
REVIEW

Biology of Cachexia

Michael J. Tisdale*

About half of all cancer patients show a syndrome of cachexia, characterized by loss of adipose tissue and skeletal muscle mass. Such patients have a decreased survival time, compared with the survival time among patients without weight loss, and loss of total body protein leads to substantial impairment of respiratory muscle function. These changes cannot be fully explained by the accompanying anorexia, and nutritional supplementation alone is unable to reverse the wasting process. Despite a falling caloric intake, patients with cachexia frequently show an elevated resting energy expenditure as a result of increases in Cori cycle (i.e., catalytic conversion of lactic acid to glucose) activity, glucose and triglyceride-fatty acid cycling, and gluconeogenesis. A number of cytokines, including tumor necrosis factor- α , interleukins 1 and 6, interferon γ , and leukemia-inhibitory factor, have been proposed as mediators of the cachectic process. However, the results of a number of clinical and laboratory studies suggest that the action of the cytokines alone is unable to explain the complex mechanism of wasting in cancer cachexia. In addition, cachexia has been observed in some xenograft models even without a cytokine involvement, suggesting that other factors may be involved. These probably include catabolic factors, which act directly on skeletal muscle and adipose tissue and the presence of which has been associated with the clinical development of cachexia. A polyunsaturated fatty acid, eicosapentaenoic acid, attenuates the action of such catabolic factors and has been shown to stabilize the process of wasting and resting energy expenditure in patients with pancreatic cancer. Such a pharmacologic approach may provide new insights into the treatment of cachexia. [J Natl Cancer Inst 1997;89:1763-73]

The word "cachexia" is derived from the Greek "kakos" meaning "bad" and "hexis" meaning "condition." It occurs in a number of disease states, including cancer, acquired immunodeficiency syndrome (AIDS), major trauma, surgery, malabsorption, and severe sepsis. Cachexia is characterized by weight loss involving depletion of host adipose tissue and skeletal muscle mass. Weight loss in cancer patients differs from that found in simple starvation. During the first few days of starvation, glucose utilization by the brain and erythrocytes necessitates depletion of liver and muscle glycogen and an increased glucose production by the liver, using gluconeogenic amino acids derived from catabolism of muscle. This early phase is replaced in long-term starvation by the use of fat as a fuel, in which free

fatty acids released from adipose tissue are converted into ketone bodies, which are utilized for energy by peripheral tissues and eventually to a great extent by the brain. This leads to conservation of muscle mass. In anorexia nervosa, more than three quarters of the weight loss arises from fat and only a small amount from muscle. In contrast, in cancer cachexia, there is equal loss of both fat and muscle, so that for a given degree of weight loss there is more loss of muscle in a patient with cachexia than in a patient with anorexia nervosa (1). Thus, although anorexia is common in cancer patients, with reports of occurrence in 15%–40% of subjects at presentation (2), the body composition changes suggest that anorexia alone is not responsible for cachexia. Also, in malnourished cancer patients, the measured food intake fails to correspond with the degree of malnutrition (3), and loss of both muscle and adipose tissue has been reported to precede the fall in food intake (4). In contrast to simple starvation, it is not possible to reverse the body composition changes seen in patients with cancer cachexia by the provision of extra calories. Attempts to increase energy intake in cancer patients through dietary counseling failed to reverse the cachexia (5). Trials of total parenteral nutrition in cachectic cancer patients also failed to show benefit in terms of increased median survival time or long-term weight gain (6). Although a short-term weight gain was observed, this weight was subsequently lost, suggesting the retention of water (7). Analysis of body composition indicated that patients receiving total parenteral nutrition temporarily maintained body fat stores, but there was no evidence for preservation of lean body mass. Thus, the cause of wasting in cancer cachexia is more complex than that in simple starvation.

Anorexia and Cachexia

Although anorexia alone is unlikely to be responsible for the wasting seen in cancer patients, it may be a contributing factor. In addition, its presence is an extremely distressing syndrome because appetite and the ability to eat have been reported to be the most important factors in the physical and psychological aspects of a patient's quality of life (8). Anorexic cancer patients often report early satiety, which together with a reduced appetite has been postulated to be caused by the production of factors by the tumor that exert their effects by acting on the hypothalamic

*Correspondence to: Michael J. Tisdale, Ph.D., D.Sc., Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET, U.K.

See "Note" following "References."

sensory cells. Possible candidates for such a factor are the satietins (9). Satietyins have been purified from human plasma and found to consist of two proteins that copurify until they are purified from one another by affinity chromatography. The larger protein has been characterized as an extensively glycosylated α 1-acid-glycoprotein of a molecular mass of 64 kd, which is probably a vehicle for satietin D, a 41-kd glycoprotein. When injected into rats, satietin D has been shown to produce a long-lasting anorectic effect, although its role in the development of anorexia is not known. There may also be some dysfunction of the hypothalamic neuropeptide Y feeding system in the tumor-bearing state, since rats bearing a methylcholanthrene-induced sarcoma were found to be refractory to the intrahypothalamic injection of neuropeptide Y; normal rats, by contrast, increased their food intake by more than 50% in response to such an injection (10).

