Molecular Pathways Leading to Cancer Cachexia

Loss of body weight in cancer patients strongly influences morbidity and mortality. Recent studies have suggested that both tumor and host factors play a major role in tissue catabolism in cachexia, leading to upregulation of degradative pathways in both skeletal muscle and adipose tissue.

Cachexia is derived from the Greek “kakos hexis,” literally meaning “bad condition,” and involves all of the effects produced by the tumor on the host that are not the direct result of mechanical interference with vital organs. One of the most devastating effects of cachexia is a progressive loss of body weight, resulting in severe depletion of both adipose tissue and skeletal muscle, but unlike the situation in starvation, visceral protein reserves are preserved. Loss of adipose tissue reaches 85% and loss of skeletal muscle proteins reaches 75% when the patient has lost 30% body weight (22), a situation in which death is likely to occur fairly quickly. Loss of protein from skeletal muscle is probably the most important factor regulating survival, since at this level of lean tissue loss physiological functions, such as respiratory muscle function, are significantly impaired (66). Cachexia has been suggested to be responsible for at least 20% of cancer deaths (30) and also plays an important part in the compromised immunity leading to death from infection. Asthenia is also directly related to the substantial muscle atrophy in cancer cachexia, reducing the quality of life of the cancer patient. Loss of protein from skeletal muscle has been shown to reduce the performance status (activity level) of cancer patients (16). In home-living cachectic patients with advanced pancreatic cancer, resting energy expenditure (REE) is increased compared with the predicted values for healthy individuals, whereas total energy expenditure and physical activity level are reduced (44). An elevated REE has also been observed in patients with cachexia from lung cancer, whereas patients with gastric or colorectal cancer show no elevation in REE (23). The increased REE probably represents an increase in futile metabolic cycles leading to an increase in energy expenditure.

About 50% of all cancer patients lose weight, but the incidence of cachexia is not distributed equally among all tumor types. Thus patients with pancreatic or gastric cancer have the highest frequency of weight loss (83–87%), patients with unfavorable non-Hodgkin lymphoma, colon, prostate, and lung cancer form an intermediate group (48–61% of patients losing weight), and patients with favorable subtypes of non-Hodgkin lymphoma, breast cancer, acute nonlymphocytic leukemia, and sarcomas have a low frequency of weight loss (31–40%) (16). This suggests that cachexia-inducing tumors may have an altered genetic expression that allows them to produce factors that degrade triglyceride stores in adipose tissue and myofibrillar proteins in skeletal muscle. Certainly cachexia bears no simple correlation to tumor burden, metastasis, or anatomic site of involvement. Cachexia can arise in a patient with a tumor comprising <0.01% of the host weight, although some large tumors do not produce cachexia.

Anorexia and Cachexia

Anorexia, defined as the loss of appetite and early satiety, often accompanies cachexia and has been suggested to play a role in the loss of body weight. However, in a study of 297 cancer patients with generalized malignant disease due to solid tumors, weight loss could not be accounted for by diminished dietary intake, since the energy intake in absolute amount was not different between weight-losing and weight-stable patients (8). In fact, the intake per kilogram of body weight was actually higher. However, weight loss and hypermetabolism were not compensated for by an increase in spontaneous food intake, suggesting a defect in orexigenic signals such as neuropeptide Y (NPY) and leptin. In mice bearing cachexia-inducing tumors, plasma leptin levels were found to decrease, with weight loss mirroring the loss of adipose tissue, while hypothalamic NPY mRNA was raised (5). Therefore, at least in this model, suppression of hunger is probably due to tumor products that inhibit NPY transport or release or that interfere with neuronal targets downstream of NPY.

Appetite stimulants such as megestrol acetate fail to restore the loss of lean body mass, and any gained weight has been shown to be due to an accumulation of both adipose tissue and water (37). Loss of skeletal muscle is not prominent in anorexia, since the brain adapts to use ketone bodies derived from the metabolism of fat, reducing the requirement for gluconeogenesis from amino
acids derived from muscle proteins. This suggests that the metabolic changes in anorexia and cachexia are different. In anorexia, a decreased nutrient intake is normally associated with a decreased REE, whereas in cachexia, as seen above, REE is often increased or remains at normal levels. This is due to metabolic changes specific to the tumor-bearing state.

