

# The Role of Tumor Necrosis Factor- $\alpha$ in Muscle Wasting Disorders

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## Abstract

The present review discusses the role of tumor necrosis factor- $\alpha$  (TNF) in muscle weight loss and protein mobilization during cancer and infection, with special emphasis on the acquired immunodeficiency syndrome (AIDS). It is basically concluded that the most important biochemical event associated with muscle wasting is an enhanced protein degradation through the ubiquitin-dependent proteolytic pathway. TNF is an important activator of this proteolytic system, and future therapeutic approaches for wasting should consider either blocking TNF action or inhibiting the activity of the referred proteolytic system.

**Key words:** TNF- $\alpha$ , cancer cachexia, AIDS, muscle wasting, cytokines.

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## Cancer Cachexia and Muscle Waste

Cancer cachexia is one of the worst effects of malignancy, accounting for nearly a third of cancer deaths. It is a pathological state characterized by weight loss together with anorexia, weakness, anemia and asthenia. The complications associated with the appearance of the cachectic syndrome affect both the physiological and biochemical balance of the patient and have effects on the efficiency of the anticancer treatment, resulting in a considerably decreased survival time. At the metabolic level, cachexia is associated with loss of skeletal muscle protein together with a depletion of body lipid stores. The cachectic patient, in addition to having practically no adipose tissue, is basically subject to an important muscle wastage manifested as an excessive nitrogen loss. The metabolic changes are partially mediated by alterations in circulating hormone concentrations (insulin, glucagon and glucocorticoids in particular) or in their effectiveness [109]. On the other hand, a large number of observations point towards cytokines (TNF in particular), polypeptides released mainly by immune cells, as the molecules responsible for the above referred metabolic derangements.

Asthenia or lack of strength is one of the main characteristics of cancer cachexia, and it is directly related to the muscle waste observed in cachectic states. During fasting, muscle proteins are degraded to provide

amino acids which are used for gluconeogenesis; however during longer starvation periods, protein breakdown is decreased in order to conserve nitrogen and maintain lean body mass. This ability, absolutely essential for conserving nitrogen when the intake is reduced, seems to be absent in cancer-bearing states, leading to a depletion of vital host protein both skeletal and functional. The skeletal muscle, which accounts for almost half of the whole body protein mass, is severely affected in cancer cachexia [60, 81, 111] and evidence has been provided for muscle protein waste as being associated with enhanced turnover rates [10, 56, 76, 108]. Since cachexia tends to develop at a rather advanced stage of the neoplastic growth, preventing muscle waste in cancer patients is of great potential clinical interest. Whether the negative protein balance results from altered rates of synthesis or breakdown, or from changes on both sides of muscle protein turnover is still debated [25, 70, 88, 108]. Rennie *et al.* have suggested that the muscle mass is decreased during cancer cachexia as a result of a lower rate of protein synthesis, while changes in protein degradation are secondary [94]. Conversely, studies involving the release of 3-methylhistidine (a marker of myofibrillar protein degradation) from peripheral muscle in cancer patients suggest that protein degradation is clearly increased [71]. Our group has demonstrated, using several experimental models, that protein synthesis is hardly altered in skeletal muscle

during tumor growth and that there is a great increase in protein degradation both *in vivo* [17, 21] and *in vitro* [38]. In addition, we have identified the proteolytic mechanism which is involved in skeletal muscle during cancer cachexia [6, 65, 66]. A non-lysosomal, ATP and ubiquitin-dependent proteolytic system is activated in skeletal muscle of tumor-bearing animals.

The result of the enhanced proteolysis is a large release of amino acids from skeletal muscle which takes place specifically as alanine and glutamine. The release of amino acids is also potentiated by an inhibition of amino acid transport into skeletal muscle [38]. Alanine is mainly channelled to the liver for both gluconeogenesis and protein synthesis. Interestingly, liver fractional rates of protein synthesis are increased in tumor-bearing animals, accounting for the production of the so-called acute phase proteins, while there is a decrease in albumin synthesis, leading to hypoalbuminemia [55]. Glutamine is basically taken up by the tumor to sustain both the energy and nitrogen demands of the growing mass [74].