Increased serotonergic activity within the central nervous system has been proposed as a possible cause of anorexia. Such activity is secondary to the enhanced availability of tryptophan to the brain. Thus, a close relationship between elevated plasma-free tryptophan and anorexia was observed in patients with cancer and reduced food intake (11). The uptake into the brain of tryptophan is competitive with that of branched chain amino acids. An attempt to reduce tryptophan uptake by increasing plasma levels of competitor branched chain amino acids produced a decrease in the incidence of anorexia (12), but it was not reported whether such patients also regained body weight. However, although the serotonin antagonist cyproheptadine has been reported to have a weight-enhancing effect in normal subjects, a randomized, placebo-controlled, double-blinded trial found it to have no effect on progressive weight loss in cachectic cancer patients (13).

Metabolism in Cancer Cachexia

Metabolic Rate in Cancer Cachexia

In chronic starvation, the basal metabolic rate is reduced as the body adapts to conserve tissues and energy in a low-protein-low-calorie environment (14). However, when compared with control groups, cancer patients have been reported to have reduced (15), normal (16), or increased (17) energy expenditures. Because cancer patients typically have a reduced caloric intake, even a normal energy expenditure could be classified as being in excess. In one study of cancer patients with solid tumors, the basal metabolic rates of the patients were found to be elevated even before the onset of weight loss (18), thus suggesting that an elevated basal metabolic rate may be a contributing factor rather than a consequence of the condition. The elevated basal metabolic rate has been shown to be associated with an increase in heart rate, and it has been suggested that it may be due to an elevated adrenergic state (18). Certainly, many cancer patients show elevated plasma concentrations and increased urinary excretion of adrenergic substances (19), in contrast to malnourished patients without cancer who generally show a decrease in catecholamine turnover.

The tumor type appears to play an important role in determining whether or not an elevation of resting energy expenditure is observed. Thus, patients with lung cancer show an increase in resting energy expenditure compared with that in healthy control

subjects, whereas patients with gastric or colorectal cancer show no elevation in resting energy expenditure (20). Patients with pancreatic cancer have also been reported to have an increased resting energy expenditure compared with that in control subjects, and this effect was found to be more pronounced in those patients with an acute-phase response (21). This may explain why, in a mixed group of cancer patients with gastrointestinal, gynecologic, or genitourinary cancer, 33% were found to be hypometabolic, 41% had average metabolic rates, and 26% were hypermetabolic (15). It has been calculated that an elevation of 12% in the metabolic rate could account for the loss of 1–2 kg of body weight per month (17). Brown adipose tissue plays a thermoregulatory role and has been implicated as an important effector of both body temperature and energy balance in many mammals as well as in humans. Weight loss in a rat model of cancer cachexia has been attributed to a high metabolic rate produced by the activity of brown adipose tissue (22), although results from sarcoma-bearing mice with weight loss suggested that brown adipose tissue could not account quantitatively for the host wasting (23). Results in humans are sparse, although an examination of autopsy samples of periadrenal tissue by light microscopy revealed brown adipose tissue in 80% of cachectic cancer patients compared with 13% of age-matched control subjects (24). The reasons for these changes in basal metabolic rate and brown adipose tissue in patients with cancer cachexia are unknown, although both tumor and host factors, together with the various aberrations in metabolic processes observed in this group of cancer patients, probably contribute to this condition.

Carbohydrate Metabolism

Most solid tumors, either because of isoenzyme alterations or because of their poor vascularization and hence hypoxic nature, rely almost exclusively on the anaerobic metabolism of glucose as their main energy source, with most being converted to lactate (25). Glucose uptake and lactate release by human colon carcinomas have been found to exceed the peripheral tissue exchange rate by 30-fold and 43-fold, respectively (26). Selective transcriptional regulation of hexokinase isoforms by a tumor may enable it to have a growth advantage over normal cells. Thus, the type II isoform has been shown to be the dominant form expressed in AS-30D hepatoma cells in contrast to the type IV isoform in normal hepatocytes (27). The promoter activity of the type II hexokinase was found to be resistant to normal hormonal control, thus enabling tumor cells to maintain glycolysis at an optimal rate regardless of the metabolic state of neighboring healthy cells.

Since glycolysis is an inefficient method of energy production from glucose compared with oxidative phosphorylation, high levels of glucose will be consumed by the tumor. In mice bearing transplantable colon tumors, glucose utilization by the tumor was second only to that by the brain (28). This extra demand for glucose by the tumor was accompanied by a marked decrease in glucose utilization by host tissues, in particular the brain, which resembles the situation found in starvation. Despite this weight loss, cancer patients show a 40% increase in hepatic glucose production compared with control subjects, in contrast to the reduced level seen in patients with anorexia nervosa (29). The increase in glucose production in some cancer patients can be accounted for by an increase in Cori cycle activity (30).