**Energy Use in Cancer Cachexia**

Futile cycles are often increased in the tumor-bearing state. Thus nonesterified fatty acids released from adipose tissue can be immediately reesterified in what is known as the triacylglycerol/fatty acid substrate cycle. This process has been shown to be increased threefold in tumor-bearing mice, although there was no difference in animals with and without cachexia (3).

Another futile cycle that may account for an energy loss of 300 kcal/day in cancer patients is the Cori cycle (18). Tumors consume large amounts of glucose and convert it to lactate, because the oxygen tension is too low for the Krebs cycle and mitochondrial oxidative phosphorylation to operate. The lactate produced circulates to the liver and is reconverted into glucose in a process known as the Cori cycle. Although the Cori cycle is normally responsible for 20% of glucose turnover, it has been shown to be increased to 50% in cachectic cancer patients, accounting for 60% of the lactate produced (29). Gluconeogenesis uses six ATP molecules for every lactate-glucose cycle and is very inefficient for the host, contributing to the increased REE in cachectic subjects.

Another mechanism for increasing energy expenditure is through an increased expression and activity of mitochondrial uncoupling proteins (UCPs). These are proteins that translocate protons across the inner mitochondrial membrane in a process not coupled to phosphorylation of ADP, so that energy is lost as heat. The principal UCPs are UCP1, which is found only in brown adipose tissue (BAT), and UCP3, found only in BAT and skeletal muscle. BAT is not normally found in adult humans, although BAT was found to be present in periadrenal tissue in 80% of cachectic cancer patients compared with 13% of age-matched control subjects (51). In mice bearing the cachexia-inducing MAC16 adenocarcinoma, UCP1 mRNA levels in BAT and UCP3 mRNA levels in skeletal muscle were increased (4). UCP3 mRNA levels have also been shown to be significantly higher in the skeletal muscle of cancer patients with weight loss than in those that had not lost weight and in patients without cancer (13). The increase in UCP expression in cancer patients would increase REE and contribute to weight loss. The increase in UCP3 mRNA in skeletal muscle may be due to hydrolysis of triglycerides in adipose tissue, since treatment of tumor-bearing animals with nicotinic acid eliminated both the hyperlipidemia and the increase in UCP3 mRNA in soleus but not gastrocnemius muscle (11). However, certain cytokines and tumor products have been shown to directly upregulate UCP expression, suggesting a further mechanism for the control of energy expenditure in cachexia.

**Factors Governing Mass of Adipose Tissue and Skeletal Muscle**

The mass of adipose tissue is determined by the rate of synthesis of triacylglycerols from circulating lipoproteins and the rate of their hydrolysis to fatty acids and glycerol (FIGURE 1). The enzyme lipoprotein lipase (LPL) is involved in the extraction of fatty acids from plasma lipoproteins, and the rate of synthesis is determined by substrate supply from the liver. Fat has the highest caloric value of any nutrient, and demand for energy is met by the hydrolysis of triacylglycerols by the hormone-sensitive lipase (HSL), which is regulated by the intracellular level of cAMP. Hormones such as adrenaline and glucagon stimulate adipocyte adenylate cyclase, converting ATP to cAMP. The cAMP activates a protein kinase, which in turn phosphorylates and activates HSL. When the energy demand ceases, the excess fatty acids released are resynthesized into triacylglycerols in adipose tissue.

