated by a large number of protein factors named eukaryotic initiation factors (eIFs). One of these factors, eIF2, mediates the attachment of the initiator methionyl-tRNA to the 40S ribosomal subunit to form the 43S preinitiation complex. The activity of eIF2 can be modulated by the activity of another factor, eIF2B. Inhibition of the epsilon-subunit of eIF2B and a consequent decrease of translational efficiency was shown in muscle from septic rats after chronic IL-1 infusion;[84] this was prevented by treatment with IL-1ra.[85] Similarly, treatment of septic rats with TNF-binding protein, a specific TNF-α antagonist, significantly limited the decrease in gastrocnemius eIF2Bε expression observed in septic rats.[86] TNF-α is known to stimulate the secretion of IL-1 and IL-6, however, so an

effect of TNF mediated through the production of other cytokines cannot be ruled out. The ability of cytokines to directly influence muscle protein translation remains controversial. Inhibition of IL-1 expression after induction of sepsis is associated with concomitant increase in both insulin-like growth factor-I (IGF-I) and muscle protein synthesis,[87] and exposure of myoblasts to TNF-$\alpha$ completely blocked the ability of serum and IGF-I to stimulate protein synthesis;[88] this suggests the possibility that these cytokines can stimulate muscle protein synthesis at least in part through IGF-I.

Another potential mechanism that can underlie skeletal muscle depletion during different catabolic conditions is represented by impairment of repair processes. Guttridge et. al.[89] recently showed that the addition of TNF-$\alpha$ to mouse C2C12 myocytes inhibited their differentiation program by down-regulating the myogenic transcription factor MyoD through the activation of the NF-$\kappa$B subunit p65. Moreover, apoptosis was observed in incompletely differentiated myocytes in culture treated with TNF-$\alpha$ and IFN-$\gamma$.[89] Guttridge et al.[89] hypothesized that TNF and IFN-$\gamma$ could severely impair the ability to repair damaged skeletal muscle not only by suppressing the formation of new myotubes from quiescent satellite cells, but also by causing the degeneration of newly formed myotubes.

## Apoptosis

The process of programmed cell death, known as apoptosis, is important in the development and homeostasis of multicellular organisms and is characterized by a specific pattern of morphologic cellular alterations.[90] The role and the mechanisms of apoptosis in solid, differentiated, post-mitotic tissues, such as skeletal muscle, are poorly understood.[90] Apoptosis is known to occur during skeletal muscle differentiation, but was recently observed in myocytes, under different pathophysiologic conditions.[91–97] In a rat model of chronic heart failure (CHF), muscle atrophy was associated with an increase of apoptotic nuclei in myofibers of fast[91] and slow skeletal muscles.[92] Adams and colleagues found that apoptosis was present in approximately 50% of skeletal muscle biopsies obtained from patients with CHF and was associated with a lower exercise capacity.[93] Reduction of neuromuscular activity in denervating disorders,[94] and unloading in hindlimb suspension,[95] resulted in rapid skeletal muscle atrophy with the characteristic features of apoptosis. Skeletal muscle apoptosis has also been observed in rats after burn injuries[96] and in tumor-bearing animals.[97]

The signals that can trigger apoptosis in skeletal muscle tissue have not yet been elucidated. Cytokines have been shown to control apoptosis in many cell types.[98] And high levels of cytokines have been detected in the many different conditions of muscle atrophy where apoptosis was observed. However, the evidence that supports a role for cytokines in inducing apoptosis particularly in skeletal muscle tissue is incomplete and contradictory. In monocrotaline-treated rats,[91,92] the development of myocyte and interstitial apoptosis was paralleled by a dramatic increase in TNF-$\alpha$ circulating levels. In an in vitro study, the topical treatment of hamster striated muscle with TNF-$\alpha$ increased the number of apoptotic cells tenfold.[99] However, TNF-$\alpha$ concentrations of 10 ng/mL, commonly observed during inflammatory disease, failed to induce apoptosis in differentiated C2C12 myotubes and in myotubes from rat primary cultures.[65] Finally, at the molecular level the mechanisms of apoptosis in skeletal muscle still remain uncertain even though they seem to resemble what has been described for several other tissues.[90]

Although there is evidence that apoptosis is present in skeletal muscle tissue in different pathophysiologic conditions, several other issues need to be clarified: molecular signals, mechanisms of programmed cell death, functional significance of apoptosis, and methods of detection are some of them.