Patients with progressive cancer have been shown to have an increased glucose synthesis not only from lactate (31), but also from alanine (32) and glycerol (33). The Cori cycle normally accounts for 20% of glucose turnover, but it was shown to be increased to 50% in cachectic cancer patients, accounting for the disposal of 60% of the lactate produced (34). Both glucose production rates and recycling rates were found to be higher in malnourished cancer patients than in patients without cancer and with comparable weight loss (35). The increased glucose recycling equivalent to 40% of the daily glucose intake of the cancer patient has been estimated to lead to a potential loss of 0.9 kg of body fat per month.

Hypoglycemia has been reported in a number of animal models of cancer, and in humans it is associated with carcinoma of the stomach, cecum, or bile ducts, pseudomyxoma, and paraganglioma, sometimes occurring before the presence of a tumor is suspected (36). It was originally thought that hypoglycemia was due to the high glucose consumption by the tumor, but a recent study (37) suggests that it arises through the ability of some tumors, other than insulinoma, to produce insulin or insulin-like substances. Since insulin levels have been shown to be low in cases of tumor-associated hypoglycemia, the production by the tumor of an insulin-like growth factor has been suggested as the cause of enhanced peripheral glucose uptake. The most likely candidate for the pathogenesis of extrapancreatic tumor hypoglycemia is the production of a high-molecular-mass (15–25 kd) insulin-like growth factor II (IGF II) by the tumor (37).

Thus, patients with cancer have an increased glucose production and turnover, which may be enhanced by the production of IGF II. Such changes contribute to an increased energy expenditure by the host.

Lipid Metabolism

Fat constitutes 90% of the adult fuel reserves, and loss of whole-body fat is a feature of cancer cachexia. Cancer patients with weight loss have an increased turnover of both glycerol and fatty acids when compared with patients without weight loss (38). Fasting plasma glycerol concentrations have been shown to be higher in weight-losing cancer patients than in weight-stable cancer patients, thus providing evidence for an increase in lipolysis (39). Increased utilization of fatty acids as a preferred energy source has been observed even in the presence of high plasma glucose concentrations, suggesting that, in the presence of certain tumors, host tissues may increase their utilization of fatty acids as an energy source (40).

Several clinical studies [reviewed in (41)] have observed an increased mobilization of fatty acids before weight loss occurs, suggesting the production of lipid-mobilizing factors either by the tumor or by host tissues. Although normal individuals suppress lipid mobilization with administration of glucose, there is an impaired suppression in patients with malignant diseases as well as continued oxidation of fatty acids (42). Increased fatty acid oxidation in the absence of increased dietary fat intake would result in a depletion of fat stores, while increased triglyceride fatty acid cycling and gluconeogenesis from glycerol could result in an increase in metabolic rate. All of these processes, therefore, have the potential to contribute to a net loss of body weight.

Mobilization of fatty acids from host adipose tissue may be

an important factor contributing to tumor growth. Patients with ovarian or endometrial tumors were found to have lower concentrations of linoleic acid in subcutaneous adipose tissue than cancer-free subjects, suggesting mobilization to supply lipids to the tumor (43). Linoleic acid has been found to act as a stimulator of tumor growth both *in vitro* (44) and *in vivo* (45). The effect is probably due to formation of prostaglandins or products of the lipoxygenase pathways. Rat Walker 256 carcinosarcoma cells transfected with 12-lipoxygenase-specific antisense oligonucleotide or antisense oligonucleotides directed to conserved regions of lipoxygenases underwent time- and dose-dependent apoptosis (46). Also, treatment with lipoxygenase but not with cyclooxygenase inhibitors induced apoptotic cell death, which could be partially inhibited by exogenous 12(*S*)- or 15(*S*)-hydroxyeicosatetraenoic acids (46). This observation suggests that essential fatty acids from adipose tissue may be required for tumor expansion by preventing cell death by apoptosis.

Protein Metabolism

Lean body mass and visceral protein depletion are characteristics of patients with cancer cachexia, and the degree of depletion may be associated with reduced survival (47). The major site of this protein loss has been observed to be the skeletal musculature (48). An increased rate of whole-body protein turnover has been reported in cachectic cancer patients. A reduced rate of protein synthesis and an increased rate of degradation were observed in muscle biopsy specimens from 43 newly diagnosed cancer patients with weight loss (49). An increase in cathepsin D activity was observed in biopsy specimens from the rectus abdominal muscle, an increase that was associated with the rate of protein degradation. In another study (50), a decrease in muscle protein synthesis was also observed in weight-losing cancer patients, with no change in total body synthesis or degradation. Muscle protein synthesis accounted for only approximately 8% of the total body synthesis in these patients compared with 53% for healthy control subjects. The observed maintenance of the total protein synthetic rate in these patients may therefore be due to a twofold increase in nonskeletal muscle protein synthesis. This twofold increase in protein synthesis may be due to increased hepatic synthesis of secretory proteins such as acute-phase reactants. An elevated whole-body protein turnover may also be apparent in patients with small tumor burdens (51).

