

LOSS OF SKELETAL MUSCLE IN CANCER: BIOCHEMICAL MECHANISMS

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1. ABSTRACT

Patients with cancer often undergo a specific loss of skeletal muscle mass, while the visceral protein reserves are preserved. This condition known as cachexia reduces the quality of life and eventually results in death through erosion of the respiratory muscles. Nutritional supplementation or appetite stimulants are unable to restore the loss of lean body mass, since protein catabolism is increased mainly as a result of the activation of the ATP-ubiquitin-dependent proteolytic pathway. Several mediators have been proposed. An enhanced protein degradation is seen in skeletal muscle of mice administered tumour necrosis factor (TNF), which appears to be mediated by oxidative stress. There is some evidence that this may be a direct effect and is associated with an increase in total cellular-ubiquitin-conjugated muscle proteins. Another cytokine, interleukin-6 (IL-6), may play a role in muscle wasting in certain animal tumours, possibly through both lysosomal (cathepsin) and non-lysosomal (proteasome) pathways. A tumour product, proteolysis-inducing factor (PIF) is produced by cachexia-inducing murine and human tumours and initiates muscle protein degradation directly through activation of the proteasome pathway. The action of PIF is blocked by eicosapentaenoic acid (EPA), which has been shown to attenuate the development of cachexia in pancreatic cancer patients. When combined with nutritional supplementation EPA leads to accumulation of lean body mass and prolongs survival. Further knowledge on the biochemical mechanisms of muscle protein catabolism will aid the development of effective therapy for cachexia.

2. INTRODUCTION

One of the commonest paraneoplastic syndromes is cachexia, reported to be present in one-half of all cancer patients depending upon tumour type. Patients with carcinomas of the pancreas and stomach have the highest incidence of cachexia (83-87%), while patients with breast cancer, acute non-lymphocytic leukaemia and sarcomas have the lowest incidence (31-40%) (1). Cachexia is most frequently manifested by weight loss, but also includes anorexia, asthenia and anaemia. The degree of cachexia appears to be unrelated to tumour burden, anatomical site of involvement or the presence or absence of metastasis. As in simple starvation weight loss in cachexia involves loss of both muscle and adipose tissue, but whereas in the former most of the weight loss is derived from fat, in cachexia muscle mass is also depleted, such that for a given degree of weight loss there is more loss of muscle mass in cachexia than in starvation (2). The body composition analysis of lung cancer patients who had lost 30% of their pre-illness stable weight showed an 85% decrease in total body fat and a 75% decrease in skeletal muscle protein mass (3). Unlike anorexia nervosa, where loss of visceral mass is proportional to the loss of skeletal muscle, in cancer there are insignificant changes in liver, kidney and heart weight (2) and there appears to be a specific depletion of the skeletal muscle mass.

Although weight loss is often a presenting symptom in many cancers (4) progressive cachexia is usually associated with advanced disease and ultimately leads to death. Cachexia has been estimated to account for

between 10 (5) and 22% (6) of all cancer deaths, and commonly occurs when patients have lost more than 30% of their ideal body weight. Death is commonly through hypostatic pneumonia due to erosion of respiratory muscles (7). The poor nutritional status also leads to a susceptibility to infection leading to death by sepsis.

Patients with cachexia also show a decreased response to chemotherapy compared with weight stable patients (1). This probably reflects the fact that patients with cachexia have to be administered lower doses of chemotherapy, due to more frequent and more severe dose-limiting toxicity (8). Patients with weight loss receive on average one month less treatment than comparable weight stable patients and patients who stopped losing weight had a better overall survival. Thus cachexia is a progressive debilitating condition which reduces the patients quality of life, interferes with tumour therapy and eventually leads to death. An understanding of the mechanism of this syndrome is important for effective therapy.