Although skeletal muscle contains the majority of protein in the body, it does not act as an energy source like adipose tissue under normal conditions. In the young animal, protein synthesis exceeds protein degradation and muscle bulk increases. This can be enhanced by load-bearing exercise through the stimulation of a growth factor called insulin-like growth factor-I (IGF-I) (15). Branched-chain amino acids, and leucine in particular, are also known to stimulate protein synthesis in muscle by initiating signal-transduction pathways that moderate translation initiation (68). During aging, loss of muscle mass occurs, and this is clinically referred to as sarcopenia. Forced inactivity such as bed rest or space flight also results in loss of muscle protein. Although muscle mass is
fairly constant in the adult, it is subjected to continuous turnover, enabling the body to regulate enzyme systems and remove defective proteins. If the balance between anabolic and catabolic processes is not maintained, then loss of muscle nitrogen will occur. This can be achieved by an increase in breakdown or a decrease in synthesis. Muscle atrophy during fasting is associated with a reduced protein synthesis, but the major loss of contractile proteins, which constitute the bulk of muscle mass, has been suggested to result from an increased degradation (55).

In all cases of muscle atrophy, this appears to be due to an increased activity and expression of the ubiquitin-proteasome proteolytic pathway (33). In this process proteins are marked for degradation by a polyubiquitin tag, which is recognized by the 26S proteasome, a large multisubunit proteolytic complex consisting of a central catalytic core (20S proteasome) and two terminal regulatory subcomplexes (19S complex) (FIGURE 2). The protein is converted to short oligopeptides by proteolytic enzymes residing on the inner surface of the 20S core. There are three main proteolytic enzymes with specificity for acidic (peptidyl-glutamyl-peptide), basic (trypsin-like), and hydrophobic (chymotrypsin-like) residues. The 19S regulator plays a central role in the recognition and unfolding of proteins so that they can enter the catalytic core. Energy is required for this process as well as for the activation of ubiquitin by the ubiquitin-activating enzyme (E1). Two other enzymes are also involved in the conjugation of a polyubiquitin chain to the protein substrate: ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). There are a number of E3s that recognize particular protein substrates and ensure the specificity of the process. E2s also function in the degradation of different types of substrate and accept the activated ubiquitin from E1 and transfer it to the protein linked to E3. Progressive rounds of ubiquitin ligation result in the attachment of a polyubiquitin chain to the substrate. E3 is considered to be the rate-limiting step of the process, because two E3s, MAFbx/atrogin-1 and MURF1, not only increase during muscle atrophy but are absolutely required for the process to occur (7, 27). Although ubiquitin conjugation is normally required for proteasome proteolysis, a number of publications over the past few years have shown that the 26S proteasome can degrade some proteins in a ubiquitin-independent manner, e.g., ubiquitin conjugation appears not to be necessary for proteasome degradation of oxidized proteins (52).

**Changes in Adipose Tissue and Skeletal Muscle During Cancer Cachexia**

Weight-losing cancer patients show an increased turnover of both glycerol and fatty acids when compared with normal subjects or cancer patients without weight loss (50). In addition, fasting plasma glycerol concentrations have been shown to be
higher, providing evidence for an increased lipolysis (17). The fatty acids released are rapidly oxidized, and there is a 20% increase in oxidation. There is no evidence for a decreased level of LPL in adipose tissue of cancer patients, but there is a twofold increase in the relative level of mRNA for HSL, suggesting an upregulation of triacylglycerol hydrolysis (56). The released fatty acids serve as an energy source to drive futile metabolic cycles, as well as serving as an energy source for heat production in BAT and skeletal muscle.

Most studies in cancer patients provide evidence for a decreased protein synthesis in skeletal muscle (19) as well as an increased protein degradation (40). A number of studies have shown an increased activity and expression of the ubiquitin-proteasome proteolytic pathway in the skeletal muscle of weight-losing cancer patients (9, 32). Protein synthesis requires the correct balance of amino acids, and an increase in synthesis of acute-phase proteins in the liver may alter the balance of amino acids for protein synthesis, since acute-phase proteins contain relatively high levels of sulfur amino acids (46). There are also decreases in the concentrations of branched-chain amino acids in the plasma, reducing the stimulus for protein synthesis in muscle. However, because nutritional supplementation or appetite stimulants such as megestrol acetate are unable to replenish the lean body mass in cachectic patients (37), this suggests that the catabolic stimulus outweighs the decrease in anabolism, without specific defects in the protein synthesis machinery. The upregulation of catabolism in both adipose tissue and skeletal muscle in cancer cachexia suggests that specific stimuli may be involved.