There are also changes in the plasma amino acid profile in patients with cancer cachexia, and most studies have reported that such patients exhibit decreases in the concentrations of gluconeogenic amino acids. This result contrasts with the situation in subjects with severe malnutrition, in whom the concentrations of branched chain amino acids in plasma are normal or increased. Compared with normal colon tissue, human colon tumors were observed to have a specific requirement for serine and for the branched chain amino acids valine, leucine, and isoleucine (52). However, the two tissues had similar retention of total amino acids. Correlations between histidine-induced, enhanced formiminoglutamic acid excretion, elevated basal metabolic rate, and reduced serum albumin levels were observed in a study of eight weight-losing patients with small-cell carcinoma of the lung (53). Formiminoglutamic acid excretion was reduced in patients who showed a positive response to chemotherapy,

whereas there was no change in a patient with progressive disease.

The pathway responsible for breakdown of myofibrillar proteins is the adenosine triphosphate (ATP)-ubiquitin-dependent proteolytic system, which has been shown to be elevated in starvation (54), sepsis (55), and metabolic acidosis (56) and after transplantation of certain tumors such as the Yoshida ascites hepatoma in rats (57). In this process, proteins for degradation are first conjugated with ubiquitin, which serves as a signal for degradation by a large proteolytic complex, the 26S proteasome, which requires ATP to function. In skeletal muscle of rats bearing the Yoshida ascites hepatoma, a 500% increase in expression of polyubiquitin genes was observed in relation to both pair-fed (i.e., non-tumor-bearing rats fed the same amount of food as tumor-bearing rats) and *ad libitum*-fed animals (57). Treatment with clenbuterol suppressed the elevation of protein breakdown rates toward control values, and this suppression was associated with a decreased expression of polyubiquitin genes (58). Thus, understanding more about this proteolytic system and factors involved in its regulation may provide important clues for the treatment of muscle wasting in cachexia.

Factors Implicated in Production of Cancer Cachexia

Numerous cytokines, including tumor necrosis factor- α (TNF- α), interleukin 1 (IL-1), interleukin 6 (IL-6), interferon gamma (IFN γ), and leukemia-inhibitory factor (LIF), have been postulated to play a role in the etiology of cancer cachexia. Such cytokines may be produced by tumor or host tissue and are characterized by the induction of anorexia and a decrease in the clearing enzyme lipoprotein lipase. The ability to inhibit lipoprotein lipase varies among the cytokines. Thus, while LIF is twofold to 10-fold less potent than TNF- α , it is 100 times more potent than IL-6 (59). However, it is unlikely that a decrease in lipoprotein lipase alone could account for the fat cell depletion and wasting seen in cachexia, since in type 1 hyperlipidemia caused by an inherited deficiency in lipoprotein lipase, patients have normal fat stores and are not cachectic. This fact, together with the inability of the cytokines to explain all of the metabolic changes associated with cancer cachexia, has inspired investigators to search for tumor-produced catabolic factors that act directly on adipose tissue and skeletal muscle initiating the process of cachexia.

Tumor Necrosis Factor- α (TNF- α)

TNF- α was suggested as a possible cachectic factor as a result of studies on the mechanism of the weight loss observed in rabbits chronically infected with *Trypanosoma brucei brucei*. Administration of TNF- α to laboratory animals induces a state of cachexia, with anorexia and depletion of adipose tissue and lean body mass. However, tachyphylaxis rapidly develops following administration of additional TNF- α (60). Tachyphylaxis was not found to develop in immunodeficient nude mice that were given an injection of Chinese hamster ovary (CHO) cells transfected with the human TNF- α gene (61). These cells induced high serum levels of TNF- α (1.0–22.8 ng/mL) in the mice and a syndrome resembling cancer cachexia with progressive wasting, anorexia, and early death. It is interesting that, when

such cells were transplanted intracerebrally, hypophagia and weight loss were observed, and the body composition was comparable to that seen in starvation, i.e., a decrease in whole-body lipid but conservation of protein (62). When the tumor cells were transplanted intramuscularly, profound anorexia did not develop; after a long period of tumor burden (50 days), however, cachexia developed and both protein and lipid were depleted.

In addition to inhibition of lipoprotein lipase (59), incubation of cultured human fat cells with TNF- α has been shown to induce a marked dose-dependent stimulation of lipolysis by up to 400% of control values; this stimulation became apparent after a 6-hour exposure at the earliest (63). TNF- α has been shown in several studies (64–68) to activate muscle protein degradation, although not all of the reports agree that TNF- α does activate protein degradation directly. In one of the studies (65), TNF- α administration to healthy (i.e., cancer-free) rats brought about an enhanced rate of degradation of skeletal muscle protein, even though body weight loss was not apparent in the animals. In another of the studies (68), administration of anti-murine TNF- α immunoglobulin to rats bearing the Yoshida AH-130 ascites hepatoma led to decreases in the rates of protein degradation in the skeletal muscle, heart, and liver tissues, but it had no effect on weight loss in the animals. However, a direct action of TNF- α has not been demonstrated by most authors, when either tyrosine or 3-methylhistidine was used as a measure of the proteolytic rate (69). Despite this result, a recent report (67) has shown an increase in ubiquitin gene expression, with no change in the expression of the C8 proteasome subunit after incubation with TNF- α for 180 minutes *in vitro*. These results show that TNF- α has the potential to act as a modulator of the cachectic process.

