Mechanisms of Cancer Cachexia

MICHAEL J. TISDALE

Nutritional Biomedicine, School of Life and Health Sciences, Aston University, Birmingham, United Kingdom

I. Introduction 382
II. Energy Balance in Cachexia 382
   A. Anorexia 383
   B. Causes of anorexia: role of neuropeptides 384
   C. Energy expenditure 384
   D. Role of futile cycles 385
III. Adipose Tissue 386
   A. Normal control of lipogenesis and lipolysis 386
   B. Changes in adipose tissue in cachexia 387
IV. Tumor and Host Factors Influencing Adipose Mass in Cachexia 387
   A. Lipid mobilizing factor/zinc α2-glycoprotein 387
   B. Tumor necrosis factor-α 389
   C. Interleukins 1 and 6 and interferon-γ 390
V. Skeletal Muscle 391
   A. Control of protein synthesis in normal and cachectic states 391
   B. Protein degradation in cachexia 392
   C. Apoptosis in skeletal muscle 394
VI. Tumor and Host Factors Influencing Muscle Mass in Cachexia 394
   A. Proteolysis-inducing factor 394
   B. Glucocorticoids 397
   C. Tumor necrosis factor-α 398
   D. Interleukin-6 399
   E. Angiotensin II 399
VII. Treatment of Cachexia 400
   A. Agents affecting appetite 400
   B. Agents affecting cachectic mediators or signaling pathways 401
VII. Conclusions 402
I. INTRODUCTION

Cachexia is a multifactorial syndrome characterized by progressive loss of body weight, often, but not always, accompanied by anorexia (24). It includes all of the effects of the tumor on the host, which are not a direct result of mechanical interference with major organs. Cancer therapies, including surgery, chemotherapy, and radiotherapy, also induce anorexia and further weight loss (255), but the mechanism by which this occurs is likely to be different from that found in cancer cachexia. Depending on the tumor type, weight loss occurs in 30–80% of cancer patients and is severe (with loss of >10% of the initial body weight) in 15% (53). Patients with pancreatic or gastric cancer have the highest frequency of weight loss, while patients with non-Hodgkin’s lymphoma, breast cancer, acute nonlymphocytic leukemia, and sarcomas have the lowest frequency of weight loss (53). Although certain tumor types are more commonly associated with cachexia, even with the same tumor type there are variations in the extent to which patients exhibit cachexia. Thus, in pancreatic cancer, 85% of patients become cachectic, but 15% do not. This is due to variations in tumor phenotype (186), or host genotype, which contribute to the development of cachexia. In patients with pancreatic cancer, weight loss is a presenting symptom with a median weight loss of 14.2% of their illness stable weight (281) (Fig. 1). This weight loss is progressive over the next 6 mo, increasing to a median of 24.5% at the last assessment before death (Fig. 1).

Weight loss is an important prognostic factor in cancer; the higher the extent of weight loss, the shorter the survival time. The prognostic effect of weight loss is greatest in patients with a more favorable prognosis (53). A study of 109 disease-free breast cancer patients in stage II node positive, and stage III disease, showed unexplained body weight loss in 84% of patients developing recurrence, compared with 10% of patients remaining disease free (172). Weight loss as a symptom of lung cancer also predicts for treatment toxicity as well as a short survival time (216).

Weight loss in cancer patients is due to depletion of both adipose tissue and skeletal muscle mass, while the nonmuscle protein compartment is relatively preserved, thus distinguishing cachexia from simple starvation (77). The loss of both adipose tissue and skeletal muscle mass can be extensive, as illustrated in Table 1, which shows data on body composition of lung cancer patients who had lost 32% of their preillness stable weight, compared with a group of controls matched for age, sex, height, and preillness stable weight of the cancer patients. Although the overall weight loss was 32%, the cachectic patients had lost 85% of their total body fat and 75% of their skeletal muscle, and there was also a significant decrease in mineral content, suggesting erosion of bone. This marked loss of skeletal muscle explains why patients with cachexia have a reduced mobility, and thus quality of life, together with a shorter life span, since loss of respiratory muscle function will lead to death from hypostatic pneumonia (283). Death of patients occurs with 25–30% total body weight loss (281). A similar situation is found in patients with acquired immunodeficiency syndrome (AIDS), where death is imminent when they have lost 34% of their ideal body weight (140). Respiratory failure has been found to be responsible for the death of 48% of cancer patients (109).

To effectively treat patients with cachexia, it is important to understand the mechanisms leading to progressive tissue wasting. In addition, it is important to understand the role of tumor and host factors in the wasting process. Over the past 10 years, considerable information has emerged on mechanisms of cancer cachexia, so it is timely to review progress to date, and how this may influence future therapy. This review will cover factors regulating energy balance in cachexia (sect. II), normal control of adipose and skeletal muscle mass and changes in cachexia (sects. III and V), as well as tumor and host factors influencing the mass of adipose tissue (sect. IV) and skeletal muscle (sect. VI). In conclusion, the effectiveness and mechanism of action of agents affecting appetite, as well as agents affecting cachectic mediators, or signaling pathways will be discussed. The review should be comprehensible to those with an understanding of biochemistry and physiology.

II. ENERGY BALANCE IN CACHEXIA

Body mass is controlled by the balance of energy intake and energy expenditure, like all thermodynamic
Anorexia, defined as the loss of the desire to eat, is common in cancer patients. A study of 66 cancer patients nearing the end of life showed that 61% had anorexia despite the fact that they were not receiving chemotherapy (262). This suggests that anorexia can be produced by the tumor independently of that produced by treatment, which is reversible when the treatment is terminated. Early satiety is often reported by anorectic cancer patients, such that they feel full after ingestion of a small amount of food. This may be the result of an encroachment by the tumor on the gastrointestinal tract, which may hinder the passage of food. In addition, tumors may produce abnormalities in the mucosa resulting in malabsorption (139).

Although anorexia frequently accompanies cachexia, there does not appear to be a cause-effect relationship between the two. A study of 297 unselected cancer patients with solid tumors found that weight loss could not be accounted for by a diminished dietary intake, since the absolute amounts of energy intake did not differ, and the intake per kilogram of body weight was actually higher in the weight-losing patients compared with the weight-stable patients (24). Both dietary intake of energy and protein were decreased, although the micronutrient composition was not changed. However, weight loss was not compensated for by an increase in spontaneous food intake. Animal experiments have also shown that pair-feeding does not lead either to the same extent of weight loss or the metabolic abnormalities seen in tumor-bearing animals. In fact, the changes in body composition seen in cachexia resemble those found in infection and injury rather than those in starvation (250). The body composition changes in cachexia also differ from that in anorexia, where most of the weight is lost from fat, and only a small amount from muscle (185), while in cachexia there is equal loss of fat and muscle (77). Also in anorexia nervosa, loss of visceral mass occurs in proportion to loss of muscle mass, while in cachexia visceral protein is conserved, and may even increase (77). During prolonged starvation, ketone bodies derived from metabolism of fat in the liver replace glucose as an energy source for the brain, thus preventing loss of muscle through glucogenesis from amino acids, while in cachexia this does not happen, probably because the energy demands on the host are sufficiently high to prevent the build up of acetyl CoA in the liver and its subsequent conversion to acetoacetate and β-hydroxybutyrate (74). Clinical studies have also shown that it is not possible to reverse the wasting process in cancer patients by nutritional supplementation. These studies include dietary counseling (198), total parenteral nutrition (TPN) (69) or appetite stimulants such as cryoheptadine (127), a histamine antagonist with antiserotonergic and appetite stimulatory effects, or dronabinol, the active ingredient in marijuana (267). Moreover, with TPN, any weight gain is transient, and body composition analysis shows that this is fat and water rather than lean body mass (69). A similar situation is seen in patients with human immunodeficiency virus (HIV) (140) or sepsis (250). In contrast, malnutrition resulting from therapy does respond to nutritional supplementation (112). Thus patients receiving radiotherapy to the gastrointestinal tract, or head and neck, who received intensive, individualized nutrition counseling by a dietician showed smaller deteriorations in body weight, nutrition status, and quality of life compared with those receiving the usual care. Also in patients with pancreatic cancer, although nutritional supplementation is unable to reverse the loss of body weight, there is a relationship between calorie intake and survival (196). Thus survival was found to be significantly longer for the high-calorie intake groups compared with the low (50 vs. 32 days). A recent study (76) identified a reduced food intake (>1,500 kcal/day), together with weight loss (10% or greater), and a systemic inflammatory response [C-reactive protein (CRP), 10 mg/l or higher] to be important variables to identify cancer patients with both adverse function and prognosis, while weight loss alone was not a prognostic variable.

These results suggest that anorexia is an important component of cachexia, although, alone, it may not be directly responsible for the loss of body mass, especially skeletal muscle. Feeding is an important social interaction, and something in which the family feel that they can help. Before treatment can be initiated, it is important to understand the underlying cause of anorexia in cancer patients.

### Table 1. Comparison of body composition of cachectic cancer patients with normal controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal, kg</th>
<th>Cachectic, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body weight</td>
<td>65.6</td>
<td>44.9</td>
</tr>
<tr>
<td>Total fat</td>
<td>17.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Muscle protein</td>
<td>2.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Nonmuscle protein</td>
<td>8.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Intracellular water</td>
<td>19.1</td>
<td>12.9</td>
</tr>
<tr>
<td>Extracellular water</td>
<td>15.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Minerals</td>
<td>3.0</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Data from Fearon (77).
B. Causes of Anorexia: Role of Neuropeptides

In addition to any effects of the tumor on the gastrointestinal tract and psychological depression, patients with cancer frequently have a decreased taste and smell of food, resulting in increased sweet and bitter thresholds (52). Release of chemicals by the tumor, or the host immune system, may also induce anorexia. Many cytokines have an effect on appetite, including interleukin (IL)-1α, IL-1β, and IL-6 as well as tumor necrosis factor-α (TNF-α) (204). The cytokines are transported across the blood-brain barrier where they interact with the luminal surface of brain endothelial cells to release substances that affect appetite (10). Receptors for TNF-α and IL-1 are found in the hypothalamic areas of the brain, which regulate food intake. Anorexia induced by both TNF-α and IL-6 can be blocked by inhibitors of cyclooxygenase, suggesting that a prostaglandin (PG), such as PGE2, may be the direct mediator of appetite suppression (102).

Cancer anorexia may be a result of an imbalance between orexigenic signals, such as neuropeptide Y (NPY), and anorexigenic signals, such as proopiomelanocortin (POMC), which favors the latter (49). NPY neurons increase parasympathetic output and decrease resting energy expenditure, whereas POMC stimulates sympathetic activity and increases resting energy expenditure. In rats bearing a methylcholanthrene-induced sarcoma, intrahypothalamic injection of NPY was less potent in stimulating feeding than in control animals (40). This effect was observed prior to the onset of anorexia and became more severe as anorexia developed. The level or release of NPY in the paraventricular nucleus (PVN), or hypothalamus, was also found to be reduced in tumor-bearing rats, while it was increased in both fasting animals and those restricted to the same food intake as tumor-bearing animals (39). Mice bearing the MAC16 tumor develop cachexia without a drop in food intake, but food intake is not increased to suppress the drop in body weight. In these animals NPY expression is regulated appropriately in response to fat depletion, suggesting that tumor products may inhibit NPY transport, or release, or interfere with neuronal targets downstream of NPY (22). In anorectic cancer patients, NPY levels were found to be lower than controls, which correlated with the extent of anorexia (120). Leptin plays a contributing role in the control of body fat stores by inhibiting food intake and increasing energy expenditure through a feedback loop involving the hypothalamus. Serum leptin concentration depends on the total amount of body fat. Thus as fat levels decrease in cachexia, leptin levels fall correspondingly and are inversely related to the intensity of the inflammatory response (6). Hypothalamic melanocortin, α-MSH, a product of POMC, is most strongly implicated in the control of normal food intake (73). α-MSH induces anorexia by activating two distinct melanocortin receptors, Mc3r and Mc4r, which are expressed in the hypothalamus and other brain regions. Increased CNS melanocortin signaling has been implicated in the pathogenesis of cancer anorexia, since a potent synthetic Mc3r and Mc4r antagonist, when administered into the third cerebral ventricle of rats anorexic from prostate cancer, increased food intake and caused a significant gain in body weight (284). Cachexia induced by lipopolysaccharide (LPS) and tumor growth was also ameliorated by central Mc4r blockage using Mc4r knock-out mice, or mice administered the Mc3r/Mc4r antagonist agouti-related peptide (173). However, in neither study was there body composition data to determine whether there was an increase in lean body mass, or whether the weight gain was due to an increase in water and fat, as with nutritional supplementation.