Factors Involved in Tissue Catabolism in Cancer Cachexia

Several factors produced by tumors, and by host tissues in the presence of certain tumors, have been suggested to play a critical role in tissue wasting in cancer cachexia. Such factors include cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and -6, and interferon-γ (IFN-γ), which can be produced by tumor and host tissues, as well as the lipolytic factor zinc α2-glyco-protein (ZAG) and tumor-specific products such as proteolysis-inducing factor (PIF).

Cytokines

Cancer cachexia shows similarities to tissue injury, infection, or inflammation in showing an acute-phase response (APR) in which liver protein synthesis changes from synthesis of albumin to production of acute-phase proteins such as C-reactive protein (CRP), fibrinogen, and α1-antitrypsin. In patients with lung and gastrointestinal cancers, an increased CRP was associated with the rate of loss of body mass (43); in patients with pancreatic cancer, elevated levels of fibrinogen were associated with a shorter survival time (21). The APR is known to be activated by cytokines such as IL-6, IL-8, and TNF-α, suggesting that they may play a role in cancer cachexia. However, animal experiments suggest that cytokines can induce an APR without inducing weight loss. Thus IL-6, when administered daily to healthy mice, had no effect on body weight over a 7-day period, although it did induce a hepatic APR (20). In contrast, ciliary neurotrophic factor, a member of the IL-6 superfamily, when administered at the same dose level produced both weight loss and an APR. These results suggest that the APR is related to the development of cachexia, although alone it is not sufficient to cause this condition.

Cytokines can be produced by host tissues or tumor cells. Thus studies in mice bearing the Lewis lung tumor showed that when the tumor grew and cachexia was observed, splenocytes produced IL-6, IL-11, and oncostatin M (2). IL-8 has been shown to be produced by one pancreatic tumor cell line (63). Production of IL-8 was increased following exposure to IL-2 and TNF-α. Despite this, there have been difficulties in establishing elevated serum lev-
levels of TNF-α in weight-losing cancer patients (53), but other studies that have measured serum TNF-α have suggested that this may be an indicator of tumor burden (49). A study of 63 patients with pancreatic cancer found serum levels to be detectable in 36.5% of patients, with higher levels in patients with metastatic disease, and such patients had a significantly lower body weight and body mass index (31). However, other studies have shown serum levels of TNF-α, IL-1, IL-6, and IFN-γ not to correlate with weight loss in patients with advanced and terminal cancer (41). In contrast with TNF-α and IL-1, IL-6 is generally detectable in serum or plasma of cancer patients (63). The short half-life of biologically active TNF-α and formation of complexes with its soluble receptor have been suggested to contribute to its lack of detection. However, elevated concentrations of TNF-α have been reported in patients with both malaria and visceral leishmaniasis, and this has been correlated with the development of cachexia.