Some animal tumors are thought to produce the cachectic syndrome through the mediation of TNF- α . The Yoshida AH-130 ascites hepatoma induces weight loss in the host, and elevated endogenous circulating TNF- α and prostaglandin E₂ levels are observed (68). Administration of anti-TNF- α antibody before transplantation of the tumor abolished detectable TNF- α levels, while fractional rates of protein degradation in gastrocnemius muscle, heart, and liver were reduced, although treatment failed to prevent a reduction in body weight. A similar result was obtained in mice with methylcholanthrene-induced sarcomas, where anti-TNF- α monoclonal antibody delayed but did not prevent the development of anorexia and had no effect on overall body weight (70). In another model, the Lewis lung adenocarcinoma anti-TNF- α antibody partially reversed the loss of body fat, without affecting food intake or causing a change in body weight (70). This finding suggests that TNF- α may not be responsible for the changes seen in animals or humans with cancer cachexia.

Similar problems have been encountered in clinical studies on the role of TNF- α in cancer cachexia. While a short intravenous infusion of recombinant human TNF- α increased plasma triglyceride levels, glycerol turnover by more than 80% and free fatty acid turnover by more than 60% (71,72), in the case of long-term administration, the changes resolved despite the continuous administration of TNF- α (71). Similar effects were seen on protein metabolism, where whole-body protein turnover was increased as measured by ¹⁵N enrichment of urinary urea and ammonia (71). Thus, TNF- α has the potential to induce catabolism of

adipose tissue and skeletal muscle in humans, although anorexia has not been reported to be a major dose-limiting toxicity. Several studies have failed to detect elevated circulating levels of TNF- α in cachectic cancer patients (73,74) or have failed to associate the elevation with the development of cachexia (75). Elevation of serum TNF- α levels seems to be associated more with the clinical status of the patient. Thus, in a study of 91 patients with B-cell chronic lymphocytic leukemia, serum levels of TNF- α were elevated in all stages of the disease with a progressive increase in relation to the stage (76). TNF- α levels were found to be substantially higher in patients with endometrial carcinoma than in healthy postmenopausal women or in women with endometrial hyperplasia, and serum TNF- α levels were associated with advancing stage of disease (77). Increased serum levels of TNF- α receptors were found in a range of solid tumor types, and the incidence and extent of the increase also were associated with the staging of the disease (78).

The inability to associate serum levels of TNF- α with the development of cachexia may be due to the very rapid blood transit of cytokines, so that they can be transported from the sites of production to the target tissues without causing an elevated serum concentration. However, elevated concentrations of TNF- α have been reported in patients with both malaria (79) and visceral leishmaniasis (80), which has been associated with death and cachexia. In addition, although animal experiments have shown TNF- α to be a potent inhibitor of lipoprotein lipase (59), there appears to be no difference between cancer patients and control subjects in either the total lipoprotein lipase activity or the relative levels of the messenger RNA (mRNA) for lipoprotein lipase and fatty acid synthesis (74), thus raising a question of a local effect for this cytokine. In rats infected with *Escherichia coli*, pentoxifylline (a potent inhibitor of TNF- α secretion) decreased the anorexia, loss of body weight, and muscle protein observed and partially prevented the decrease in muscle protein synthesis induced by infection (81). However, in a clinical study (82), pentoxifylline failed to reduce anorexia or cachexia in 35 patients with cancer.

Interleukin 6 (IL-6)

A potential role for IL-6 in the development of cancer cachexia has mainly been provided from animal studies involving the use of the murine colon-26 adenocarcinoma model (83-85), in which increasing levels of IL-6 appear to lead to the development of cachexia (83). In addition, the administration of anti-mouse IL-6 monoclonal antibody, but not of anti-mouse TNF- α monoclonal antibody, attenuated the development of weight loss and other parameters of cachexia in the mice (83). In another study (84) in which clonal variants of the colon-26 tumor model were used, the serum concentrations of IL-6 in mice bearing a tumor clone that does not induce weight loss were lower than in mice bearing a tumor clone that does induce weight loss; however, infusion of IL-6 into mice in the former group did not lead to body weight loss. These results indicate that IL-6 was not solely responsible for the induction of cachexia. Suramin, a polysulfonated naphthylurea, has been shown to inhibit the binding of IL-6 to cell surface receptor subunits, and it has been shown to partially block cachexia in the colon-26 model, without a decrease in tumor burden (85). Since anti-IL-6 antibody treatment did not enhance the effect, this result suggests that suramin

inhibits cachexia, at least in part, by interfering with the binding of IL-6 to its receptor. IL-6 has also been identified as a mediator of cachexia by the growth of a uterine cervical carcinoma called Yomoto in nude mice (86). Administration of a neutralizing antibody against human IL-6 to mice, after the development of cachexia, was shown to reduce the loss of body weight and the wasting of adipose tissue (86).