2.1. Effect of nutritional repletion

In addition to cancer a variety of disease states including AIDS, sepsis, renal failure, burns and trauma are associated with weight loss, although the causative factors may be different in each case. As in cancer cachexia anorexia is common, resulting in a low energy intake, and therefore attempts have been made with nutritional repletion. A similar result is seen in all cases. Thus trials of total parenteral nutrition in patients with cancer cachexia showed a short-term weight gain suggesting retention of water (9). Body composition analysis showed a temporary maintenance of body fat stores, but no preservation of lean body mass. When individuals with HIV were given parenteral nutrition an increase in body weight was observed, but this also did not represent accrual of lean body mass (10). A similar situation is seen in sepsis, where aggressive nutritional support results in weight gain, but *in vivo* neutron activation measurement of body composition showed that this was adipose tissue accumulation and not lean body mass (11). Thus nutritional repletion alone is insufficient to replenish skeletal muscle protein stores, despite calculations suggesting that nitrogen intake should be sufficient to accomplish this task.

Similar results are obtained with appetite stimulants. Megestrol acetate (Megace) is a progestational agent, which was initially observed to induce a weight gain in breast cancer patients. A subset of patients with hormone-independent cancer and weight loss have been shown to have a weight gain of greater than 5% when treated with Megace with improved appetite (12), although body composition measurements indicate that the majority of the weight gain was due to an increase in adipose tissue, while an increase in body fluid may be responsible for a minority of the weight gain (13). Stimulation of appetite alone may not result in any weight gain in cachectic cancer patients. Thus medroxyprogesterone, a synthetic derivative of progesterone related to Megace caused a significant improvement in appetite in patients with advanced malignant disease, but this did not result in an increase in body weight (14). The serotonin antagonist,

cyproheptadine, also produced a small increase in appetite in cachectic cancer patients, but did not result in a significant enhancement in body weight (15). These results suggest that although anorexia may be present in cachectic cancer patients, it is not a major contributor to the loss of lean body mass. It is therefore important to understand the changes in protein flux in the skeletal muscle of cachectic cancer patients and the mediator(s) of these events.

2.2. Protein metabolism in cachexia

Patients with cachexia show abnormalities in protein metabolism, with an increased whole body protein turnover (16), an increased protein breakdown in skeletal muscle (17) and a decreased protein synthesis (18), while hepatic protein synthesis is increased (19). The major problem with studies of whole body protein kinetics in cancer patients is that the non-muscle protein compartment is much larger than the muscle protein and may obscure changes in the latter. Thus in a study by Emery *et al.* (20) no change in total body protein synthesis was observed in weight losing cancer patients compared with healthy controls. However, the contribution of skeletal muscle to whole body protein synthesis was found to be lower in cachectic patients compared to healthy controls. The maintenance of the total protein synthetic rate in these patients may be due to a two-fold increase in visceral protein synthesis, resulting from the increased hepatic production of acute phase proteins (APP). An elevated C-reactive protein (CRP) level is strongly associated with a shorter survival time in patients with advanced pancreatic cancer (21). Synthesis of APP has been suggested to be partly responsible for the catabolism of skeletal muscle proteins to provide the essential amino acids required for APP. Despite the increased synthesis of APP, hypoalbuminemia is common in cancer patients, although this does not appear to be due to a decreased hepatic albumin synthesis (22).

The catabolism of muscle proteins may have a beneficial effect on tumour growth. Glutamine and alanine represent more than 50% of the amino acids exported from skeletal muscle (23). Both amino acids are produced by the metabolism of other amino acids, mainly the branched chain amino acids and aspartate. Both alanine and glutamine are efficient vehicles for the transport of nitrogen and carbon-skeletons between the various tissues in the body. Glutamine is a prime source of nitrogen for tumours, both for the biosynthesis of purine and pyrimidine bases, and as an energy source. As such glutamine antagonists have demonstrated antitumour activity (24). Patients with progressive cancer have been shown to have an increased glucose synthesis from alanine (25), since most solid tumours rely almost exclusively on the anaerobic metabolism of glucose as the main energy source. Protein turnover, increased APP synthesis, and an increased cycling of glucose, probably contribute to an elevated resting energy expenditure seen in some cancer patients (26).