Branched-chain amino acids (BCAA: leucine, isoleucine and valine) are essential nutrients for both human and animals, making up to 40% of the minimal daily requirements of indispensable amino acids in man [2]. In studying the mechanism which leads to protein waste, particular interest has been given to the metabolism of these amino acids [83]. The carbon skeletons arising from transamination of BCAA provide a major source of metabolic fuel for skeletal muscle. In wasting disorders (including cancer and injury) plasma concentrations of BCAA are often increased and their turnover rates altered [5]. It has been previously demonstrated that *in vivo* leucine oxidation to CO<sub>2</sub> is enhanced in sepsis [37] and in tumor-bearing animals [24, 39], related to an increased turnover of the amino acid [5]. The trigger for the increased leucine oxidation remains unknown. Williams and Matthaei [117] suggested that the plasma concentration of ketone bodies could modulate the extrahepatic oxidation of BCAA, and that a decrease in their levels during cancer could result in increased oxidation. However, this hypothesis is in contrast with the observation by Rofe *et al.* [95] of increased plasma ketone bodies in tumor-bearing mice. Alternatively, uncontrolled BCAA oxidation may be due to hypoinsulinemia [16] or peripheral insulin resistance [72].

Despite the controversy about the underlying molecular mechanisms of cancer cachexia, the growing tumor has a considerable demand for essential amino acids. Attempts have been made to inhibit the development of cancer by altering the diet composition of ingested amino acids, inducing a state of amino acid imbalance [14, 84]. In particular, it has been shown that tumor growth is delayed in rats maintained on a valine-

depleted diet; however, this had a negative impact on the nutritional state of the host [85]. According to Lazo [61], a tumor can increase considerably the daily need for leucine in humans, the increase having a better correlation with the clinical deterioration of the patient than the modification of carbohydrate and energy metabolism. As a result of the demand for leucine, there is an amino acid flux from muscle to the tumor associated with muscle waste in the host. In general, however, the leucine taken up by the tumor only represents a minor part of the amino acid demand in the tumor-bearing animal [5]. It can thus be proposed that the major contribution to BCAA oxidation in cancer-bearing states is made by skeletal muscle [5]. Indeed, BCAA are the only amino acids that are extensively degraded in skeletal muscle and have been shown to stimulate protein synthesis and inhibit degradation in skeletal muscle *in vitro* [15, 79, 110] and *in vivo* [83, 102]. It could be proposed that during cancer cachexia the response of muscle protein turnover to BCAA is altered. Interestingly, the activation of muscle proteolysis in skeletal muscle always parallels that of BCAA oxidation. We have also recently described that  $\beta_2$  agonists (clenbuterol in particular) are able to suppress the activation of the ubiquitin-dependent proteolytic system during tumor growth [22]. In a similar manner,  $\beta_2$  agonists are also able to suppress the increase in BCAA oxidation in skeletal muscle during cancer cachexia [24]. Concerning possible mechanisms involved in the activation of BCAA oxidation, hormonal changes could be involved. Thus, insulin resistance is often present during cancer-bearing states but recently it has been demonstrated that it does not influence either protein or BCAA metabolism in cancer patients [48, 91]. The changes in muscle protein turnover are presumably induced by the combined actions of cytokines [21] alone or in combination with the so-called stress hormones such as glucagon [44], glucocorticoids [62] and catecholamines.

As a result of these major changes in amino acid metabolism, plasma amino acid profiles are altered during cancer-bearing states. It is observed that basically the concentrations of gluconeogenic amino acids are decreased, particularly in very cachectic tumors such as lung and gastrointestinal tract ones. Interestingly, the plasma concentration of BCAA is either normal or increased, even in the presence of severe malnutrition. This finding confirms the profound differences between cancer-induced cachexia and non-cancer malnutrition, in which both gluconeogenic and BCAA are reduced in relation to normal feeding. Another interesting finding concerning plasma amino acids in cancer relates to tryptophan. Meguid *et al.* have found great increases in free tryptophan concentrations during tumor growth and

have suggested this amino acid as a marker for neoplastic disease [75].

In conclusion, muscle protein degradation is perhaps the most important metabolic feature of the cachectic cancer-bearing host and future studies will no doubt concentrate on the discovery of compounds which are able to block the activation of the proteolytic systems responsible for the enhanced degradation.