## Skeletal Muscle Contractility

Symptoms such as weakness and fatigue in the absence of manifest cachexia are common in different clinical conditions characterized by elevated circulatory levels of cytokines, including starvation, infection, cancer chemotherapy, and overtraining.[100] Recent studies have shown that cytokines can directly influence muscle function independent of changes in muscle mass and protein content.[101–106] TNF-$\alpha$ in particular is considered to have a prominent role in the induction of muscle contractile dysfunction. In 1992, Wilcox et al.[101] showed that monocyte inflammatory products released during sepsis directly impaired hamster diaphragm contractility in vitro. The same authors subsequently reported that systemic TNF-$\alpha$ administration to anesthetized dogs was followed by a significant decrease in isotonic and quasi-isometric diaphragm contraction.[102] In another study, anti-TNF-$\alpha$ antibody treatment prevented the significant decrement of diaphragm contractility observed in septic rats, and an increase in TNF-$\alpha$ mRNA expression in diaphragm was detected after endotoxin injection.[103] In the report by Diaz et al.,[107] however, the incubation of isolated rat diaphragm strips with TNF-$\alpha$ did not lead to significant differences in contractile function compared with control preparations. Methodological considerations may explain the negative results of this study: as shown by Wilcox et al.,[104] only diaphragm tissue incubated with high doses of TNF-$\alpha$ is associated with changes in muscle function. Recently, other in vitro studies, even though conducted in different muscle groups and under

different experimental conditions, have further supported the role of TNF-$\alpha$ in muscle function.[105,106]

Indirect mechanisms may partially explain the effects of TNF-$\alpha$ on muscle contractility: a reduction in diaphragm perfusion and energy substrate supply owing to alterations in systemic or regional muscle blood flow and or disturbances in serum electrolytes are very common during sepsis. However, direct effects of TNF-$\alpha$ on myocytes can be recognized as well. Tracey and colleagues showed significant reductions in skeletal muscle resting transmembrane potential after TNF infusion in an isolated muscle model.[108] Similarly, Wilcox and colleagues more recently found a significant decrease in diaphragm action potential in response to supramaximal phrenic stimulation after TNF-$\alpha$ infusion in dogs.[102] Nevertheless, it seems possible that mechanisms other than alterations in neuromuscular function could explain the effect of TNF-$\alpha$ on muscle contractility.

Only recently have the intracellular signaling pathways that mediate the effects of TNF on myocytes been partially clarified. Skeletal muscle cells produce ROS, nitric oxide (NO), and the related redox form ($NO_x$) at a low rate under resting conditions.[100] In skeletal muscle function oxidant signaling appears to be critical: ROS can reduce muscle contractility both in vivo[109] and in vitro;[110] moreover, ROS-induced contractile depression can be reversed by treatment with antioxidant agents.[110] Recently Li and colleagues evaluated oxidant levels in diaphragm of transgenic mice that constitutively overexpressed a cardiac-specific transgene for secreted TNF-$\alpha$.[106] In both transgenic animals and wild-type mice treated with exogenous TNF-$\alpha$, the observed reduction in diaphragm function was related to a significantly higher intracellular oxidant level than in control animals and was partially restored after antioxidant incubation.[106] These data suggest that TNF-$\alpha$ can compromise muscle function directly through an increase in mitocondrial or cytosolic production of ROS. The role of nutritional antioxidant intake in modulating this effect is unknown. In another recent study, Alloatti showed that the negative effects of TNF-$\alpha$ on pig extensor digitorum longus muscle are mediated by NO production, which may be largely dependent on the synthesis of platelet activating factor.[105] NO generation in this experimental model is critical in inducing the negative inotropic effect of TNF-$\alpha$ because treatment with L-NAME, an NO synthesis inhibitor, completely prevented the alterations observed.[105] In single mouse muscle fibers, NO exposure was shown to decrease myofibrillar $Ca^{2+}$ sensitivity.[111] Alternatively, intracellular NO could impair muscle contractility giving rise to a cascade of $NO_x$ that could increase intracellular oxidant levels in muscle as described for TNF-$\alpha$ in other experimental conditions.[106] ROS probably induce oxidative modifications of target proteins of structures that appear to be critical for muscle function such as sarcolemma, sarcoplasmatic reticulum, and myofilaments.

## Conclusion

Several relevant clinical conditions are characterized by alterations in protein metabolism and muscle function, and in most of them muscle wasting has been associated with increased mortality. Nutritional factors per se cannot fully explain these conditions: even though anorexia is frequently present, nutritional supplementation alone is ineffective in the prevention and/or in the treatment of muscle protein loss. Humoral mediators, such as cytokines, appear to influence protein nutritional status, not only with a systemic anorectic effect, but mainly by directly impairing the regulation of skeletal muscle protein turnover.

More research is needed to develop a comprehensive understanding of potential key regulatory points in muscle protein metabolism, and to find pharmacologic therapies capable of suppressing muscle protein catabolism and apoptosis and stimulating muscle protein synthesis and repairing processes. The effect of nutrition on cytokine production and the role of specific micronutrients, such as vitamin and non-vitamin antioxidants, in regulating the catabolic function of cytokines have not been the object of extensive studies and seem to represent other important lines of future research.


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