Agents that regulate cytokines, such as interleukin 12 (IL-12), reduced the serum levels of IL-6 in mice bearing the murine colon-26 carcinoma and alleviated the loss of body weight, adipose tissue wasting, and hypoglycemia associated with cachexia (87). IL-12 also substantially increased IFN γ levels in the tumor, and IFN γ administered intraperitoneally also prevented the cachexia, although it did not reduce IL-6 levels. It is interesting that IFN γ has been reported to be a possible mediator of cachexia in other animal model systems (88). Interleukin 10 (IL-10) was originally identified as a cytokine synthesis inhibitory factor. A reduction in serum IL-6 levels was observed in mice bearing the colon-26 tumor transfected with the IL-10 gene, although these levels did not reach baseline values and such mice did not develop cachexia (89). Since cachexia was completely prevented with an incomplete reduction of serum IL-6 levels, the authors suggested that an additional unknown factor was responsible for the development of cachexia in this model. This suggestion was also made in another study (90) on mice bearing the colon-26 tumor. An antibody to the IL-6 receptor reduced the loss of weight of the gastrocnemius muscle and suppressed the enzymatic activity of cathepsins B and L and mRNA levels of cathepsin L and poly-ubiquitin, but it had no effect on the overall loss of body weight or wasting of adipose tissue, suggesting that the latter two may not be influenced by IL-6.

Certainly, IL-6 has the potential to act as a cachectic factor. Atrophy of muscles is observed in IL-6 transgenic mice; this atrophy is completely blocked by anti-mouse IL-6 receptor antibody (91). The muscle atrophy is associated with increased mRNA levels for cathepsins (B and L) and mRNA levels of ubiquitins (poly- and mono-). Administration of IL-6 to rats has also been shown to acutely activate both total and myofibrillar protein degradation in skeletal muscle (92). In an *in vitro* study (93) using murine C₂C₁₂ myotubes grown in cell culture, exposure of the cells to 100 U/mL recombinant human IL-6 was found to shorten the half-life of long-lived proteins and to increase the activity of the 26S proteasome and lysosomal (cathepsins B and L) proteolytic pathways. This result suggests that IL-6 is capable of directly up-regulating pathways of protein degradation.

Unlike the results obtained with TNF- α , statistically significant increases in IL-6 and C-reactive protein, as a measure of the acute-phase response, have been found in weight-losing patients with non-small-cell lung cancer, when compared with patients with the same tumor, but without weight loss (94). An elevated level of serum IL-6 has also been reported in patients with colon cancer and an acute-phase response (95); however, since all patients had lost weight, it is difficult to associate this elevation with the induction of cachexia.

The results of these animal and human studies strongly implicate IL-6 in the cachectic process. However, IL-6 probably

does not act alone but may either induce or act in synergy with other cachectic factors.

Interleukin 1 (IL-1)

IL-1 has been shown to have many effects similar to those of TNF- α ; these similar effects include suppression of lipoprotein lipase and enhancement of intracellular lipolysis. Administration of recombinant IL-1 was observed to induce anorexia, weight loss, hypoalbuminemia, and elevated amyloid P levels in the mouse (96). Indeed, this cytokine was observed to have a greater anorexigenic effect than TNF- α when administered in isomolar quantities. However, in the MCG 101 sarcoma tumor model of cachexia in mice, IL-1 mRNA was present at low levels as determined by northern blot analysis in the spleen, liver, intestine, and brain and at elevated levels in the spleen (97). IL-1 and TNF- α protein were both detected in tumor tissue, but at levels similar to those in normal tissue. Neutralizing antibodies against the IL-1 receptor were observed to cause statistically significant, but minor, inhibitory effects on cachexia and anorexia in this model (98). Administration of the IL-1 receptor antagonist to rats bearing the Yoshida ascites hepatoma was also found to be completely ineffective in preventing tissue depletion and protein hypercatabolism (99). Transfection of a cachectic tumor cell line (colon-26) with the gene for the IL-1 receptor antagonist also failed to abolish the capacity of the tumor to produce cachexia (100). These results cast doubt on a role of this cytokine in the induction of tissue wasting in cancer cachexia.

Interferon Gamma (IFN γ)

Interest in the role of IFN γ in the pathogenesis of cancer cachexia developed as a result of observations confirming that it had properties similar to those of TNF- α with respect to fat metabolism *in vitro* (101). Weight loss in mice bearing the Lewis lung tumor is associated with IFN γ production, and administration of an anti-IFN γ antibody reduced the depletion of body fat but had no effect on total body protein (102). In rats that had received transplants of the MCG 101 sarcoma, anti-IFN γ antibody reduced weight loss and anorexia and increased survival, but the treatment was partial and short-lived, suggesting that IFN γ may not be the sole mediator (103). Inoculation of CHO cells transfected with the IFN γ gene into mice resulted in a dose-related development of anorexia and marked weight loss due to fat and muscle depletion, not wholly attributable to the reduction in food intake (104). Such a result should not be interpreted to mean that IFN γ by itself can induce cachexia, since both IFN γ release and the presence of the tumor cells were found to be required. While serum TNF- α levels of patients with multiple myeloma did not differ from those found in healthy control subjects, IFN γ was found to be raised in 53% of the patients (105). However, no association was observed between the level of IFN γ and clinical parameters of the disease. These results suggest that IFN γ alone may not be responsible for the induction of cachexia.