The main support for a role for cytokines in the induction of cachexia comes from studies in animals bearing cachexia-inducing tumors. Thus Chinese hamster ovary cells transfected with the gene for human TNF-α produced a state of cachexia in nude mice (45). These mice had serum levels of TNF-α of 1.0–22.8 ng/ml, ~1,000-fold higher than that found in cancer patients. Interestingly, the effect of these cells on weight loss was dependent on the site of transplantation (60). Thus when these cells were transplanted intracerebrally, hypophagia and weight loss were observed and the body composition was comparable with starvation, i.e., a decrease of whole-body lipid but conservation of protein. When transplanted intramuscularly, profound anorexia did not develop, but 50 days after cell implantation cachexia developed, with depletion of both protein and lipid. IL-6 has been implicated in the development of cachexia in mice bearing colon-26 adenocarcinoma, since it was associated with increasing serum levels of IL-6 and since a monoclonal antibody to mIL-6, but not mTNF-α, significantly suppressed progression of cachexia (54). However, it is unlikely that IL-6 acts alone to induce cachexia, since a clonal variant of colon-26, which did not induce cachexia, also produced elevated serum levels of IL-6 when transplanted into mice (24), and administration of IL-6 alone to mice does not always result in loss of body weight (20). IFN-γ also appears to be responsible for some of the symptoms of cachexia in certain experimental tumors. Thus production of weight loss by the Lewis lung tumor is associated with production of IFN-γ, and anti-IFN-γ antibody administration reduced the loss of body fat but had no effect on muscle protein loss (42). Thus the cytokine involved in the induction of cachexia appears to vary with tumor type, and each may not act alone but may be responsible for the induction of other cytokines or factors responsible for the induction of cachexia.

Suggested mechanisms for host tissue catabolism by cytokines are as follows:

- **Adipose tissue.** All cytokines inhibit the enzyme LPL, albeit with different potencies. This was originally suggested as a mechanism by which cytokines could induce loss of adipose tissue by preventing the resynthesis of triglycerides. However, inhibition of LPL is unlikely to have a major effect on fat stores, since in patients with type 1 hyperlipidemia, caused by an inherited deficiency in LPL, fat stores are normal and there is no cachexia. In addition, in cancer patients there is no evidence for inhibition of LPL in adipose tissue (56). Some cytokines, such as TNF-α, have been shown to stimulate lipolysis, although this required prolonged incubation (12–24 h) (69). The mechanism involves cAMP, but this is not induced by the classical route through stimulation of adenylate cyclase; rather, it involves activation of mitogen-activated protein kinase and extracellular signal-regulated kinase. TNF-α also stimulates thermogenesis in rats, possibly due to the increase in expression of both UCP2 and UCP3 mRNA in skeletal muscle (10), providing a route for oxidation of any fatty acids mobilized.

- **Skeletal muscle.** Atrophy of muscles, associated with increased levels of mRNA for cathepsins (B and L) and ubiquitins, is observed in IL-6 transgenic mice (61). The effect is completely blocked by anti-mouse IL-6 receptor antibody. Treatment of rats bearing the Yoshida AH-130 hepatoma with either pentoxyfilline, an inhibitor of TNF-α synthesis, or with suramin, which blocks the peripheral action of several cytokines including TNF-α and IL-6, prevented the depletion of muscle mass and significantly reduced the increased proteasome- and calpain-dependent proteolysis (14). Acute treatment of rats with TNF-α caused an enhanced proteolytic rate and depression of protein synthesis in soleus muscle while having no effect in extensor digitorum longus muscle (25). Interestingly, some studies (20, 24) suggest that IL-6 has no direct effect on muscle protein balance when administered to mice. Also, pentoxyfilline, at dose levels shown to suppress synthesis of TNF-α in humans, was shown to be
ineffective in the treatment of anorexia and cachexia in a small patient group (26).

For some time, investigators found it difficult to demonstrate a direct effect of cytokines on protein degradation in vitro when either tyrosine or 3-methylhistidine was used as a measure of the proteolytic rate. However, TNF-α has been shown to produce an increase in ubiquitin gene expression after incubation with rat soleus muscle in vitro for 180 min (36) and directly induces loss of the myofibrillar protein myosin in a proteasome-mediated process in skeletal muscle myocytes (34). The transcription factor nuclear factor-κB (NF-κB) mediates the protein loss induced by TNF-α (35). Evidence has been presented that cytokines synergize to regulate gene expression. Thus, although TNF-α or IFN-γ alone had minimal effects on the expression of myosin heavy chain in murine myotubes, addition of both cytokines reduced myosin heavy chain mRNA in a cooperative fashion (1). Expression of troponin, tropomyosin, and actin genes were unchanged by cytokine treatment. These results suggest that cytokines have the potential to act synergistically to induce protein catabolism in skeletal muscle.