A transforming growth factor (TGF)-β superfamily member macrophage inhibitory cytokine-1 (MIC-1) has recently been implicated in the process of anorexia and weight loss in cancer patients (124). In patients with advanced prostatic cancer, there was a direct correlation between serum levels of MIC-1 and weight loss, while in mice transplanted with prostate tumor xenografts, there was a marked weight loss that was mediated through a decreased food intake. This is mediated through central mechanisms of which the hypothalamic TGF-β receptor II, ERK 1/2, STAT3, NPY, and POMC have been implicated.

Melanin concentrating hormone (MCH) is leptin sensitive and stimulates food intake (150). It works by binding the G protein-coupled receptors MCH1R and MCH2R in the brain. With the use of immunocytochemistry, a significant 1.6 times increase in the number of MCH1R was found in the infundibular nucleus in postmortem brain material of cachectic patients compared with matched controls (265). This is consistent with a function of MCH as an orexigenic neuropeptide in the human brain.

C. Energy Expenditure

An increased energy expenditure would also contribute to the wasting process. About 70% of the total energy expenditure in sedentary people arises from the resting energy expenditure (REE). The REE in cancer patients is strongly determined by the type of tumor. Thus REE is elevated in patients with both lung (82) and pancreatic cancer (71), while there is no increase in REE in patients with gastric and colorectal cancer (82). These observations may reflect how close the patients were to death at the time of measurement, since malnourished patients near death show an increased REE, which could relate to the utilization of the last skeletal muscle mass (214). In patients with pancreatic cancer, despite the increase in REE, the total energy expenditure (TEE) is reduced, due
to a reduction in the physical activity level (PAL) (190). The decrease in PAL reflects a lower quality of life. In patients with pancreatic cancer, REE is significantly higher in those with an elevated acute phase response (APR) (71). The APR is a series of changes in liver protein synthesis, which shifts from production of albumin to acute phase proteins (APP), such as CRP, fibrinogen, serum amyloid A, 2-macroglobulin, and α-1 antitrypsin, in response to tissue injury, infection, or inflammation. There is an association between the development of an APR and the rate of loss of body mass in lung and gastrointestinal cancers (181), and in patients with non-small-cell lung cancer (NSCLC), serum levels of IL-6 were found to correlate with concentrations of circulating CRP (180). This distinguishes loss of body mass in cancer from that in simple starvation. In pancreatic cancer, an elevated level of APP is associated with a shorter survival time (72).

One reason for an increased REE in some cancer patients may be due to an increased thermogenesis in brown adipose tissue (BAT), or skeletal muscle. BAT plays an important role in the control of both body temperature and energy balance in many mammals, including humans, but generally there is little BAT in adult humans. However, a single study in which autopsy samples of peri-adrenal tissue were examined by light microscopy showed BAT to be present in 80% of cachectic cancer patients, compared with only 13% of age matched controls (239). The thermogenic effect of BAT and skeletal muscle is due to the presence of uncoupling proteins (UCP), which mediate proton leakage across the inner mitochondrial membrane, thus decreasing the level of coupling of respiration to ADP phosphorylation. There are three UCPs: UCP1 found only in BAT, UCP2 found in most tissues, and UCP3 found only in BAT and skeletal muscle (211). Of the three, UCP1 is considered to be most important, although UCP3 may also play an important role in energy balance and lipid metabolism. UCP2 may be more important in the control of reactive oxygen species (ROS) produced by mitochondria (146). In mice bearing a cachexia-inducing tumor, levels of mRNA for UCP1 in BAT were significantly elevated over controls, while expression levels of UCP2 and -3 did not change in BAT, but were significantly increased in skeletal muscle (20). Similar results were obtained in rats with experimentally induced cancer cachexia (21). Troglitazone, a thiazolidinedione, which selectively activates peroxisome proliferator-activated receptor γ (PPARγ), strongly decreased UCP2 and -3 mRNA levels in murine myotubes (35), suggesting that PPARγ ligands could decrease energy expenditure in cachexia. This may also be applicable to cancer patients, since UCP3 mRNA levels were found to be five times higher in rectus abdominis muscle of cancer patients with weight loss compared with controls and cancer patients that had not lost weight (43). There was no significant difference in UCP2 mRNA levels between groups. It is suggested that the increase in UCP3 mRNA could enhance energy expenditure and contribute to tissue catabolism. The mechanism for the increase in levels in skeletal muscle is complex. In a rat model of cachexia, the increase in UCP was associated with a twofold increase in circulatory fatty acid, and reduction of hyperlipidemia with nicotinic acid also reduced UCP3 expression in soleus but not in gastrocnemius muscles (31). However, in a murine model of cachexia, the increased UCP2 and UCP3 gene expression in skeletal muscle was not linked to a rise in circulatory fatty acids (30).

There is evidence that some cytokines and tumor lipid mobilizing factors (LMF) can increase levels of UCP in both BAT and skeletal muscle.

D. Role of Futile Cycles

Most cancer cells use glycolysis as the principal method to generate ATP, and this phenomenon is called the Warburg effect (201). The increased glucose uptake by tumors is the basis of the [18F]fluorodeoxyglucose positron emission tomography (FDG-PET) tumor diagnostic method, which is based on the assumption that cancer tissue has a higher rate of glucose uptake than normal tissue (29). In addition, glycolytic inhibitors have been suggested as being useful to specifically target the slow-growing cells of a tumor, which would complement currently used chemotherapeutic agents and radiation, which target rapidly growing cells (151). Several reasons have been suggested to explain this phenomenon including dysfunctional mitochondria, which exhibit frequent mutations in the DNA (44) which would prevent their use of the tricarboxylic acid cycle, preventing the total combustion of pyruvic acid (123). Since mitochondrial DNA codes for 13 components of the respiratory chain, it is likely that such mutations would cause malfunctions in respiration. Indeed, respiration-deficient cells with deletions in mitochondrial DNA show an increased dependency on glycolysis, increased NADPH and activation of the Akt survival pathway, resistance to antitumor drugs, and a survival advantage in hypoxic conditions (202). Other alterations include overexpression of the “low $K_m$” form of hexokinase, type II hexokinase, due to gene demethylation, resulting in tumor glucose utilization at normal blood sugar levels (89), oncogenic signals, such as ras and src, which increase dependence on glucose (212), and tumor hypoxia due to growth beyond the vascular supply (299). Hypoxia activates a transcription factor called hypoxia-inducible factor 1 (HIF-1), which increases the transcription of the cell-surface glucose transporter GLUT1, and at least one isoform of nearly all the core enzymes of glycolysis (237) (Fig. 2). In addition, pyruvate dehydrogenase kinase (PDHK), which phosphorlates and inacti-
vates the pyruvate dehydrogenase (PDH) complex, which converts pyruvate into acetyl-CoA in mitochondria is activated by HIF-1 (134), causing the accumulation of pyruvate, which is then converted into lactate by another HIF-1 target lactate dehydrogenase (LDH) (28). The net result is the conversion of glucose into lactic acid, an energy-inefficient process, which means that growth of tumors requires ~40 times more glucose than if it was fully oxidized through the tricarboxylic acid cycle. In addition, the lactate passes from the tumor to the liver, where it is resynthesized into glucose, another energy-inefficient process. This process is known as the Cori cycle and requires 6 mol ATP to generate 1 mol glucose from 2 mol lactic acid. Significantly higher rates of glucose production and recycling were observed in weight-losing patients with metastatic colorectal cancer than in control subjects without cancer (107). The Cori cycle may account for an additional loss of energy in cancer patients of 300 kcal/day (60). In addition, tumor lactate levels have been found to positively correlate with the likelihood of metastasis and tumor recurrence and negatively with patient survival (268). Other substances also contribute to the increased gluconeogenesis in cancer patients, including glycerol released by hydrolysis of triglycerides in adipose tissue, and amino acids formed by breakdown of myofibrillar proteins in skeletal muscle. The increased hepatic glucose production is also partially due to a lack of inhibition of gluconeogenesis by insulin (292). Resistance to insulin occurs in patients with cancer and is negatively correlated with the APR, suggesting the involvement of inflammatory reactions (291). However, since translocation is not a consistent feature of lipolysis, a second model has been proposed whereby perilipin acts as a scaffold protein, with phosphorylation leading to an altered structure, and recruitment of HSL increasing lipase access to the lipid surface (95).

Another enzyme, adipose triglyceride lipase (ATGL), which specifically hydrolyzes long-chain FA TAG, has recently been described and is also considered to be rate-limiting for TAG catabolism at least in rodents. In humans, ATGL is of less importance than HSL in regulating catecholamine-induced lipolysis, but both lipases regulate basal lipolysis (228). PKA also phosphorylates perilipin (PLIN), which coats the intracellular lipid droplet, resulting in translocation away from the surface of the lipid droplet (154), and enables HSL to access the lipid surface for TAG hydrolysis. The G protein-coupled receptors can also activate mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase pathways (ERK). Activated ERK also increases lipolysis and phosphorylates HSL at Ser600, increasing its activity (96).

In addition to the Cori cycle, energy can also be lost by the esterification of nonesterified fatty acids (NEFA), released by lipolysis in adipose tissue, back into triacylglycerols (TAG). This is referred to as the TAG/FA substrate cycle. There have been few measurements of this cycle in cancer patients or animal tumor models. In one of the few studies in murine tumor-bearing animals, the rate of TAG/FA cycling was increased over that found in non-tumor-bearing animals, irrespective of the development of cachexia (16). However, cachectic animals did show an elevated de novo synthesis of TAG/FA.

III. ADIPOSE TISSUE

A. Normal Control of Lipogenesis and Lipolysis

FA are stored in adipose tissue as TAG and constitute 90% of adult fuel reserves. An enzyme lipoprotein lipase (LPL) hydrolyzes fatty acids from plasma lipoproteins, and these are transported into adipocytes for synthesis of TAG (Fig. 3). Lipolysis is mediated by hormones such as epinephrine, glucagon, and adrenocorticotropic hormone (ACTH), through a cAMP-mediated process. Hormone production of cAMP is stimulated as a consequence of GTP-binding protein (G protein)-coupled receptors, acting through adenylyl cyclase, which converts ATP into cAMP (122). cAMP activates a protein kinase (PKA), which in turn activates hormone-sensitive lipase (HSL), a key rate-limiting step in the conversion of one molecule of TAG into three molecules of NEFA and one molecule of glycerol. HSL is phosphorylated on several serine residues, but Ser659 and Ser660 are responsible for the increased activity (7). Phosphorylation of HSL triggers its translocation to the lipid droplet (61). However, since translocation is not a consistent feature of lipolysis, a second model has been proposed whereby perilipin acts as a scaffold protein, with phosphorylation leading to an altered structure, and recruitment of HSL increasing lipase access to the lipid surface (95).

Another enzyme, adipose triglyceride lipase (ATGL), which specifically hydrolyzes long-chain FA TAG, has recently been described and is also considered to be rate-limiting for TAG catabolism at least in rodents. In humans, ATGL is of less importance than HSL in regulating catecholamine-induced lipolysis, but both lipases regulate basal lipolysis (228). PKA also phosphorylates perilipin (PLIN), which coats the intracellular lipid droplet, resulting in translocation away from the surface of the lipid droplet (154), and enables HSL to access the lipid surface for TAG hydrolysis. The G protein-coupled receptors can also activate mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase pathways (ERK). Activated ERK also increases lipolysis and phosphorylates HSL at Ser600, increasing its activity (96).
Adipocyte differentiation is controlled by a number of transcription factors, which are activated in a sequential manner (168). The process starts with the transient activation of CCAAT/enhancer binding protein (C/EBP) β and δ, which in turn stimulate C/EBPα expression. This synergizes with PPARγ in controlling terminal differentiation, and this is enhanced by sterol regulatory element binding protein-1c (SREBP-1c), which activates transcription of PPARγ (70). Deficiency of C/EBPα leads to greatly reduced body fat in mice, with adipocytes accumulating less lipid and a complete absence of insulin-stimulated glucose transport.