### **Tumor Necrosis Factor- $\alpha$**

It was in 1893 that Coley introduced the idea that tumor regression in human cancer patients could be accomplished by challenging them with bacterial toxins [18]. Much later, Old identified a protein in the serum of endotoxin-treated rabbits, which was responsible for the hemorrhagic necrosis of tumors [86]. It was then named tumor necrosis factor (TNF) and later on TNF- $\alpha$  after the discovery of lymphotoxin or TNF- $\beta$ . It was more or less coincidentally that Kawakami and Cerami identified a molecule responsible for the wasting syndrome seen in many chronic diseases, such as chronic infection [52]. This molecule was named cachectin, since it was responsible for the induction of cachexia, and later on proved to be identical to TNF [11].

TNF is synthesized mainly by macrophages, in response to invasive stimuli, as a 26 kDa membrane-bound precursor that is cleaved proteolytically to a mature 17 kDa form with the prosequence polypeptide remaining associated to the membrane [51]. The peptide is bioactive as a 51 kDa trimer, which can be recognized by two different receptors, TNFR1 (55 kDa) and TNFR2 (75 kDa). TNF is a pleiotropic factor that exerts a variety of effects such as growth promotion, growth inhibition, angiogenesis, cytotoxicity, inflammation, and immunomodulation [3].

### **TNF and Cancer Cachexia**

Episodic TNF administration has proved unsuccessful at inducing cachexia in experimental animals [73, 82, 103, 106]. Indeed, repetitive TNF administrations initially induce a cachectic effect while tolerance to the cytokine soon develops and food intake and body weight return to normal. Others have shown that escalating doses of TNF are necessary to maintain the cachectic effects [114]. However, a very elegant approach involving the implantation of CHO cells which were transfected with the human TNF gene in nude mice seems to indicate that TNF may have a clear and important role in the induction of cachexia [87].

Raised concentrations of TNF have been detected in the serum of about 70% of patients with parasitic infections such as leishmaniasis and malaria [42, 98] and in patients with septicemia [115], all of which are pathological states where a high degree of cachexia is achieved. The increase in plasma TNF in septicemia is likely to be due to increased concentrations of endotoxin

which can elicit a transitory rise in plasma TNF when administered to healthy control subjects [12, 78, 113]. In contrast, evidence for increased TNF in the plasma of cancer patients is controversial. Balkwill *et al.* found that 50% of fresh serum samples from cancer patients had a positive response for TNF with an enzyme-linked immunosorbent assay [9], and more recently the presence of this cytokine has been observed in the serum of children with acute lymphoblastic leukemia [97]. By contrast, other studies have reported no increase [80, 104, 115], even in patients with small-cell lung cancer [99]. Similarly, in tumor-bearing mice, no TNF could be detected in plasma with bioassays [80]. In contrast, other studies have found considerable amounts of TNF in the blood of tumor-bearing rats [21, 105]. These divergent findings may be due to different sensitivities or specificities of the assay methods, stability of TNF on storage, short half-life of TNF *in vivo* or localized paracrine production of TNF [1].

During cachexia there is a dramatic loss of white adipose tissue, basically due to a fall in lipoprotein lipase (LPL) activity [69] and an increase in the activity of hormone-sensitive lipase (the rate-limiting enzyme of the lipolytic pathway). In addition to these metabolic events associated with cachectic states, there is an inhibition of glucose transport and *de novo* lipogenesis in the tissue. TNF has been shown to decrease LPL activity in 3T3-L1 cells [93], associated with a decrease in LPL mRNA [20]. Fried and Zechner reported that TNF produced a dose-dependent marked suppression of LPL activity in human adipose tissue maintained in organ culture [33]. *In vivo* administration of TNF results in a decrease of adipose tissue LPL activity in rat, mouse and guinea pig [20, 100]. This decreased activity has been shown to depress the uptake of exogenous [ $^{14}$ C]lipid by adipose tissue and to increase circulating triacylglycerols in the rat [26]. Such elevation may, in part, be the result of stimulation of lipolysis in adipose tissue with subsequent increased secretion of very low density lipoproteins from the liver [27, 59]. In contrast to these observations, in human primary cultures of isolated adipocytes the cytokine was unable to decrease LPL [54]. The addition of TNF to 3T3-L1 cells increased lipolysis [53], which has been confirmed by others using fully differentiated adipocytes [28]. TNF and interleukin-1 (IL-1) have both been shown to inhibit glucose transport in adipocytes [45] and consequently decrease the availability of substrates for lipogenesis. Conversely, no direct action of TNF has been shown on *de novo* lipogenesis in adipose tissue of starved rats [27]. However, TNF decreased acetyl-CoA carboxylase during preadipocyte differentiation by a decrease in its mRNA; this did not occur in fully differentiated adipocytes [89]. Using a polyclonal rat anti-TNF antibody, we have demonstrated that TNF is involved in