Leukemia-Inhibitory Factor (LIF)

LIF has also been suggested to play a role in the cancer cachexia syndrome through its ability to decrease lipoprotein lipase activity. Nude mice implanted with the human cell line

SEKI, which expresses large amounts of LIF, develop a severe cachexia (106). LIF mRNA has also been shown to be present in two types of melanoma xenograft that induce weight loss in transplanted animals, whereas none was detected in non-cachexia-inducing xenografts (106). Although inhibition of lipoprotein lipase has been suggested to account for the cachectic effect of LIF, it is unlikely that a decrease in lipoprotein lipase alone could account for the fat cell depletion, and no mechanism has been proposed to explain skeletal muscle catabolism. It seems unlikely that any of the cytokines alone are able to explain the complex mechanism of wasting seen in cancer cachexia, and other factors must be involved. This view is substantiated in a study of the factors responsible for the cachectic syndrome in nude mice bearing human tumor xenografts (107). In four of the eight models, a cytokine such as LIF or IL-6 produced by the cancer cells may be responsible; however, in the remaining four cancer cell lines, the inducing factor was unknown. In these cases, the inducing factors may be catabolic factors, which act directly on host tissues.

Lipid-Mobilizing Factors (LMFs)

LMFs act directly on adipose tissue with the release of free fatty acid and glycerol in a manner similar to that of the lipolytic hormones. Like the induction by hormones, the induction of lipolysis is associated with an elevation of intracellular cyclic adenosine monophosphate (108), possibly as a result of stimulation of adenylate cyclase. Evidence for the production by tumors of an LMF was provided by Costa and Holland (109), who showed that nonviable preparations of the Krebs-2 carcinoma, when injected into mice, were able to induce the early, rapid stage of fat depletion, which represented true cachexia in this model. Ascites serum from rats transplanted with the Walker 256 carcinoma increased stimulation of lipolysis in an *in vitro* assay (110), whereas serum from mice bearing a thymic lymphoma when injected into non-tumor-bearing controls produced massive fat loss (111), providing further evidence for an LMF. This latter factor was also detected in extracts of the tumor, in tissue culture medium, and in the sera of patients with adenocarcinomas of the cervix and stomach, thus suggesting that the LMF was tumor derived and circulatory. Other studies have shown that the level of LMF in the sera of cancer patients was proportional to the extent of weight loss (112) and was reduced in patients responding to chemotherapy (113).

Most studies provide evidence that the LMF is an acidic protein, although there appears to be variations in the molecular weight. A heat-stable protein of molecular mass around 5 kd was isolated from a thymic lymphoma (114), and another heat-stable protein of molecular mass 6 kd was isolated from the conditioned medium of the A375 human melanoma cell line (115). A heat-labile material of molecular mass between 65 kd and 75 kd was isolated from the ascites fluid of patients with hepatoma and mice with sarcoma 180 (116). Tryptic digestion of the active material produced a low-molecular-weight material that was still active.

These studies provide strong evidence for the production of LMF by tumors. The production of this material appears to be related to the process of cachexia, since LMF is absent or present in reduced amounts in tumors that do not induce cachexia (117) and is absent from normal serum, even under conditions of star-

vation (118). No report to date has provided sequence information on these LMFs; therefore, further studies are required.

Protein-Mobilizing Factors (PMFs)

Using bioassays to detect protein degradation, investigators have found evidence for the existence of PMF(s) in the sera of both animals (119) and humans (120) with cancer cachexia. The bioactivity appears to be associated with the loss of skeletal muscle mass and is absent from the sera of healthy control subjects. This material has now been purified from a cachexia-inducing murine tumor (MAC16) and from the urine of patients with cancer cachexia by use of affinity chromatography with a monoclonal antibody derived from mice bearing the MAC16 tumor (121). The PMF from both murine and human sources appeared to be identical and was characterized as a sulfated glycoprotein of a molecular mass of 24 kd and of unique amino acid sequence (122,123). Although the PMF was readily detected in the urine of cachectic cancer patients, irrespective of the tumor type, it was absent from the urine of cancer patients with little or no weight loss, from the urine of normal subjects, or from the urine of patients with weight loss due to trauma or sepsis (122). When the PMF was injected into non-tumor-bearing mice, rapid weight loss (about 10% in 24 hours) was observed, without a reduction in food and water intake, and body composition analysis showed selective depletion of the lean body mass. Evidence for a direct effect of the PMF was provided by the induction of protein degradation in isolated gastrocnemius muscles (121). The conservation in structure of this material between murine and human sources suggests that production of a PMF may be important in the growth and survival of some tumors.

Treatment of Cancer Cachexia

Weight loss is associated with psychologic distress and a lower quality of life. In addition, patients with weight loss have a shorter survival time and a decrease in response to therapy (124). About half of all patients with cancer show some weight loss (124), but those with pancreatic cancer show it at the highest frequency (125); in the latter study, the investigators found that all patients at the time of diagnosis had lost weight (median, 14.2% of pre-illness stable weight), and this weight loss was progressive, increasing to a median of 24.5% just before death. Patients with more than 15% weight loss are likely to have substantial loss of total body protein, and at this level of lean tissue depletion, physiologic function (e.g., respiratory muscle function) is markedly impaired (126). Thus, such patients need effective therapy if death from cachexia is not to occur.