B. Changes in Adipose Tissue in Cachexia

Loss of adipose tissue in cachexia is primarily due to an increased lipolysis, since there is an increased turnover of both glycerol and FFA compared with normal subjects or cancer patients without weight loss (238). Fasting plasma glycerol concentrations are also much higher in weight-losing cancer patients compared with weight-stable subjects, as are concentrations of NEFA and triglycerides, and they also show an increased sensitivity to the lipolytic effects of epinephrine (56). Lipolysis was found to be increased by 40% in patients in whom complete TAG hydrolysis without reesterification was observed, and there was a 20% increase in FFA oxidation (147). Adipocytes from cachectic subjects also show a two- to threefold increase in response to natriuretic peptide, which is attenuated by inhibition of HSL, but there is no increase in the basal lipolytic rate (5). Similar changes in response to lipolytic stimuli have also been observed in adipocytes from cachectic mice, and arise from an enhanced stimulation of adenylyl cyclase, due to an increased expression of the stimulatory G protein, Goαs, and a decrease in the inhibitory form, Goαi (114). Expression levels of HSL mRNA and protein are increased by 50 and 100%, respectively, in human adipocytes from cachectic subjects, while there is no change in total LPL, or the relative level of mRNA for LPL, although serum triglycerides and NEFA are elevated twofold (256). There is no effect of cachexia on ATGL expression, and expression of this enzyme does not correlate with lipolysis (5).

These changes lead to loss of body fat, which is lost more rapidly than lean tissue in progressive cancer cachexia (81). In cachectic mice, the adipocytes are shrunken and heterogeneous in size, and there is increased fibrosis in white adipose tissue (WAT) (21). There is extensive delipidation in adipocytes, and modifications in cell membrane conformation, with the mitochondria differing from typical WAT mitochondria, being electron dense, and with increased cristae. There are major reductions in the levels of both mRNA and protein of adipogenic transcription factors, including C/EBPα and -β, PPARγ, and SREBP-1c, while levels of UCP-2 increase (21). These changes suggest an impairment not only of the lipid storage function of adipocytes in cancer cachexia, but also in differentiation.

There may be changes in lipid metabolism in cancer patients even when there is no sign of overt cachexia. Thus, in patients with gynecological cancer, which is not generally considered to be associated with cachexia, there is an increased lipolysis promoting activity in the serum, which caused an elevation in the level of HSL in normal adipocytes (86). These results suggest that the changes in lipid metabolism seen in cancer cachexia are due to lipid mobilizing substances produced by the host or the tumor and present in the circulation. Early studies (194) used parabiotic transfer to establish the humoral transmission of a cachectic factor into a second animal, for which there was no evidence of metastasis, so potential cachectic factors should be present in the circulation.

IV. TUMOR AND HOST FACTORS INFLUENCING ADIPOSE MASS IN CACHExIA

A. Lipid Mobilizing Factor/Zinc α2-Glycoprotein

Costa and Holland (45) were the first to report in 1962 that nonviable preparations of Krebs-2 carcinoma cells were able to induce weight loss, and in particular fat depletion, in mice in a manner similar to viable preparations. This suggested that tumor metabolism alone was not responsible for the atrophy of adipose tissue. Further studies (138) showed that serum from lymphoma-bearing mice, when injected into normal mice, also produced an immediate fat mobilization. Purification of the LMF showed it to be a heat-stable protein of molecular mass 5 kDa, although later studies suggested a requirement for aggregation to a high-molecular-mass material for activity (137). Another LMF of molecular mass 6 kDa, which was acidic in nature, was isolated from the conditioned medium of the human melanoma cell line A375 (253). This material was also heat stable and resistant to degradation by proteolytic enzymes. A LMF of molecular mass 70–75 kDa, which was also acidic, and converted by trypsin into a low-molecular-weight material, which was still active, has been isolated from the ascites fluid of patients with hepatoma and mice with sarcoma 180 (176, 177). This material was called toxohormone L and was shown to induce anorexia when injected into mice.

Evaluation of the effect of human tumors in nude mice showed that depletion of carcass lipid was a function of tumor type, and not tumor burden, suggesting that some tumors produce LMF, which induces lipid depletion in cachexia (106). Such material was detectable by biossay and was present in the serum of cancer patients in levels proportional to the extent of weight loss (15). This
material was also acidic in nature, and the activity was found to decrease in patients responding to chemotherapy, but did not change if there was no response.

A LMF has been purified, both from a cachexia-inducing murine tumor (MAC16), and from the urine of cancer patients with weight loss, using a combination of ion exchange, exclusion, and hydrophobic interaction chromatographies (259). Both sources identified a material of apparent molecular mass 43 kDa, the amino acid sequence and immunoreactivity of which were identical to a known protein zinc α2-glycoprotein (ZAG). Only those tumors that produced a decrease in carcass lipid expressed mRNA for ZAG, and, like LMF, ZAG was shown to stimulate glycerol release from isolated murine epididymal adipocytes with a comparable dose-response profile (104). Both LMF and ZAG induced lipolysis by the classical cAMP-mediated mechanism (Fig. 3) through stimulation of adenylyl cyclase in a GTP-dependent process, and activation of HSL. In vivo studies showed LMF to decrease body weight of both exbreeder mice, with a 42% reduction in carcass lipid, and also ob/ob mice, with a 19% reduction in carcass fat, without a change in body water or nonfat mass, and without a change in food or water intake (103). A similar effect was produced by ZAG purified from normal human plasma (225). LMF produced similar increases in plasma glycerol to that found in cachexia, but NEFA levels were only elevated in ob/ob mice, suggesting an increased utilization, and this was confirmed by an increased oxygen uptake by BAT (104). In addition, LMF has been shown to increase overall lipid oxidation, as determined by the production of 14CO2 from [14C-carboxy]triolein (223). An increased oxidation of lipids has also been observed in mice bearing a cachexia-inducing tumor, as well as in cachectic cancer patients (147). The increased lipid oxidation is probably related to an increased expression of UCP1 in BAT induced by ZAG (226). This is likely to be mediated through a β3-adrenoreceptor, since both LMF and ZAG have been shown to stimulate adenylyl cyclase in murine WAT through a β3-adrenoreceptor (221, 226). Treatment of mice with the specific β3-adrenoreceptor agonist CL 316,243 has been shown to markedly increase the expression of UCP1 in BAT (293). In vitro studies have shown that ZAG was able to increase UCP1 expression directly in primary cultures of BAT and that this effect was attenuated by the β3-adrenoreceptor antagonist SR59230A (231). In addition, ZAG also increased UCP2 expression in murine myotubes through a β3-adrenoreceptor-mediated process, as well as a dose-dependent increase in UCP3, in a process requiring MAPK. LMF also increased UCP2 expression in tumor cells through a mechanism involving the β3-adrenoreceptor, and this appeared to be important in the detoxification of free radicals, since it antagonized the antiproliferative effect of chemotherapeutic agents working through a free radical mechanism (232). LMF has also been shown to increase the sensitivity of WAT to the lipolytic effects of catecholamines, as happens in adipocytes from patients with cancer cachexia (5), through an increased expression of Gaα, and a decreased expression of Gαi (114). Treatment of mice with LMF has also been shown to increase glucose utilization in brain, heart, BAT, and skeletal muscle, thus accounting for its ability to decrease blood glucose levels. LMF has also been shown to deplete liver glycogen through stimulation of hepatic adenylyl cyclase in a GTP-dependent manner (105).

Thus LMF/ZAG increase lipid mobilization, but also substrate utilization, by increasing mitochondrial oxidative pathways in BAT, and possibly also skeletal muscle. Since LMF/ZAG acts through a β-adrenoreceptor, and since β-agonists stimulate skeletal muscle hypertrophy in animals, it is not surprising that LMF has been shown to stimulate protein synthesis in murine myotubes through a cAMP-mediated process, as well as decrease protein degradation, by decreasing proteasome activity (115). The β2-adrenergic agonist formoterol has been shown to effectively reverse muscle wasting in tumor-bearing rats by decreasing protein degradation and increasing the rate of protein synthesis in skeletal muscle (33). The main anti-proteolytic effect was based on inhibition of the ubiquitin-proteasome pathway. These results suggest that LMF/ZAG may protect skeletal muscle from atrophy and explain why loss of fat precedes loss of protein in cachectic cancer patients (5).

In addition to expression by certain cachexia-inducing tumors, ZAG is also expressed in normal tissues, including lung, BAT, heart, and all types of WAT (19). Studies on the ontogeny of ZAG expression during postnatal development in the mouse suggest that it may be involved in the development of adipose tissue mass (264). ZAG is not only expressed, but also secreted by human adipocytes (11). In mice bearing the MAC16 tumor, and with a 61% decrease in fat mass, ZAG mRNA levels were

![Fig. 3. Mechanisms for controlling triglyceride (TG) content of adipocytes by lipid mobilizing factor (LMF)/zinc α2-glycoprotein (ZAG) and tumor necrosis factor (TNF-α).](https://www.prv.org)
increased 10-fold in WAT and 3-fold in BAT, while leptin mRNA levels in WAT were decreased 33-fold, and the adiponectin mRNA level was unchanged (19). ZAG protein levels were also increased 10-fold in WAT and 20-fold in BAT, when the weight loss reached 24%. Interestingly, ZAG expression in human WAT in obese subjects is reduced by 70%, suggesting that ZAG expression is inversely related to the mass of WAT (48). These results suggest that ZAG is a new adipokine and may influence adipose tissue metabolism locally.

In human adipocytes, the PPARγ agonist rosiglitazone induced a threefold increase in ZAG mRNA level, while TNF-α led to a fourfold decrease (11). ZAG expression was also increased by a β3-agonist, BRL37344, and the glucocorticoid dexamethasone (19). Glucocorticoids may be responsible for the increased ZAG expression seen in mice with cachexia, since the glucocorticoid receptor agonist RU38486 attenuated both the loss of body weight and ZAG expression in WAT (225). This suggests that glucocorticoids stimulate lipolysis through an increase in ZAG. An increased cortisol secretion is one of the earliest hormonal changes in cachexia, and in a murine cachexia model serum cortisol concentrations increased in parallel with weight loss (225). An increased 24-h urinary excretion of cortisol has also been found in malnourished cancer patients compared with malnourished controls (57), and there was also an increased urinary excretion of epinephrine and norepinephrine. With the use of 3T3-L1 adipocytes, ZAG was shown to stimulate its own expression, and this was attenuated by the β3-adrenoreceptor antagonist SR59230A, suggesting that it is mediated through the β3-adrenoreceptor (225). Interestingly, eicosapentaenoic acid (EPA), which has had some success in the treatment of cachexia, since the glucocorticoid receptor antagonist RU38486 attenuated both the loss of body weight and ZAG expression in WAT (225). This suggests that EPA could preserve adipose tissue in cancer cachexia (224). These pathways are illustrated in Figure 4.

Further evidence for a role of ZAG in lipolysis was provided by experiments in mutant mice in which ZAG was inactivated by homologous recombination in embryonic stem cells by mutating the second and third exons (215). ZAG−/− mice gained significantly more weight than ZAG+/+ control animals over a 25-wk period, and this was more pronounced when they were fed a high-fat diet. Adipocytes from ZAG knockout animals showed a decreased lipolytic response to isoprenaline, a nonselective β-adrenergic agonist, and CL316243, a specific β3-adrenergic agonist, as well as forskolin and isobutylmethylxanthine, both of which increase cAMP levels. There was no effect on basal lipolysis. Thus adipocytes from ZAG knockout animals show an inverse response to lipolytic stimulation as do adipocytes from cachectic cancer patients (5). These results strongly suggest that an increased ZAG expression may be responsible for the increased lipolytic response of adipose tissue in cancer cachexia.