the abnormalities in lipid metabolism found in tumor-bearing rodents [17]. In conclusion, it may be suggested that TNF, together with perturbations in the hormonal homeostasis, is likely to play an important role in forcing the metabolic balance of the adipocyte towards the catabolic side. It has to be pointed out, however, that other cytokines are also likely to be involved in lipid changes since some of them such as IL-1, interleukin-6 (IL-6) and  $\gamma$ -interferon ( $\gamma$ -IFN) have also been shown to decrease LPL activity [77].

Perhaps the most dramatic consequence of the cachectic state is the loss of skeletal muscle protein. A large body of evidence suggests that TNF participates in the protein wasting and loss of nitrogen associated with cachectic situations [21, 64]. Chronic treatment of rats with recombinant TNF resulted in a depletion of body protein compared with pair-fed control animals [32]. Indeed, chronic treatment with either recombinant TNF or IL-1 $\beta$  resulted in a body protein redistribution and a significant decrease in muscle protein content, associated with coordinate decreases in muscle mRNA levels for myofibrillar proteins [32]. Studies involving administration of recombinant TNF *in vivo* have shown an increase in nitrogen efflux from skeletal muscle of non-weight losing humans with disseminated cancer [116]. Flores *et al.*, by infusing  $^{14}$ C-leucine to rats, showed that chronic recombinant TNF administration significantly enhanced muscle protein breakdown [31]. Goodman, measuring both tyrosine and 3-methylhistidine release by incubated rat muscles of animals acutely treated with the cytokine, concluded that TNF was involved in activating muscle proteolysis [40]. The mechanisms underlying such actions still remain obscure. Our research group has clearly demonstrated that TNF treatment enhances protein degradation measured *in vivo* in rat skeletal muscle [63, 64]. In addition, we have described that, at least during tumor growth, muscle wasting is associated with the activation of non-lysosomal ubiquitin-dependent proteases [65, 66], and that this activation seems to be mediated via TNF [34-36]. Ubiquitin, a 8.6 kDa peptide, is involved in the targeting of proteins undergoing cytosolic ATP-dependent proteolysis. In the cell, ubiquitin can be found free or conjugated in an isopeptide linkage to other cellular proteins. Proteins with multiple ubiquitins are the ones targeted for degradation by an ATP-dependent protease [29, 30, 47]. However, it has been suggested that the activity of this system, which is integrated in a supramolecular structure called the proteasome can also be related to the turnover of long-lived proteins, such as those found in skeletal muscle [49]. We have also reported that *in vivo* administration of TNF to rats results in an increased skeletal muscle proteolysis associated with an increase in both gene expression and higher levels of free and

conjugated ubiquitin [34, 36]. In addition, the *in vivo* action of TNF during cancer cachexia does not seem to be mediated by IL-1 [23] or glucocorticoids [67]. Concerning a possible direct action of TNF on muscle proteolysis, the presence of both TNFR1 and TNFR2 receptors has been described in muscle tissue [107] and we have very recently demonstrated that the action of the cytokine on the induction of ubiquitin-dependent proteolysis can be direct [68]. In conclusion, TNF, alone or in combination with other cytokines, seems to mediate most of the changes concerning nitrogen metabolism associated with cachectic states. In fact, therapeutic approaches directed against TNF have already been used in the treatment of cachexia in experimental models [21].

It is by no means intended here to give the idea that TNF is the only molecule involved in cachexia. Many other cytokines (i.e. IL-1, IL-6 and  $\gamma$ -IFN) have been reported to have an important role in the cachectic state [7].