As previously discussed, nutritional support in the form of total parenteral nutrition has failed to replete lean body mass. Even worse, a meta-analysis of the published trials on patients receiving total parenteral nutrition while undergoing chemotherapy showed a decreased survival, a poorer tumor response, and a significantly significant increase in infectious complications (127).

An improvement in appetite alone does not fully reverse the cachectic syndrome. Thus, patients with advanced malignant disease receiving medroxyprogesterone acetate (100 mg taken orally three times a day) showed a great improvement in appe-

tite, but this effect did not result in weight gain or an improvement in performance status, energy levels, mood, or relief from pain (128). Results with the appetite stimulant megestrol acetate look more promising in terms of weight gain. A number of clinical studies (129,130,131) have been performed, all of which report an increase in appetite and weight gains of up to 6.8 kg over baseline values in 16% of patients treated. However, body composition analysis, as determined by use of dual-energy x-ray absorptiometry and tritiated body water methodologies measured at the time of maximum weight gain, showed that the majority of patients gained weight from an increase in adipose tissue, while an increase in body fluid was responsible for a small portion of the weight gained (131). An increase in lean body mass was not observed. Such body composition changes are similar to those observed in patients receiving total parenteral nutrition (7).

Pharmacologic approaches to the treatment of cancer cachexia have been more successful. Hydrazine sulfate, an agent that inhibits the enzyme phosphoenolpyruvate carboxykinase, has been demonstrated to favorably influence the abnormal glucose and protein metabolism in cachectic cancer patients (132) and to maintain or even increase body weight (133). Ibuprofen, a cyclooxygenase inhibitor, has been shown to reduce the resting energy expenditure in patients with pancreatic cancer, suggesting that it may have a role in abrogating the catabolic processes that contribute to weight loss (134). Serum C-reactive protein levels were also reduced. The polyunsaturated fatty acid eicosapentaenoic acid, another cyclooxygenase inhibitor, has also been shown to counteract the weight loss in patients with pancreatic cancer with stabilization of protein and fat reserves (135). This result was accompanied by a temporary reduction in acute-phase protein production and stabilization of resting energy expenditure. The effect appears to be specific for eicosapentaenoic acid, since patients receiving a related polyunsaturated fatty acid, gammalinolenic acid, continued to lose weight. A similar structure-activity relationship was observed in mice with cachexia induced by the MAC16 tumor (136). Eicosapentaenoic acid appears to act by attenuating the action of catabolic factors in cachexia. Induction of lipolysis by an LMF was inhibited by eicosapentaenoic acid, and the effect appeared to be due to prevention of the rise in adipocyte cyclic adenosine monophosphate levels (108). Administration of eicosapentaenoic acid also led to statistically significant reductions in protein degradation *in vivo* (136), possibly as a result of the ability to inhibit prostaglandin E₂ production in skeletal muscle by a PMF (119).

Summary

Although cancer cachexia superficially resembles starvation, nutritional intervention alone is unable to reverse the condition. Tremendous progress has been made in the last 10 years in elucidating the role of various factors in host tissue catabolism, and the results of these studies are now being translated into treatment regimens for the benefit of patients with cachexia. Cachexia is an important cause of mortality in cancer patients, accounting directly for between 10% (137) and 22% (138) of all cancer deaths, as well as death from other causes such as infection. Thus, an effective therapy for cachexia not only should improve the quality of life of cancer patients, but also may be

expected to extend the survival time. In addition, since some tumors may depend on the products from host tissue catabolism for survival, such therapy may also have an antitumor effect. Considering that cachexia is common in those cancers for which therapy is currently limited, this could prove to be of great clinical benefit.

Appendix: Methodology

Purpose

Our goal was to review the metabolic processes that contribute to cancer cachexia-related tissue wasting and to critically assess the role of cytokines and catabolic factors as mediators of these processes; studies of this condition in humans were emphasized as much as possible.

Information Source

Our sources of information were Medline (National Library of Medicine, Bethesda, MD), *Current Contents* (Institute for Scientific Information, Philadelphia, PA), and a large reprint file built up over 15 years. No study has been excluded on the basis of the date of publication if the results are still appropriate, although emphasis has been placed on the most recent studies whenever possible. Where several authors report the same result, only one study is included, usually the first published.

Criteria for Evaluating Validity

Data from studies on humans rather than on experimental animals have been used whenever possible, particularly if results from the latter contradict the human evidence. Results from animal experiments that involved use of models not appropriate to the human condition have been excluded; these included studies of tumors that are rapidly growing or where the weight of the tumor was large in relation to the weight of the animal. Since cachexia is an *in vivo* phenomenon, priority was given to studies in which whole animals were used.

Methods for Summarizing Evidence

There was insufficient data in this area to warrant highly structured quantitative techniques, and a simple narrative approach has been used to summarize the evidence.

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Note

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