**Lipid-mobilizing factor/ZAG**

A search for a tumor product with direct lipid-mobilizing activity (called lipid-mobilizing factor, or LMF) led to the purification of a glycoprotein of 43 kDa from both an experimental cachexia-inducing tumor and from the urine of cachectic cancer patients (57). The glycoprotein was found to be homologous with the plasma protein ZAG in amino acid sequence, electrophoretic mobility, and immunoreactivity. Both LMF and ZAG directly stimulated lipolysis in murine epididymal adipocytes through an increase in cAMP, resulting from the stimulation of adenylate cyclase in a GDP-dependent manner (28). Both LMF (28) and ZAG (47) produced specific loss of body fat when administered to mice, with a tendency to increase lean body mass. The effect appears to be due to interaction with a β3-adrenergic receptor. Loss of adipose tissue was coupled with an increase in expression of UCP1 in BAT (47). This appears to be a direct effect, since ZAG increased UCP1 expression in primary cultures of BAT, as well as increasing expression of UCP2 and UCP3 in murine myotubes (48). Induction of UCP1 and UCP2 has been shown to be mediated through a β3-adrenergic receptor, whereas induction of UCP3 appears to require mitogen-activated protein kinase. Recent results (6) suggest that ZAG is not only produced by certain tumors (28) but also by white adipose tissue and BAT and that the induction of cachexia is accompanied by major increases in ZAG mRNA and protein levels in both types of adipose tissue. This suggests a local role of adipocyte-derived ZAG in the induction of both lipolysis and UCP expression.

**PIF**

PIF was first detected and purified due to its reaction with an antibody present in the serum of mice bearing the cachexia-inducing MAC16 tumor but absent from serum of those with a related tumor (MAC13) that does not induce cachexia (58). The antibody was reactive to a similar material detectable in the urine of patients with cancer cachexia due to a range of solid tumors but absent if the tumor did not induce cachexia (12). PIF is detectable in the urine of 80% of patients with pancreatic cancer, and these patients have a significantly greater total weight loss and rate of weight loss than those in which PIF was undetectable (64). A study of patients with advanced cancer stemming from a variety of primary gastrointestinal tumors showed that over time, patients positive for PIF experienced weight loss, whereas those with a negative test gained weight (65).

Administration of PIF to mice produced a profound depression of body weight (~13%) over a 24-h period without a reduction in food and water intake (38). The major contribution to the decrease in body weight was a decrease in lean body mass, which was accounted for by a decrease (by 50%) in protein synthesis and an increase (by 50%) in protein degradation. PIF was shown to be a novel sulfated glycoprotein of 24 kDa with extensive glycosylation at serine and asparaginel residues of a small peptide core (59). The biological effect of PIF is due to the sulfated oligosaccharide chains, and the peptide core is unable to initiate protein degradation in skeletal muscle. Skeletal muscle from mice treated with PIF, as well as murine myotubes treated with PIF in vitro, show an increased activity and expression of key components of the ubiquitin-proteasome proteolytic pathway (39). Treatment with proteasome inhibitors attenuated the enhanced protein degradation, suggesting this to be the main mechanism for degradation of myofibrillar proteins. Like with TNF-α (35), induction of proteasome expression by PIF is associated with rapid degradation of IκB and increased nuclear migration of NF-κB, which appears to be essential, since inhi-
References


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Conclusion

These studies show myriad factors with the potential to mediate loss of adipose tissue and skeletal muscle protein in cancer cachexia (FIGURE 3). Although PIF appears to be restricted to cancer cachexia, other factors such as cytokines and ZAG may mediate tissue loss in other catabolic conditions. There is also the potential for both cytokines and PIF to induce expression of each other. Despite their different origins and chemical composition, both cytokines and PIF may activate a common intracellular signaling pathway leading to activation of NF-κB. Since the primary mediators of the cachectic process in humans have not been established beyond doubt, therapies aimed at such signaling systems may be more effective in preventing tissue atrophy.