**B. Tumor Necrosis Factor-α**

There is considerable experimental evidence that TNF-α can induce lipid depletion in WAT, either through inhibition of LPL, by suppressing transcription (208), or by stimulation of lipolysis (227). TNF-α has been shown to selectively decrease LPL mRNA levels and activity in 3T3-L1 adipocytes (18), which would prevent adipocytes from extracting FFA from plasma lipoproteins for storage and result in a net flux of lipid into the circulation (Fig. 3). However, studies in human adipocytes isolated from cancer patients have shown no decrease in either LPL mRNA or LPL enzyme activity (256). TNF-α also stimulates lipolysis in adipocytes, although, unlike LMP-ZAG, which acts immediately, this occurs after prolonged (6–12 h) incubation. In human adipocytes, this occurs through activation of the MAPK, ERK, and elevation of intracellular cAMP (296). TNF-α-mediated lipolysis requires the activation of the MAPK p44/42 and c-jun-NH2-terminal kinase (JNK), but unlike rodent cells it does not affect Gα signaling (227). Instead, cAMP levels are increased due to inhibition of a specific cAMP phosphodiesterase (PDE), called PDE3B (296) (Fig. 3). Upregulation of the MAPK pathway leads to downregulation of PLIN expression, which is paralleled by an increase in basal lipolysis (227), while PLIN phosphorylation is also increased by TNF-α through the p44/42 MAPK (296). Downregulation of PLIN may be the main mechanism by which TNF-α induces lipolysis, since PLIN has been proposed to act as a barrier to lipolysis, and constitutive overexpression of PLIN by adenovirus infection of 3T3-L1 adipocytes blocks the ability of TNF-α to increase lipolysis (248). Activation of NFκB
also appears to be important in TNF-α-induced lipolysis in human adipocytes, and while TNF-α reduced mainly PLIN protein expression, when in combination with an inhibitor of NFκB both HSL and PLIN protein levels were reduced (145). Activation of p44/42 MAPK and JNK would lead to phosphorylation of PPARγ, which is known to block PPARγ-induced transcriptional activation (110), and would lead to inhibition of preadipocyte differentiation (168), as well as decreasing ZAG expression (11). The ability of TNF-α to decrease ZAG expression in WAT may provide a mechanism for its role in obesity (108). This suggests that MAPK-induced phosphorylation of PPARγ is both a negative and positive regulator of lipid accumulation. Like LMF/ZAG, TNF-α may also stimulate thermogenesis, since a single injection of TNF-α into rats has been shown to induce a significant increase in the expression of mRNA for both UCP2 and UCP3 in skeletal muscle (34).

There is some debate on the role of TNF-α in human cancer cachexia. Thus, while some studies show TNF-α to be detectable in the serum of 36.5% of patients with pancreatic cancer, with serum levels of TNF-α inversely correlated with body weight and body mass index, and serum protein and albumin levels (126), other studies in patients with advanced and terminal cancer found no correlation between circulating TNF-α levels and weight loss and anorexia (163). There are a number of other studies which reflect this disparity, and serum levels of TNF-α may correlate better with the stage of the disease, reflecting tumor size rather than the extent of weight loss. The disparities may reflect differences in sensitivity of measuring techniques, diurnal fluctuations in the TNF-α serum levels, a short half-life, or the cytokine could have an auto- or paracrine role in adipose tissue. Also the significance of individual cytokines in the process of cachexia may be difficult to determine, since there is a pronounced state of redundancy among networks of cytokines. Alternatively, the production of TNF-α may rest in peripheral blood polymorphonuclear leukocytes, which show higher spontaneous production of TNF-α when isolated from cancer patients than from normal subjects (3), and may be transported to the liver to induce an APR. However, tumor-bearing animals do not show higher serum levels of TNF-α after endotoxin administration than non-tumor animals, and the dose-related reduction of body weight in mice after administration of TNF-α is directly proportional to the decreased food and water intake (164).

C. Interleukins 1 and 6 and Interferon-γ

TNF-α induces IL-6 secretion and synergizes with it in many of its actions, e.g., both stimulate other cytokines in a cascade, which has both proinflammatory and anti-inflammatory components. The proinflammatory cytokines such as IL-6 may lead to an APR and trigger tissue catabolism. A significant positive association has been found between the level of the APR and serum levels of IL-6 and soluble TNF-α receptors 55 and 75 (12). Evidence for a role of IL-6 in the development of cancer cachexia has come mainly from studies using the murine colonic-26 adenocarcinoma, where increasing levels of IL-6 correlated with the development of cachexia, and treatment with a neutralizing antibody to IL-6, but not TNF-α or interferon (IFN)γ, attenuated the development of weight loss and other key parameters of cachexia (249). However, further studies with this tumor suggested that IL-6 alone could not be responsible for production of cachexia, since serum levels of IL-6 were raised equally in mice bearing clones of the colon-26 tumor, which were and were not capable of inducing cachexia (245). As with TNF-α, IL-1 can induce body weight loss and anorexia in mice (184), but IL-6 was found to have no effect on food intake or body weight, although it produced an hepatic APR (68). However, another member of the IL-6 superfamily ciliary neurotrophic factor (CNTF) produced profound anorexia and tissue wasting when administered at the same dose level as IL-6. While results using IL-6 transgenic mice have been equivocal, mice implanted with C6 glioma cells genetically modified to secrete CNTF exhibited rapid catabolism of adipose tissue and skeletal muscle, depressed levels of glucose and triglyceride, and death over a period of 7–10 days (103). Studies in weight-losing patients with NSCLC have found significant increases in serum IL-6, when compared with patients with the same tumor, but without weight loss. In contrast, another study found that serum levels of TNF-α, IL-1, IL-6, and IFNγ did not correlate with weight loss in 61 patients with advanced and terminal cancer (165). However, raised serum IL-6 levels and low IFNγ were found to be related to a shorter survival in lung cancer patients (174), and IL-6 levels showed a sharp elevation 1 wk before death (116). It was concluded that IL-6 increases gradually during the early stages of cachexia and then shows a sudden and steep rise just before death. IFNγ has also been suggested to play a role in cancer cachexia from the ability of neutralizing antibodies to attenuate weight loss in experimental animal tumors, but no significant correlation between serum levels and weight loss has been observed in cachectic cancer patients (165).

Like TNF-α, IL-1, IL-6, and IFNγ have all been shown to inhibit expression of LPL mRNA and, like TNF-α, IL-1, and IFNγ, have been shown to directly stimulate lipolysis, while IL-6 has no effect. However, it is unlikely that a decrease in LPL alone could account for the fat cell depletion seen in cancer cachexia, and the increased expression and activity of HSL is probably the major factor (5).
V. SKELETAL MUSCLE

In the adult, muscle mass remains fairly constant in the absence of stimuli such as exercise so that protein synthesis and degradation remain in balance. However, in cachexia muscle atrophy occurs, which must result either from a depression in protein synthesis, an increase in protein degradation, or a combination of both. The relative importance of protein synthesis and degradation to muscle atrophy varies between various studies. Thus a study by Emery et al. (66) suggested that muscle mass in cancer cachexia is regulated primarily by alterations in protein synthesis and that changes in protein degradation are likely secondary. Another study by Lundholm et al. (160) came to the same conclusion, since the release of 3-methylhistidine from leg tissue of cachectic cancer patients was insignificant, while both well-nourished controls and acutely ill patients showed significant release of 3-methylhistidine. Since 3-methylhistidine cannot be re-utilized for protein synthesis, this provides a direct measure of protein degradation, and this is the only study in the literature that has tried to quantify the degradation rate of myofibrillar proteins in vivo. However, other studies (195) have attributed the exceptionally high protein turnover rates in patients with hepatocellular carcinoma to be the result of an elevated rate of protein breakdown, with oxidation of the released amino acids. Studies in a number of experimental models of cachexia suggest both processes are occurring simultaneously so that effective treatment of this condition will need to address both the depression in protein synthesis in skeletal muscle as well as the increase in protein degradation. Fast-twitch type II-containing muscles, such as tibialis anterior and gastrocnemius, are lost faster than slow-twitch type I muscle, such as soleus in cachexia, and this is due to an increased protein oxidation and degradative protein expression in response to cachetic stimuli in type II fibers (295). Antioxidant gene expression is also lower in type II fibers as is nitric oxide (NO) production and inducible NO synthase (iNOS). A change in muscle myosin isoform expression also occurs, with a decrease in type I and an increase in type II (fast) isoform expression (54). Muscle wasting in cancer has been shown to be linked to a dysfunctional dystrophin glycoprotein complex (DGC), a membrane structure associated with muscular dystrophy (1). In muscles from mice with cachexia and in patients with gastrointestinal cancers, there were reduced levels of dystrophin and increased glycosylation on DGC proteins. Furthermore, tumor-induced muscle wasting was enhanced in dystrophin null mice, but attenuated in dystrophin transgenic animals. These results suggest that DGC dysfunction plays an important role in cachexia-induced muscle atrophy.

A. Control of Protein Synthesis in Normal and Cachectic States

Protein synthesis in skeletal muscle is primarily regulated at the initiation phase of protein translation, a highly complex and conserved process involving at least 13 initiation factors, many of which are assembled from numerous subunits. There are two points of control (Fig. 5). The first is the binding of initiator methionyl-tRNA (met-tRNA) to the 40S ribosomal subunit, which is regulated by eukaryotic initiation factor 2 (eIF2), and mediates ribosomal binding in a GTP-dependent manner (207). eIF2 binds to GTP, and the ternary complex eIF2.GTP.met-tRNA binds to the 40S ribosomal subunit, together with eIF3, forming the 43S preinitiation complex. Following start codon recognition, the GTP is hydrolyzed to GDP, and to return to the GTP-bound form, the GDP is exchanged with GTP in a reaction catalyzed by the guanine nucleotide exchange factor eIF-2B (200) (Fig. 5). The recycling of GTP by eIF-2B can be inhibited by phosphorylation of eIF2 on the α-subunit leading to inhibition of translation initiation (218). Mammalian cells possess four different eIF2α kinases: double-stranded RNA-dependent protein kinase (PKR), heme-regulated inhibitor kinase...
(HRI), PERK, and the yeast GCN2 (209). Each of these kinases responds to distinct stress conditions that affect transcription and protein synthesis.

The second important control point in translation initiation is mediated by the eIF4F triad of translation initiation factors, which recruits the 40S ribosomal subunit to mRNA through the 5’-cap structure recognition (m7GpppX) (210). The eIF4F complex consists of three subunits: eIF4E which binds the 5’-mRNA, eIF4A, an ATP-dependent RNA helicase, and eIF4G, a scaffold protein for assembly of eIF4E and 4A into the eIF4F complex. eIF4E is one of the main regulatory initiation factors and is therefore present in low molar amounts in the cell. The concentration of eIF4E is also regulated by its association with its binding protein (4E-BP1) (163). Hypophosphorylation of 4E-BP1 blocks assembly of the eIF4F complex because it competes with eIF4G for binding to eIF4E. Phosphorylation of at least two of the binding proteins, 4E-BP1 and 4E-BP2, is regulated through a signal transduction pathway involving phosphatidylinositol 3-kinase (PI3-K) and the mammalian target of rapamycin (mTOR) (209) (Fig. 5). The mTOR signaling pathway also regulates the activity of the 70-kDa ribosomal protein S6 kinase (p70^s6k), which is activated by phosphorylation, and was thought previously to confer selective translation of mRNAs that contain a 5’-poly-pyrimidine tract (5’-TOP) as a common feature, although mTOR may regulate 5’-TOP mRNAs independently of p70^s6k. Thus the role of p70^s6k phosphorylation is presently unclear. The transcripts from these mRNAs encode proteins that are involved in the translational apparatus, such as eukaryotic elongation factor 2 (eEF-2), which mediates the translocation step of elongation. Phosphorylation of eEF-2 inhibits elongation by decreasing its affinity for the ribosome by 10–100 times (38).

While studies in some animal models suggest that the depressed protein synthesis in skeletal muscle is related to the anorexia, protein synthesis is also depressed in other animal models of cachexia where anorexia is absent (244), suggesting an underlying defect in the protein synthetic machinery. Thus there are changes to the phosphorylation of initiation factors in cancer cachexia, which would lead to a depression in protein synthesis. Gastrocnemius muscle from mice bearing the cachexia-inducing tumor MAC16 show activation (autophosphorylation of PKR) when the weight loss is >16% and a corresponding increase in phosphorylation of eIF2α (66). There is no change in the total amount of PKR or eIF2α. In weightlosing patients with esophagogastric cancer, levels of both phospho-PKR and phospho-eIF2α are also significantly enhanced, compared with healthy controls (65), and this is independent of the extent of weight loss. There is a linear relationship between phosphorylation of PKR and phosphorylation of eIF2α, suggesting that phosphorylation of PKR led to phosphorylation of eIF2α. The increased phosphorylation of eIF2α appears to be at least partly responsible for the loss of myofibrillar proteins, since there is a linear relationship between myosin expression and phosphorylation of eIF2α.