2.3. Mechanism of catabolism of muscle proteins

There are three major proteolytic pathways responsible for the catabolism of proteins in skeletal muscle

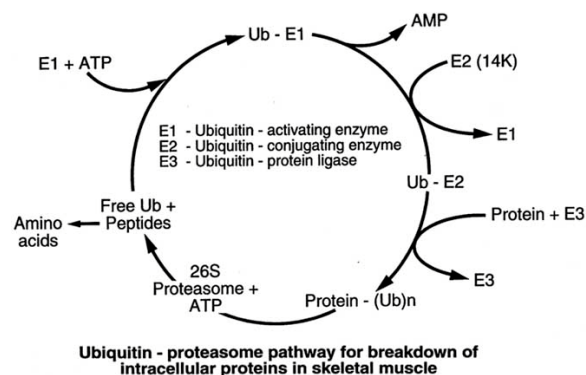


Figure 1. Ubiquitin - proteasome pathway for breakdown of intracellular proteins in skeletal muscle.

(i) lysosomal system, including the cysteine proteases cathepsins B, H and L and the aspartate protease cathepsin D. This system is mainly concerned with the proteolysis of extracellular proteins and cell surface receptors (27). (ii) a cytosolic calcium-activated system, calpains I and II, which is independent of ATP and plays an important role in tissue injury, necrosis and autolysis (28). (iii) ATP-ubiquitin-dependent proteolytic pathway, which is believed to be involved in the catabolism of the majority of the intracellular proteins in skeletal muscle (27). This pathway is thought to be responsible for the accelerated proteolysis seen in a variety of wasting conditions including fasting, sepsis, metabolic acidosis, acute diabetes, weightlessness and cancer cachexia (28).

In this process proteins are marked for degradation by the attachment of ubiquitin, a small protein, which is first activated by ubiquitin-activating enzyme (E1) (Figure 1). This transfers the ubiquitin to a carrier protein (E2), which either ligates the ubiquitin directly to the target protein or does so in the presence of ubiquitin protein ligase. The conjugation mediated by E2 has been regarded as the rate-limiting step in this pathway (29). Proteolysis occurs within a large multisubunit complex known as the proteasome, consisting of a central catalytic chamber (20S proteasome) together with two terminal regulatory subunits, the 19S complex (or PA700) and the 11S regulator (or PA28). These are attached at both ends of the central chamber in opposite orientations to form the 26S active proteasome (Mr ~ 2000kDa) in a process requiring ATP. MSS1 and P45 are ATPase subunits of the 19S complex, which are thought to provide energy to inject the substrate into the proteolytic chamber of the 20S proteasome. There are at least 6 ATPases associated with the 26S proteasome, the function of which is to provide a continuous supply of energy for protein degradation (30). The 20S proteasome has a molecular mass of 700-750kDa, and is a tube-like structure, consisting of a stack of four rings, two outer alpha-rings and two inner beta-rings in the order alpha beta beta alpha. The proteolytic sites are found on the inner surface of the beta-subunits. There are three types of proteolytic activity, one of which is 'chymotrypsin-like' in specificity, one of which is 'trypsin-like', and one of which cleaves after acidic residues and is usually termed

'peptidylglutamyl-peptide hydrolyzing'. The proteasome releases short oligopeptides having mean lengths of 6-9 residues (31). The majority of the peptides generated by the proteasome are rapidly degraded into amino acids by cytosolic peptidases.

In experimental cachexia models in rats transplanted with the Yoshida ascites hepatoma (32), Yoshida sarcoma (33) and in mice transplanted with the MAC16 colon carcinoma (34) there is a co-ordinate increase in expression of ubiquitin, the 14kDa ubiquitin carrier protein E2, as well as proteasome subunits, providing evidence that this pathway plays a major role in muscle atrophy in cancer cachexia. This process is independent of the amount of protein consumed and therefore it is not surprising that simple nutritional supplementation is unable to prevent muscle catabolism. To do this specific drugs are required, which inhibit the proteasome, eg the peptide aldehyde MG132 blocks several peptidase activities of the 20S proteasome and reduces protein degradation in skeletal muscle without altering protein synthesis (35). Cachexia induced in mice transplanted with the Lewis lung carcinoma transfected with cDNA for IL-6 was found to be associated with an increased ubiquitination of muscle proteins in comparison with non tumour-bearing mice. This elevation was found to be down-regulated in mice treated with either of the polyunsaturated fatty acids, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), which were also shown to attenuate the development of cachexia in this model (36). In rats bearing the AH-130 Yoshida ascites hepatoma muscle wasting was observed and was associated with a 500% increase in polyubiquitin gene expression (37). Treatment with clenbuterol suppressed the elevation of protein breakdown rates to close to control values and this was concomitant with a decrease in the expression of polyubiquitin genes (38).