### The AIDS-Wasting Syndrome

Weight loss is a common manifestation of both malignancy and infection. Since the acquired immunodeficiency syndrome (AIDS) is produced by chronic infection with HIV, it is at times characterized by significant weight loss which contributes to morbidity and mortality [57]. The pathogenic mechanisms leading to weight loss in AIDS patients are very poorly understood. The metabolic disorders that develop during the course of the disease and secondary infections lead to a manifest wasting state. A major problem concerns the definition of the wasting syndrome associated with the pathological state. While the Centers for Disease Control (CDC) in 1987 named it *the HIV-wasting syndrome* it was conceived as "an involuntary weight loss of >10% baseline body weight plus either chronic diarrhea (at least two loose stools per day for >30 days, intermittent or constant) or chronic weakness and documented fever (>30 days, intermittent or constant) in the absence of a concurrent illness or condition other than HIV infection that would explain the findings" [19]. This definition is too narrow and tends to oversimplify the problem since patients with AIDS may be subject to weight loss due to the viral growth, opportunistic infections or other invasive stimuli, such as tumor growth. It may be a much better proposal to term it AIDS-wasting syndrome and include in it just the idea of weight loss associated with either altered food intake or malabsorption or with increased energy expenditure. This broader term would avoid the problems associated with determining if the patients belong to the referred HIV-wasting syndrome and would be more representative of the real course of events during AIDS.

The viral infection at the various stages is characterized by a number of nutritional, metabolic and endocrinologic abnormalities. Thus, weight loss can be caused by inadequate dietary intake, decreased intestinal absorption, increased nutrient excretion or increased energy expenditure. Any one or a combination of these can produce weight loss in AIDS patients, depending on the stage of the HIV disease and associated infections. Gastrointestinal dysfunction, especially malabsorption, is prevalent in HIV infection with or without identifiable pathogens. This has been demonstrated by abnormal D-xylose absorption tests, Schilling tests and  $^{14}\text{C}$ -glycocholate absorption and by the presence of steatorrhea [41].

### **TNF and Wasting in AIDS**

When analyzing the possible role of TNF in the development of cachexia in AIDS patients, two very distinct aspects have to be considered. The first of these refers to the alterations in food intake or malabsorption of nutrients. While it is quite clear that a large percentage of patients suffer from diarrhea [46], which is a common sign of malabsorption, others have a marked anorexia which leads to a reduced food intake [43]. TNF can be one of the factors responsible for both phenomena [8]. It has been described that the cytokine reduces gastric emptying [4, 90] and also induces hemorrhagic lesions in the intestinal walls [112], this leading to malabsorption. TNF also can induce anorexia [13], especially in combination with IL-1 [118], a cytokine which is also elevated in a large percentage of AIDS patients.

Secondly, in spite of changes in food intake or nutrient assimilation, the hypermetabolism (increased energy expenditure) observed in AIDS patients may be a more important factor leading to the cachectic state. In favor of this interpretation, total parenteral nutrition to AIDS patients, while serving to increase body weight (mainly fat stores) in anorectic patients or those suffering from malabsorption [101] does apparently not stop the development of protein wasting [58].

Several studies have reported increased resting energy expenditure in asymptomatic HIV infection with a further increase observed later in AIDS complications; however, the increase in resting energy expenditure remains quite moderate [50]. In spite of this, there is an important point to take into consideration. Since total energy expenditure is the sum of resting metabolic, expenditure and diet-induced thermogenesis, very interestingly, Poizot-Martin *et al.*, using indirect calorimetry, have reported an increased in postprandial dietary thermogenesis during the course of HIV infection and this mechanism could be involved in the starvation observed during the course of HIV infection [92]. Cytokines, TNF in particular, have been shown to influence diet-induced thermogenesis in experimental

animals [96] and one should take them into consideration when trying to explain the changes in thermogenic responses found in the AIDS patient.

### **Conclusion**

One of the key biochemical features associated with muscle wasting is an enhanced protein degradation through the ubiquitin-dependent proteolytic pathway. TNF is an important activator of this proteolytic system, and future therapeutic approaches for wasting should consider either blocking TNF action or inhibiting the activity of the referred proteolytic system.

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