Weight loss in mice bearing the MAC16 tumor is also associated with an increased amount of eIF4E bound to 4E-BP1 in gastrocnemius muscle, due to hypophosphorylation of 4E-BP1, resulting in a progressive decrease in the concentration of the active eIF4G-eIF4E complex (64). This would also contribute to a depression in protein synthesis, as would also a decrease in phosphorylation of mTOR and p70^s6k. There is also a fivefold increase in the phosphorylation of eEF2, which would also decrease protein synthesis through a decrease in translation elongation.

In addition to their role as substrates for protein synthesis in skeletal muscle, branched-chain amino acids (BCAA) are uniquely able to enhance protein synthesis by initiating signal transduction pathways that modulate translation initiation (291). Of the BCAA, leucine has been found to be the most potent and has been shown to attenuate the loss of body weight in mice bearing the MAC16 tumor, with an increase in muscle mass which was attributed to an increase in protein synthesis and a decrease in protein degradation (64). Leucine attenuated the increased phosphorylation of PKR by increasing expression of protein phosphatase 1 (PP1) (Fig. 5), which is known to dephosphorylate PKR (251). The decreased phosphorylation of PKR led to a decreased phosphorylation of eIF2α, allowing conversion of eIF2.GDP to eIF2.GTP. Leucine also caused increased phosphorylation of mTOR and p70^s6k. It is unlikely that leucine activates the protein kinase activity of mTOR directly, but more likely modulates activity by interaction with other proteins, such as Raptor, which regulates the activity of mTOR, and its sensitivity to rapamycin (98). Leucine also caused hyperphosphorylation of 4E-BP1, probably through activation of mTOR, resulting in the release of eIF4E from the inactive 4E-BP1.eIF4E complex, which was then able to associate with eIF4G to form the active eIF4F complex (Fig. 5). Leucine also caused a reduction in phosphorylation of eEF2, possibly by stimulating the mTOR pathway. These results provide compelling evidence for the inclusion of leucine in nutritional supplements for the treatment of muscle atrophy in cachectic cancer patients.

**B. Protein Degradation in Cachexia**

There are three major proteolytic pathways responsible for the degradation of proteins in skeletal muscle. These are 1) the lysosomal system including the cysteine proteases cathepsins B, H, and L as well as the aspartate protease cathepsin D. This is mainly responsible for the degradation of extracellular proteins and cell receptors.
2) The calcium-activated system includes calpains I and II, which is mainly involved in tissue injury, necrosis, and autolysis. 3) The ubiquitin-proteasome pathway, which requires ATP and works in harmony with the calpain system to disassemble and degrade muscle myofilaments (100). The ubiquitin-proteasome pathway has been extensively reviewed (88). Studies in animal models of cancer cachexia, as well as in cancer patients, suggest that the ubiquitin-proteasome pathway plays the predominant role in the degradation of myofibrillar proteins, particularly in patients with a weight loss of >10% (131). Recent studies (167) suggest that the transcription factor Fox03 controls both the ubiquitin-proteasome and lysosomal pathway in muscle but by a different mechanism. For patients with a low weight loss (2.9%), muscle biopsies showed no change in components of the ubiquitin-proteasome pathway, but an increased expression of mRNA for cathepsin B (117). However, it is not clear whether patients with such a low weight loss show muscle atrophy, and if so whether the increased expression of cathepsin B is responsible for muscle protein degradation, since at least in the rat, lysosomes have been shown not to be involved in the degradation of myofibrillar proteins (158). Newly diagnosed lung cancer patients do show an elevated protein turnover, but this is also seen in noncachectic as well as cachetic subjects (180). An early study (234) showed an increase in lysosomal activity as measured by cathepsin D and acid phosphatase in skeletal muscle of cancer patients, which in five subjects appears to correlate with weight loss, although there have been no further studies. About half of the total muscle protein is myofibrillar protein, which is lost at a faster rate than other proteins during atrophy. Myosin heavy chain is selectively targeted by the ubiquitin-proteasome pathway in the cachectic state, while other core myofibrillar proteins including troponin T, tropomyosin (α-and β-forms), and α-sarcomeric actin remain unchanged (2).

The problem faced by the cell in degrading intracellular proteins is to maintain specificity, since if the proteases were to mix with the intracellular contents, nonspecific degradation would occur. In the ubiquitin-proteasome pathway, the proteases are confined to an intracellular structure, the proteasome, and the specificity of the process is ensured by tagging proteins for degradation with a polyubiquitin chain (Fig. 6). Ubiquitin is a 76-amino amino protein, which is covalently linked to an ε-amino group in a lysine residue of the substrate protein. The proteasome is a barrel-like structure composed of four rings, two outer α-rings and two inner β-rings, in the order αββα. The α- and β-rings are made up of seven subunits, and the proteolytic enzymes are located on the inner surface of the β-rings, facing the inner cavity of the cylinder (252). The β5-subunits contain two chymotrypsin-like sites, which cleave after basic residues. The β1-subunits contain two sites often called peptidylglutamyl-peptide hydrolyzing sites, which cleave after acidic residues. These latter two sites have caspase-like specificity (136). A cyclical mechanism has been suggested for protein breakdown, in which the chymotrypsin-like site initially cleaves the substrate and stimulates caspase-like sites, which accelerates further cleavage of the fragments, while the chymotrypsin-like activity is temporarily inhibited. When further caspase-like cleavage is not possible, the chymotrypsin sites are reactivated and the cycle is repeated. The final products of protein degradation by the proteasome are peptides containing six to nine amino acid residues (135), and these are degraded by the giant protease tripeptidyl peptidase II (TPPII) and various aminopeptidases (Fig. 6). TPPII cleaves peptides generated by the proteasome into triptides (9). Although this step is not rate-limiting for proteolysis, it is important because the accumulation of abnormal peptides may be injurious to the cell. Both proteasome proteolytic activity and TPPII activity increased in parallel with weight loss in cancer cachexia.
mice bearing the cachexia-inducing MAC16 tumor, reaching a maximum at 16% weight loss, after which there was a progressive decrease in activity for both proteases with increasing weight loss (41).

The 26S proteasome consists of the 20S core proteasome and two 19S subunits, which mediate the binding and unfolding of the substrate protein before its transfer to the interior of the 20S core (285). MSS1 and P45 are ATPase subunits of the 19S complex, thought to provide energy to inject the substrate into the chamber of the 20S proteasome. mRNA for MSS1 but not P45 was found to be increased in wasting muscle of rats bearing the Yoshida sarcoma (8), and expression of mRNA for both α- and β-proteasome subunits was increased in gastrocnemius muscle of weight-losing mice bearing the MAC16 adenocarcinoma (132). The increased expression of MSS1 was normalized in cachectic rats administered pentoxifylline, but not tobafylline, although both block TNF-α production and suppress the enhanced proteolysis (8).

The ubiquitin chain is attached to the protein substrate through a reaction sequence consisting of a series of enzymes involving ubiquitin activation (E1), the ubiquitin carrier protein (E2), which is able to recognize the ubiquitin protein ligase (E3), which recognizes both the protein substrate, and catalyzes the transfer of ubiquitin from the E2 thioester intermediate. The E3s are the primary determinants of substrate specificity and recognize several structural motifs. Two E3s, muscle atrophy F box (MAFbx)/atrogin 1 and muscle RING finger 1 (MuRF1), are highly expressed during muscle atrophy in a range of catabolic conditions including cancer cachexia (23, 91).

Overexpression of MAFbx in myotubes was shown to induce atrophy, while mice deficient in either MAFbx or MuRF1 were found to be resistant to atrophy (23). The substrate for one of these E3s, MuRF1, has been confirmed as myosin heavy chain protein, as depicted in Figure 6 (42). MuRF1 also catalyzes the ubiquitination of troponin I in cardiac myocytes (128). The expression of another E3 (E3α-II) has also been shown to be significantly induced at the onset and during the progression of muscle wasting (141). E3α-II was shown to be induced in myotubes by treatment with TNF-α or IL-6.

Expression of the mouse ether-a-go-go related gene (Mergla), a voltage-gated K+ channel, has been shown to be upregulated in skeletal muscle of mice undergoing atrophy as a result of both tumor implantation and disuse (270). Moreover, ectopic expression of Mergla in skeletal muscle induces proteolysis through the ubiquitin-proteasome pathway resulting in atrophy, while ectopic expression of a dysfunctional dominant negative mutant of Mergla, or treatment with astemizole, a Mergla channel blocker, inhibits atrophy and decreases proteolysis through the ubiquitin-proteasome pathway.

Evidence has been presented for a role for PPAR isoforms, particularly the γ and δ, in muscle wasting induced by the Yoshida AH-130 ascites hepatoma in rats (83). Increases in mRNA expression of both PPARγ and δ were observed in atrophying skeletal muscle, which were related to increases in the expression of several genes involved in fatty acid transport, oxidation, and activation, suggesting a metabolic shift to a more oxidative phenotype, thus acting as a sink for some of the lipids released from adipose tissue. GW1929, a PPARγ agonist, has been shown to specifically protect against loss of the white muscle extensor digitorum longus (EDL), while having no effect on gastrocnemius, soleus, or tibialis muscles (188).

C. Apoptosis in Skeletal Muscle

In addition to the proteasome, apoptosis of muscle cells may play a role in muscle atrophy. Ishiko et al. (113) proposed two mechanisms for muscle depletion during tumor growth: apoptosis in the early stages and metabolic abnormalities in the late stage. An increased activity of caspases-1, -3, -6, -8, and -9 was observed in gastrocnemius muscle of mice bearing the cachexia-inducing MAC16 tumor (17). Fragmentation of poly(ADP-ribose) polymerase (PARP) was also observed, which is cleaved during apoptosis by caspases -3 and -7, although there was no evidence for DNA fragmentation into a nucleosomal ladder typical of apoptosis. However, enhanced laddering of DNA was observed in the skeletal muscle of rats bearing the Yoshida AH-130 ascites hepatoma, mice bearing the Lewis lung carcinoma (266), and rabbits bearing the UX2 tumor (113), indicative of apoptosis. The expression of Bax, which promotes apoptosis, was also increased in the early stages of weight loss.

Despite these observations in experimental animals with cancer cachexia, an initial study found no evidence for an increase in apoptosis in skeletal muscle of gastric cancer patients compared with controls (25). However, a recent study (32) employing muscle biopsies from weight-losing patients with upper gastrointestinal cancer found a significant (3-fold) increase in muscle DNA fragmentation compared with control subjects, associated with an increased PARP cleavage and a decrease in MyoD protein content. The reason for the discrepancy between the two studies may be related to differences in both tumor type and staging, as well as differences in the rate of muscle atrophy.

VI. TUMOR AND HOST FACTORS INFLUENCING MUSCLE MASS IN CACHEXIA

A. Proteolysis-Inducing Factor

Proteolysis-inducing factor (PIF) is a 24-kDa molecular mass sulfated glycoprotein, originally isolated from
the cachexia-inducing MAC16 tumor, using an antibody cloned from splenocytes of mice bearing the same tumor, but with a delayed cachexia (261). The antibody was also reactive to a similar material in the urine of cancer patients with cachexia, which was absent from the urine of patients with the same tumor type but without cachexia (37). This substance was evident in the urine of weight-losing patients with a variety of tumor types, including pancreatic, lung, breast, ovary, rectum, colon, and liver, but absent in urine from patients without weight loss (37). Biosynthetic labeling studies of PIF produced by the MAC16 cell line, and subsequent degradation, indicated that ~85% of the molecule was carbohydrate, with a short (2–4 kDa) central polypeptide chain, with phosphate residues that may be attached to the polypeptide, or a short oligosaccharide chain (257). The polypeptide chain also has attached to it one O-linked sulfated oligosaccharide chain containing glucosamine (molecular mass 6 kDa), and one N-linked oligosaccharide chain (molecular mass 10 kDa), also containing glucosamine. A recent study (187) suggested that the polypeptide chain could not be glycosylated by human and murine tumors. Tumor lines were transfected with plasmids containing the gene for the core peptide of PIF, and although the protein was secreted, there was no glycosylation. However, the antibody used for the detection of glycosylated PIF was raised to amino acids 44–62 of the peptide chain, which are excised on glycosylation, and so therefore a glycosylated product would be undetectable. Also to form such a complex sulfated glycoprotein as PIF would require the presence of both the glycosylated sugars, and the relevant conjugating enzymes, and it is unlikely that its formation would be limited by the concentration of the polypeptide chain. To ensure correct glycosylation, previous authors (274) have used a cell line such as G361 human melanoma, which is known to produce PIF (258), and therefore must possess the relevant glycosyltransferases. However, Monitto et al. (187) used MCF7 breast carcinoma for transfection, which does not produce cachexia or PIF, and therefore does not contain the relevant enzyme capacity.

Studies by other groups (271, 282) confirmed PIF excretion in the urine was related to weight loss in patients with prostatic and primary gastrointestinal tumors. However, another study has questioned the role of PIF in weight loss in patients with metastatic gastric/esophageal and lung cancer (119, 276). It is unlikely that the material classified as PIF from mass spectrometry in these studies was the correct material, since it was the major peak in the spectrum of crude urine, whereas PIF only represents 5 × 10⁻⁴% of the urinary proteins (37). In addition, only one measurement of urinary PIF was made during the course of weight loss (276), while Williams et al. (282) made four repeated measurements during a 2.5-yr follow-up. They observed a change in the PIF status in 41% of the patients, with 19% changing from negative to positive, 8% changing from positive to negative, and 14% varying during the course of the study. Also since the antibody used was directed at the oligosaccharide chains of PIF, there is a possibility of cross-reactivity with other materials containing similar structures. To confirm that the material in urine that is being measured is PIF, Western blotting should also be carried out with antibodies to the core peptide, as demonstrated by other authors (271). Biosynthetic labeling studies and enzymatic deglycosylation showed PIF produced by the human melanoma G361 was identical in molecular weight to the mouse material and contained the same sized N- and O-linked sulfated oligosaccharide chains (258). Other mouse tumors, such as the colon 26 adenocarcinoma, which has been used extensively to evaluate a role of IL-6 in cachexia (249), have also been shown to produce PIF (111). Interestingly, unlike IL-6, PIF could not be detected in a variant of this tumor which did not produce cachexia (111).

The polypeptide chain of PIF arises at a single gene locus on human 12q3.1, which codes for three products: dermicidin (DCD), an antimicrobial peptide isolated from human sweat (235); diffusible survival evasion peptide (DSEP or Y-P30), a product of the same region of DCD as the PIF core peptide (47); and a third peptide which has been mapped to the DCD gene locus that may function as an oncogene in breast cancer, with survival-promoting properties (206). Unlike PIF, none of these peptides is glycosylated. Both the N- and O-linked sulfated oligosaccharide chains have been shown to be important for the biological activity of PIF (257). Unlike PIF, the core peptide is present in both normal and tumor tissue in patients with gastroesophageal malignancy, and its expression does not relate to prognosis or cachexia (50). An important function of this protein in normal tissue may be to promote survival in the presence of oxidative stress.

Intravenous injection of PIF isolated from either the MAC16 tumor (261), or from the urine of cachectic patients with pancreatic carcinoma (37), induced an immediate and profound loss of body weight in mice, reaching ~10% loss of body weight over a 24-h period (Fig. 7). Unlike TNF-α, this occurred without a depression in either food or water intake and was the result of specific depletion of the lean body mass (37). There were specific reductions in the weight of gastrocnemius (64%) and soleus muscles (17%), but not heart or kidney, and an increase in weight of the liver (10%). The effect on skeletal muscle was due to a depression in protein synthesis (by 50%) and an increase in protein degradation (by 50%) (157). PIF produced a specific increase in mRNA levels for ubiquitin, E2₁₄k, and the C9 proteasome subunit in gastrocnemius muscle, but not heart (156), suggesting that protein degradation was mediated through an increased expression of the ubiquitin-proteasome pathway.
PIF has also been shown to inhibit protein synthesis and stimulate protein degradation directly in isolated murine myotubes, which has facilitated the identification of signaling pathways leading to muscle atrophy (Fig. 8). In this system, PIF has been shown to induce specific depletion of myosin, while actin levels remained unchanged (290). This effect is similar to that produced by a combination of TNF-α and IFN-γ, which has been suggested as occurring through an RNA-dependent mechanism (2). PIF has also been shown to induce an increased expression of components of the ubiquitin proteasome pathway in murine myotubes, including the 20S proteasome α-subunits, MSS1, and p42, another ATPase subunit of the 19S regulator, as well as an increased chymotrypsin-like enzyme activity of the β5-subunits of the proteasome (290). These effects were completely attenuated in myotubes transfected with mutants of the inhibitor protein IκB, which were incapable of phosphorylation and subsequent degradation leading to the release and nuclear accumulation of nuclear factor κB (NFκB) (290). This suggests that the ubiquitin-proteasome pathway is induced by activation of the transcription factor NFκB in response to PIF.

Other studies confirm the importance of IκB kinase β (IKKβ)/NFκB to the induction of the ubiquitin-proteasome pathway (36). Thus activation of NFκB through muscle-specific transgenic expression of activated IKKβ in mice caused profound muscle wasting that resembled clinical cachexia. Expression of the E3 ligase MuRF1 in muscle was increased 3.3-fold, and there was also a 2.4- to 2.8-fold increase in mRNA for the C2 and C9 subunits of the proteasome, while mRNA for E214k, atrogin1/MAFbx, and lysosomal and calcium-dependent proteases were normal (36). Activation of NFκB has also been shown to suppress expression of mRNA for the myogenic transcription factor MyoD, causing a reduction in synthesis of myosin (95). The importance of activation of NFκB to muscle wasting in cachexia was confirmed by treatment of mice bearing the MAC16 tumor with resveratrol, which inhibits activation of NFκB through inhibition of IKK. Resveratrol was found to significantly attenuate weight loss and protein degradation in muscle through the ubiquitin-proteasome pathway (289).

In addition to increasing proteasome expression and activity, PIF has also been shown to produce a parallel increase in TPPII (41). This suggests that expression of both proteasome components and TPPII are induced by the same transcription factor, which is possibly NFκB.

Activation of NFκB by PIF involves a signaling cascade involving the formation of ROS (220), and this may be a common step in atrophy induced by a number of agents (Fig. 8). Mice lacking the major antioxidant enzyme Cu/Zn-superoxide dismutase (SOD) show a dramatic acceleration of age-related loss of skeletal muscle mass through an elevated oxidative stress (191). Oxida-

![FIG. 7. Effect of anti-proteolysis-inducing factor (PIF) affinity-purified urine from a cachectic patient with pancreatic carcinoma and a healthy control on body weight of female mice (weight 20 g) either pretreated or not with two doses of 800 μg anti-PIF antibody. [From Cariuk et al. (37).]
tive stress by hydrogen peroxide has been shown to induce protein degradation in murine myotubes through an increased expression of the ubiquitin-proteasome pathway (92). It is suggested that PIF induces a transient increase in ROS formation through activation of NADPH oxidase by arachidonic acid (AA), formed by the phospholipase A2 (PLA2)-catalyzed release from membrane phospholipids (PL), or that AA may contribute to mitochondrial generation of ROS. Activation of protein kinase C (PKC) is also important in activation of NADPH oxidase and has been shown to be essential in the PIF-induced expression of the ubiquitin-proteasome pathway, through activation of NFkB (243). Activation of PKC could arise directly from AA, but most likely involves conversion of AA by 15-lipoxygenase (15-LOX) to 15-hydroxyeicosatetraenoic acid (15-HETE), since inhibitors of 15-LOX have been shown to attenuate muscle atrophy in a murine cachexia model (288). AA has been shown to induce direct interaction between the NADPH oxidase subunits p47phox and p22phox, while phosphorylation of p47phox by PKC partly replaces the effect of AA (211). The increased ROS activates IKK, leading to phosphorylation and degradation of IkB, and to increased nuclear accumulation of NFkB (Fig. 8).

Activation of PLA2 is mediated through PKR, which becomes phosphorylated (activated) in response to PIF, and serves as a link between the inhibition of protein synthesis and the increased protein degradation in skeletal muscle (66). Thus activation of PKR will lead to phosphorylation of eIF2 on the α-subunit, inhibiting translation initiation (217). In addition, activation of PKR leads to an increased expression and activity of the ubiquitin-proteasome pathway, through activation of NFkB, either by direct interaction or through formation of ROS (66). The importance of this process to cancer cachexia is shown by the ability of a PKR inhibitor to attenuate skeletal muscle atrophy in a murine model of cachexia, through an increased protein synthesis and reduction of the increased protein degradation down to basal levels (63). Interestingly, inhibition of the activation of PKR also inhibited tumor growth. Both the BCAA (64) and insulin-like growth factor I (IGF-I) (219) inhibit activation of PKR, by increasing expression of PP1, which dephosphorylates PKR. This effect is important in the attenuation of protein degradation in cachexia by these agents.

A receptor for PIF with a molecular mass of 40 kDa has recently been identified in skeletal muscle and liver, but not on adipose tissue and kidney (260). Antibera to the NH2-terminal portion of the receptor blocked the action of PIF in vitro and also muscle atrophy in the MAC16 model by attenuating the depression of protein synthesis and the increase in protein degradation.

In addition to its direct effect on skeletal muscle, PIF may also act directly by induction of cytokine production by the liver. Using recombinant human PIF, both a human liver endothelial cell line and umbilical vein endothelial cell line responded by the release of increased amounts of IL-6 and IL-8 (274), and this may contribute to the APR seen in cachexia. The effect occurs through the NFkB and STAT3 transcriptional pathways. A similar effect was observed in human Kupffer cells and monocytes resulting in the production of TNF-α, IL-6, and IL-8 (273). PIF also induced syndecan shedding from human vein endothelial cells, which has been suggested to be related to metastasis, as well as patient mortality. Thus PIF may play a role outside of the cachexia process.

B. Glucocorticoids

Although glucocorticoids are useful adjuvants in the treatment of cachexia because of their beneficial effects on symptoms, such as appetite, food intake, and sensation of well-being, their use should be confined to the end-stage of disease, and limited to a few weeks, because of their ability to induce atrophy of skeletal muscle, which primarily affects the type II muscle fibers. As previously described (225), glucocorticoids may play a role in the development of cancer cachexia, although adrenalectomy has been shown not to alter the course of cachexia in other animal models (254). The effect of glucocorticoids on muscle atrophy is mediated by upregulation of the ubiquitin-proteasome pathway (101), but this occurs through the forhead type (FOXO) transcription factors rather than NFkB (223). Transgenic mice specifically overexpressing Foxo1 in skeletal muscle were found to weigh less than wild-type control mice and had a reduced skeletal muscle mass, with loss of both type I and type II fibers, and the muscle was paler in color (125). There was increased expression of atrogin 1, but not MuRF1, together with an increased expression of the lysosomal proteinase cathepsin L. Downregulation of Foxo-1 expression using a specific RNA oligonucleotide led to an increase in skeletal muscle mass in cachetic mice, with an increased level of MyoD and decreased levels of the muscle regulator myostatin (150). Also constitutively active Foxo 3 acts on the atrogin-1 promoter increasing transcription and causing massive atrophy of myotubes and muscle fibers (233). In murine myotubes, activated Foxo3 stimulates protein degradation by activating both lysosomal and proteasome pathways, with the former contributing the major effect (297). Activated Foxo3 stimulates lysosomal proteolysis by activating autophagy through a decreased activity of the IGF-I/PI3K/Akt signaling pathway through both mTOR and a transcription-dependent mechanism. Glucocorticoids induce activation of Foxo by decreasing the activity of the PI3K/Akt pathway preventing phosphorylation of Foxo, which would leave it inac-
tive in the cytosol (Fig. 8). Conditional activation of Akt induces a rapid and significant skeletal muscle hypertrophy in vivo, accompanied by activation of the downstream Akt/p70S6K protein synthesis pathway (143). IGF-I has been shown to attenuate muscle atrophy induced by dexamethasone through both the PI3K/Akt/Foxo and PI3K/Akt/mTOR pathways (144).

In addition to its effect on protein degradation, activation of Foxo1 also inhibits protein synthesis (247). Both murine myotubes and mice constitutively expressing Foxo1 showed increased total 4E-BP1 through an increase in mRNA expression by blocking the T. However, phosphorylation of 4E-BP1 was reduced, associated with a reduction in the abundance of Raptor and mTOR proteins. Hypophosphorylation of 4E-BP1 was associated with an increased binding of eIF4E and a reduction in the concentration of the eIF4F complex. This, together with a reduced phosphorylation of p70S6K through a decrease in mTOR signaling, acts to inhibit protein synthesis in skeletal muscle (247).

Other transcription factors are also activated by glucocorticoids in skeletal muscle during sepsis. Among these is CCAAT/enhancer binding protein (C/EBP)-β and -δ, and although a causative role in the wasting process was not established, it was noted that binding sites for C/EBP are present in the promoter regions of the rat ubiquitin gene and the genes for the C3 proteasome subunit and E214k (203). Another transcription factor, activator protein-1 (AP-1), is also upregulated in skeletal muscle during sepsis and has also been shown to play an important role in muscle wasting during cancer cachexia. Thus injection of a virus containing the TAM67 protein, a blocker of the AP-1 protein, resulted in a significant recovery of muscle mass in rats bearing the AH-130 Yoshida ascites hepatoma (189).

Calcium may also be involved in the regulation of glucocorticoid-induced muscle proteolysis, since treatment of L6 myotubes with the calcium chelator BAPTA, or the calmodulin kinase II inhibitor KN-62, significantly reduced the increase in protein degradation induced by dexamethasone (275). Calcium plays an important role in regulating the binding of calpastatin to calpain, resulting in inhibited calpain activity. Evidence for a calcium-dependent mechanism in muscle protein degradation in a rat cachexia model was provided by a decrease in activity of calpastatin, while total calpain activity remained unchanged, resulting in an imbalance of the calpain-to-calpastatin ratio (46). The increased calpain activity has been suggested to be an early and possibly rate-limiting step in disassembly of myofilaments through degradation of the Z-band-associated proteins titin and α-actinin, with release of actin and myosin (99) (Fig. 6). Other investigations have suggested that caspase-3 plays a similar role (58), thus providing a link between muscle protein degradation and apoptosis.

Glucocorticoid-induced muscle atrophy is also associated with increased intramuscular myostatin expression, and myostatin gene deletion prevented glucocorticoid-induced muscle atrophy (87). This suggests an important role of myostatin in muscle atrophy caused by glucocorticoids. Myostatin is a TGF-β superfamily member, which is a negative regulator of muscle growth, and systemic overexpression in mice induces profound muscle and fat loss similar to that seen in cancer cachexia (298). Its expression is increased by glucocorticoids, and glutamine, a conditional essential amino acid during catabolic states, prevents glucocorticoid-induced muscle atrophy by suppressing myostatin expression (229). The mechanism for the effect of glutamine is not known, although it could increase the processing or stability of myostatin. In vitro myostatin results in a reduction in the size and number of myotubes, and also causes loss of body mass in vivo (179). Myostatin was found to reduce the expression of the myogenic genes MyoD and pax3, while the ubiquitin associated genes atrogin1 MuRF1 and E214k were upregulated. Myostatin also inhibited the phosphorylation of Akt, thereby increasing the levels of active Foxo1, but had no effect on NFκB. There have been no reports of changes in myostatin levels in cancer cachexia. These results suggest that glucocorticoids induce muscle atrophy by a different mechanism from PIF (Fig. 8).

C. Tumor Necrosis Factor-α

There is considerable evidence from animal studies that TNF-α plays a role in muscle loss in cancer cachexia, although its role in the human condition may be more questionable. Thus transplantation of Chinese hamster ovary cells transfected with the human TNF-α gene produced a syndrome resembling cachexia, with progressive wasting, anorexia, and early death (197). Transplantation of the Lewis lung carcinoma into mice engineered to be deficient in the TNF-α receptor protein type I showed reduced wasting of skeletal muscle compared with wild-type mice despite there being equal levels of serum TNF-α in both groups (153). Muscle waste in wild-type mice was associated with an increased fractional rate of protein degradation, which was not seen in the transgenic animals, while there was no change in protein synthesis in either group. However, acute treatment of rats with recombinant TNF-α was found to enhance protein degradation and decrease protein synthesis in soleus muscle (red), but not in EDL (white) (85). TNF-α induces muscle protein degradation through the formation of ROS in a similar manner to PIF (Fig. 8), although the molecular mechanisms may not be totally identical. Thus TNF-α has been shown to induce oxidative stress and NOS in skeletal muscle of mice, and treatment with antioxidants or
NOS inhibitors prevented the decrease in body weight, muscle wasting, and skeletal muscle molecular abnormalities (27). Like PIF, TNF-α induces activation of NFκB, leading to induction of the ubiquitin-proteasome pathway (149). Activation of NFκB has been shown to occur in a biphasic manner: a first transient phase, which is terminated within 1 h of cytokine addition, and a second phase persisting for 24–36 h (142). The second phase appears to be most important, since inhibition also inhibits cytokine-mediated loss of muscle proteins. TNF-α has been shown to cause increased expression of the 1.2- and 2.4-kb transcripts of ubiquitin (152) and the ubiquitin ligase atrogin I/MAFbx in skeletal muscle (149). The latter occurs through p38MAPK, which is activated by ROS (149). p38MAPK has been identified as a potential regulator of muscle catabolism and is essential for the expression of muscle-specific genes (130). Although the mechanism of ROS formation in skeletal muscle has not been determined, in other systems TNF-α induced ROS generation is dependent on the synthesis of AA and formation of LOX metabolites, as with PIF (Fig. 8) (287).

However, oxidative stress can induce muscle atrophy through mechanisms not involving NFκB activation, as in rats bearing the Yoshida AH-130 ascites hepatoma, despite the involvement of TNF-α (175). In contrast, diabetic rats show an increase in NFκB activation due to oxidative stress, but this did not lead to hyperexpression of MuRF1. Administration of dehydroepiandrosterone, which has multitargeted antioxidant properties, partially restored normal levels of NFκB DNA-binding activity in diabetic rats and reduced the hyperexpression of MuRF1. This suggests that ROS can independently interfere with the NFκB and proteasome systems.

TNF-α also inhibits myogenesis in vitro through a mechanism in which NFκB activation leads to degradation of MyoD transcripts (97). NO production may be responsible for MyoD loss in muscle by a combination of TNF-α and IFN-γ (171). A downstream target of NFκB is the iNOS gene. The RNA binding protein HuR, localized in the nucleus, associates with iNOS mRNA through its AU-rich element (ARE), mediating stability and export to the cytoplasm. iNOS will induce enzymatic conjugation of NO with superoxide to form peroxynitrite (OONO⁻), the release of which leads to downregulation of MyoD mRNA. This mechanism could explain the ability of NOS inhibitors to prevent muscle wasting induced by TNF-α (27).

D. Interleukin-6

Muscle atrophy is seen in IL-6 transgenic mice that overexpress IL-6, which is completely blocked by IL-6 receptor antibody and is associated with increased mRNA levels for cathepsins (B and L) and ubiquitins (poly and mono) (263). However, other studies have been unable to induce a wasting effect by recombinant IL-6 in mice, even with repeated administration (68). A recent study (14) used the ApcMin/+ mouse, an established model of colorectal cancer and cachexia, to determine the role of circulating IL-6 and polyp burden for the development of cachexia. Mice with the highest circulating IL-6 levels had the most severe cachectic symptoms and the highest polyp burden, while ApcMin/+/IL6−/− mice did not show wasting and had a lower tumor burden. Systemic IL-6 overexpression in such mice induced wasting and polyp formation, but did not induce wasting of skeletal muscle in non-tumor-bearing mice. This suggests that IL-6 induces cachexia in this model by increasing tumor burden. In contrast, administration of IL-6 to rats acutely activated both total and myofibrillar protein degradation in muscle (93). In vitro studies in murine myotubes show that IL-6 decreased the half-life of long-lived proteins by increasing the activity of the 26S proteasome, together with cathepsins B and L (59). This suggests that IL-6 increases protein degradation in muscle by activating both the nonlysosomal (proteasome) and lysosomal (cathepsin) proteolytic pathways. However, unlike TNF-α, IL-6 produced no change in the expression of ubiquitin when administered intravenously to rats (152). The reason for these conflicting results with IL-6 is not known, but further research is required to establish a role for IL-6 in muscle wasting in cachexia.

E. Angiotensin II

The idea that ANG II may be catabolic towards skeletal muscle originated from clinical studies in patients with congestive heart failure (CHF), where treatment with an angiotensin converting enzyme (ACE) inhibitor caused an increase in both subcutaneous fat and muscle bulk in cachectic subjects (4). Infusion of ANG II into rats produced a significant decrease in body weight, with the loss of lean body mass being the major contributor to the weight loss (26). This was attributed to an acceleration of total protein breakdown, and in vitro studies using murine myotubes showed ANG II to directly induce muscle protein catabolism through an increase in activity and expression of the ubiquitin-proteasome pathway (230). In vivo studies in rats showed that some of the weight loss derived from an anorexigenic response to ANG II, together with a catabolic effect (26). In vivo studies showed that ANG II also inhibited protein synthesis in murine myotubes (222). Infusion of ANG II into rats reduced levels of circulating and skeletal muscle IGF-I, which was suggested as the mechanism for the enhanced protein degradation (26). This was confirmed with IGF-I transgenic mice, which overexpress IGF-I in muscle, where wasting was not seen after infusion of ANG II, or the increased mRNA for the ubiquitin ligases atrogin-1 and...
MuRF1 seen in wild-type mice (246). IGF-I was also effective in attenuating the increased protein degradation and activation of the ubiquitin-proteasome pathway by ANG II in murine myotubes (230), as well as the depression in protein synthesis (222).

Like PIF, ANG II induces activation of PKR (Fig. 8), and this has been shown to be responsible for the depression of protein synthesis and increase in protein degradation through the ubiquitin-proteasome pathway (66). Many of the other signaling steps induced by ANG II in activation of the ubiquitin-proteasome pathway are the same as PIF, including formation of ROS (220) by activation of NADPH oxidase by PKC, leading to activation of NFκB (Fig. 8). IGF-I was shown to attenuate activation of PKR by ANG II through the induction of expression of PP1, which dephosphorylates PKR, preventing activation of NFκB, and induction of the ubiquitin-proteasome pathway, and also attenuating phosphorylation of eIF2 on the α-subunit, preventing the depression in protein synthesis (219). In vivo studies suggest that IGF-I blocks the increased protein degradation induced by ANG II in skeletal muscle through an alternative signaling pathway involving Akt/mTOR/p70S6k (246). These results suggest two common signaling pathways for induction of the ubiquitin-proteasome pathway by glucocorticoids and by PIF/TNF-α/ANG II (Fig. 8).

VII. TREATMENT OF CACHEXIA

A. Agents Affecting Appetite

Since cachexia is strongly associated with anorexia, the early attempts at treatment used either caloric supplementation or appetite stimulants. The most widely employed appetite stimulant is megestrol acetate (megace), a synthetic progestin, which may stimulate appetite via NPY in the ventromedial hypothalamus (170) or by down-regulating the synthesis and release of proinflammatory cytokines (169). A systematic review of 15 randomized clinical trials of high-dose progestin therapy showed a statistically significant improvement in both appetite and body weight. However, body composition analysis of patients who gained weight showed that the weight gain was due to an increase in fat and not lean body mass (155). Similar results were obtained with another progestin, medroxyprogesterone acetate (MPA) (241), and also with nutritional supplementation (69). A recent study of insulin treatment of cancer cachexia also showed patients increased whole body fat, with no effect on fat-free lean tissue (162). In this study, insulin has no effect on body mass or food intake, although it did stimulate carbohydrate intake, but there was a significant increase in survival and no evidence that insulin stimulated tumor growth. The inability of megestrol acetate to cause accretion of lean body mass would explain why patients show no significant improvement in the Karnovsky index (performance score) or quality of life. Despite its widespread use, patients receiving megestrol acetate show an increase in thromboembolic phenomena, more edema, an inferior response rate to chemotherapy, and a trend for inferior survival duration (217).

Appetite stimulants do not invariably result in weight gain. Thus cyproheptadine, a histamine antagonist with antiserotonergic and appetite-stimulating effects, produced only a slight improvement in appetite and did not significantly prevent progressive weight loss in anorectic cancer patients (127). Marijuana is known to stimulate appetite and weight gain, but a clinical study of dronabinol, the active ingredient, failed to halt the progressive loss of body weight of cachectic cancer patients, although a subjective improvement in mood and appetite was observed (267). Similar results have been obtained using dietary counseling to increase food intake. Corticosteroids such as dexamethasone, prednisolone, and methylprednisolone are used clinically to enhance appetite and sensation of well-being and performance, usually at the end-stage of cancer, because of their catabolic effect on skeletal muscle. However, despite improvements in the quality of life, they have no beneficial effect on body weight (205).

Several neuropeptides regulate appetite and are currently undergoing trials to establish their efficacy in the treatment of cancer anorexia/cachexia. Among these is ghrelin, a neuropeptide released from the stomach in response to fasting, stimulating food intake. Interestingly, a study of 40 cancer patients found that the mean plasma ghrelin levels were higher among cachectic, compared with noncachectic, subjects, suggesting a defect in the mechanism by which ghrelin stimulates appetite in cachexia (286). Despite this, ghrelin has been shown to stimulate energy intake by ~30% in patients with cancer anorexia without any side effects (192). The period of infusion was too short to measure changes in body weight, so further clinical studies are required to establish if long-term administration of ghrelin causes a significant increase in body weight, particularly lean body mass. However, clinical evaluation of RC-1291, a ghrelin mimetic, in 60 cachectic cancer patients showed an increase in handgrip strength and lean body mass when compared with placebo, although there were no differences in body weight or quality of life (84). Certainly in mice bearing the cachexia-inducing MCG101 tumor, high-dose ghrelin (40 µg/day) increased food intake and body weight, but body composition analysis showed this to be due to an increase in whole body fat (269). However, administration of ghrelin and a synthetic ghrelin analog by continuous infusion using subcutaneous osmotic minipumps to rats bearing a cachexia-inducing tumor caused a significant increase in food consumption and weight gain through
maintenance of lean body mass (51). Ghrelin-treated animals exhibited a significant increase in expression of the orexigenic peptides agouti-related peptide and NPY and a significant decrease in the expression of the IL-1 receptor transcript. The results of clinical studies with other neuropeptides are also anticipated.

B. Agents Affecting Cachectic Mediators or Signaling Pathways

1. EPA

This is an n-3, 21-carbon atom, polyunsaturated fatty acid, with five double bonds, found in oily fish, such as salmon, mackerel, and sardine. EPA was originally identified for clinical evaluation through its ability to attenuate weight loss, particularly loss of skeletal muscle mass, in the murine MAC16 cachexia model, through its ability to downregulate the increased expression and activity of the ubiquitin-proteasome proteolytic pathway (276). This effect is achieved through the ability of EPA to attenuate activation of NFκB by PIF by stabilizing the IκB/NFκB complex through inhibition of upstream signaling pathways, particularly the release of AA from phospholipids (PL) and its metabolism by 15-LOX to 15-HETE (Fig. 8) (277). Another inhibitor of 15-LOX, CV-6504 (Fig. 8), also shows anticachectic activity in the MAC16 model, and a clinical study in patients with advanced pancreatic cancer showed that CV-6504 was well-tolerated, with few side effects, and that it produced stabilization of body weight during the 12-wk study period (80). EPA also downregulates ZAG expression through interference with glucocorticoid signaling, which may be responsible for its ability to preserve adipose tissue in cachexia (224).

Very few clinical studies have used pure EPA, and most have used fish oil as a source. Most of these studies have been uncontrolled but have shown stabilization of body weight in cachectic subjects with pancreatic cancer. Since EPA has no effect on the depression of protein synthesis in muscle (276), EPA has been combined with a nutritional supplement rich in protein and energy, since amino acids, particularly the BCAA, stimulate protein synthesis. An initial controlled clinical evaluation of the combination showed a significant weight gain (2 kg after 7 wk of treatment), that was attributed to an increase in lean body mass, with no change in fat mass (13) (Fig. 9). However, a randomized control trial failed to find an increase in body weight of the EPA/nutrient supplement compared with the nutrient supplement alone (75). In this study, there was poor compliance, with only 70% of the intended dose being consumed, and measurement of plasma EPA levels showed that 18% of the control patients were taking a fish oil supplement. A secondary analysis showed that increased plasma levels in the experimental group were associated with an increase in weight and lean body mass. In addition, patients receiving the EPA supplement showed an increased PAL, which may reflect an improved quality of life (190).

Although the primary effect of EPA may be to attenuate muscle atrophy, it has also been shown to be an appetite stimulant, though slightly inferior to megestrol acetate (121). Using a primary end point of weight gain of 10%, or more, the authors concluded that the EPA/nutritional supplement was inferior to megestrol acetate and that combinations were no more effective than megestrol acetate alone. However, body composition was not measured, so it is difficult to exclude fluid retention for the superior response to megestrol acetate. Also, compliance with the EPA supplement was not measured, which was a confounding factor in the previous study (75).

These results suggest that further trials are required to establish the anticachectic activity of EPA. Such trials should be placebo-controlled, and compliance should be monitored and of sufficient duration (at least 4 wk) for changes in body composition to become evident. These results with EPA should be compared with those of bortezomib, a proteasome inhibitor, which showed no effect on appetite or weight loss in patients with metastatic pancreatic cancer (118). This suggests that inhibitors of the increased proteasome expression in skeletal muscle rather than the proteasome itself may provide better prospects for the alleviation of muscle atrophy.

2. β-Hydroxy-β-methylbutyrate

β-Hydroxy-β-methylbutyrate (HMB) is similar to EPA in that it attenuates PIF-induced protein degradation in muscle, by downregulating the increased expression and activity of the ubiquitin-proteasome pathway (242). Like
EPA, it prevents activation of NFκB through inhibition of activation of PKC, resulting in stabilization of the IκB/NFκB complex. Unlike EPA, HMB also attenuates the depression of protein synthesis, both in a murine model of cachexia and in murine myotubes in response to PIF (62). This is achieved through increased phosphorylation of mTOR, p70S6k, and 4E-BP1, reducing the affinity for eIF4E, and increasing the concentration of the active eIF4G.eIF4E complex (Fig. 5). HMB also attenuated phosphorylation of PKR and eIF2α and reduced phosphorylation of eEF2. These effects would act to stimulate protein synthesis. Since HMB is a metabolite of leucine, it is not surprising that it acts by a similar mechanism.

HMB has undergone a placebo-controlled clinical trial in patients with cancer cachexia (178). This showed that in patients with advanced (stage IV) cancer, HMB together with L-glutamine and L-arginine increased body weight, and this was attributed to an increase in lean body mass, with no changes in fat mass. Similar results have been reported in patients infected with HIV. These results suggest that HMB should receive more extensive testing and use for the treatment of muscle atrophy in cancer.

3. Thalidomide

Thalidomide was evaluated as a treatment for cachexia due to its ability to reduce production of TNF-α, by increasing the degradation rate of TNF-α mRNA, but it also blocks NFκB-regulated genes through suppression of IKK activity (129) (Fig. 8). Clinical evaluation of thalidomide in the treatment of cancer cachexia is in its infancy, but the results are encouraging. Thus a small study in 10 patients with nonobstructing and inoperable esophageal cancer, in which the patients received an isocaloric diet for 2 wk followed by 2 wk on thalidomide, found that the patients lost both body weight and lean body mass while on the diet alone, but they gained both weight and lean body mass when receiving thalidomide (133). A larger study evaluated thalidomide in 50 patients with advanced pancreatic cancer, who had lost at least 10% of their body weight (94). Like EPA, patients receiving thalidomide did not lose body weight, while the placebo group lost 3.62 kg in the 8-wk trial period. Arm muscle mass was also stabilized, suggesting that thalidomide may prevent loss of lean body mass.

Further studies are required to confirm a beneficial effect of thalidomide in the treatment of cancer cachexia. Although thalidomide was originally identified as an anti-cachectic agent, because of its effect on TNF-α production, it is unlikely that its biological activity is manifested by this mechanism, since pentoxyfylline, which has been reported to decrease TNF-α mRNA levels, had no effect on either appetite or body weight in a double-blind, controlled trial in cachectic cancer patients (90). In addition, infliximab, a more specific inhibitor of TNF-α than thalidomide, has been reported to have no statistically significant change in lean body mass or Karnovsky performance status compared with gemcitabine (278). However, since thalidomide can potentially inhibit activation of NFκB, it could also function to attenuate the signaling cascade initiated by PIF, ANG II, or TNF-α, downregulating the increased expression of the ubiquitin-proteasome pathway (Fig. 8).

4. Nonsteroidal anti-inflammatory agents

The majority of patients with gastrointestinal cancer have an APR, which has been suggested to contribute to weight loss, and therefore, if this is downregulated, weight loss should also be attenuated. Thus administration of ibuprofen to patients with unresectable pancreatic cancer reduced REE and serum CRP levels (280). In patients with advanced gastrointestinal cancer and weight loss, a combination of megestrol acetate and ibuprofen produced an increase in body weight and improvement in the quality of life, while patients on the megestrol acetate/placebo arm showed a decrease in body weight (182). Unfortunately, there was no body composition analysis and no follow up to these clinical trials.

Although it had no effect on body weight, indomethacin, another nonsteroidal anti-inflammatory agent, prolonged the mean survival time in cancer patients with weight loss compared with placebo (from 250 ± 28 to 510 ± 28 days) (161). Further studies on cyclooxygenase inhibitors are underway. The mechanism of action of these agents is unclear, although prostaglandins have been postulated as mediators of cachexia (240). Alternatively, ibuprofen has been shown to inhibit constitutive activation of NFκB and IKK in prostate cancer cells (199) and thus has the potential to inhibit induction of expression of both MuRF1 and proteasome subunits in skeletal muscle (Fig. 8).

VII. CONCLUSIONS

The past decade has seen enormous steps in our understanding of the mechanisms of loss of both adipose tissue and skeletal muscle in cancer cachexia, but this is only just beginning to be translated into clinical therapy. Hopefully new agents will be developed against the myriad of signaling pathways that are essential for atrophy of adipose tissue and skeletal muscle mass. Despite the fact that cachexia has been estimated to be responsible for the death of up to 22% of cancer patients (272), progress in this area has been slow to date. This was basically due to poor experimental models of this condition and a lack of understanding of the mechanisms involved. Cachexia is seen not only in cancer patients, but in those with sepsis, CHF, diabetes, severe trauma, and renal failure leading to metabolic acidosis, denervation atrophy, and weightloss-
ness. There is certainly an overlap between the mechanisms of tissue loss, particularly muscle atrophy, in these conditions and those seen in cancer patients. Thus agents developed for the treatment of cachexia in cancer may also be effective in these other conditions, thus enlarging the potential patient database.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: M. J. Tisdale, Nutritional Biomedicine, School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK (e-mail: m.j.tisdale@aston.ac.uk).

REFERENCES


cachectic effects of formoterol: a drug for potential treatment of muscle wasting. 


36. Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HGW, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoel- 

37. Carlberg U, Nilsson A, Nygard O. Functional properties of phos- 


39. Chance WT, Balasubramanian A, Thompson H, Mohapatra B, 


46. Cunningham TJ, Hodge L, Speicher D, Rheim D, Tyler-Polsz 

47. Cunningham TJ, Hodge L, Speicher D, Rheim D, Tyler-Polsz 


54. Diffee GM, Kalfos K, Al-Majid S, McCarthy DO. Altered expres- 

55. Differenziati- 


59. Ebisu C, Tsuchinaka T, Morimoto T, Kan K, Injima S, Yano M, Komimani E, Tanaka K, Monden D. Interleukin-6 induces pro- 


61. Egann JJ, Greenberg AS, Chang MK, Wek SA, Moos MC Jr, 

62. Eds 

63. Eley HL, Russell ST, Baier JI, Mukherji P, Tisdale MJ. Signaling pathways initiated by β3-hydroxy-β-methylbutyrate to at- 


66. Eley HL, Skipworth RJ, Deans DAC, Fearon KCH, Tisdale MJ. Increased expression of phosphorylated forms of RNA-depend- 


72. Falconer JS, Fearon KC, Plester CE, Ross JA, Carter DC. Cytokines, the acute phase response, and resting energy expendi-


MECHANISMS OF CANCER CACHEXIA