Some studies suggest that increased gene expression of proteasomal subunits are rate limiting for protein degradation (33). Thus a small tumour mass (less than 0.3% of body weight) of the Yoshida sarcoma in rats produced a significant reduction in protein mass of the extensor digitorum longus (EDL) muscle close to the tumour, compared with the contralateral muscle. Protein loss resulted totally from increased proteolysis and not depressed protein synthesis. Neither lysosomal or calcium-dependent proteolysis were increased, and the ATP-ubiquitin-dependent proteolytic pathway was thought to be responsible for muscle atrophy, since mRNA levels for ubiquitin, 14kDa ubiquitin carrier protein E2, and the C8 and C9 proteasome subunits, increased in atrophying muscles. In tibialis anterior muscles, where protein mass was not reduced, mRNA levels for ubiquitin and E2 were also increased, but not for the proteasome subunits. Therefore increased expression of ubiquitin and E2 alone are not sufficient to induce proteolysis, but seem to be induced prior to increased proteasome expression. Ubiquitin has roles other than in proteolysis such as DNA repair (39) and mitosis (40) and thus an increased expression alone may not be related to ATP-ubiquitin-dependent proteolysis. These results suggest that the

proteasome catalytic activity rather than substrate ubiquitination is the rate-limiting step in the pathway.

These changes in protein catabolism in cachexia have lead to the search for mediators of the process. This is essential to mount an effective therapeutic approach. Mediators are of two types; those produced by host tissues, the cytokines including tumour necrosis factor alpha (TNF), the interleukins -1 and -6 (IL-1 and IL-6) and interferon gamma (IFN) and those produced by tumour cells such as proteolysis-inducing factor (PIF).

2.4. TNF

TNF was first identified as a cachectic factor as a result of studies in rabbits chronically infected with *Trypanosoma brucei brucei* (41). Studies with CHO cells transfected with the cDNA for human TNF confirmed the catabolic activity of this cytokine (42). Interestingly the effect produced depended on the site of implantation of the cells. Intracerebral transplantation produced hypophagia and weight loss comparable with starvation, ie loss of body fat with conservation of protein, while intramuscular transplantation produced depletion of both lipid and protein without anorexia (43). Muscle wasting was associated with increased levels of aldehyde products of lipid peroxidation and increased nitric oxide synthase, which were suggested as mediating the changes, since the decreased body weight, muscle wasting and skeletal muscle abnormalities were prevented by treatment with the anti-oxidants D-alpha-tocopherol or BW755c or the nitric oxide synthase inhibitor nitro-L-arginine (44).

Elevated TNF production by tumour or spleen cells have been suggested to be responsible for body weight loss and muscle wasting in experimental cachexia models induced by rodent tumours (45,46) and a human squamous cell carcinoma xenograft (47). Weight loss induced by the Yoshida AH-130 ascites hepatoma was associated with increased concentrations of circulating TNF (45). Daily administration of anti TNF-immunoglobulin was shown to decrease protein degradation rates in skeletal muscle, heart and liver compared to animals receiving a non-specific immunoglobulin. However, this treatment did not prevent a reduction in body weight. Anti-TNF-antibody treatment partially reversed the loss of body fat in mice bearing the Lewis lung adenocarcinoma, but did not cause a change in body weight (46), while in the human MH-85 model normalization of body weight was not observed (47). These results suggest that other mediators of cachexia may be involved in these models in addition to TNF.

Administration of TNF to rats has been shown to result in an enhanced protein degradation in skeletal muscle, even though body weight loss was not apparent (48). Protein accumulation was reduced due to a decreased protein synthesis in addition to the enhanced proteolytic rate. Changes in protein metabolism were only observed in red-type muscles, such as soleus, while little effect was seen in white-type muscle, such as EDL (49). In man TNF infusion was also shown to increase whole-body protein turnover, as measured by ¹⁵N enrichment of urinary urea and ammonia (50). Doses of TNF greater than 100 micrograms per square meter also increased total amino acid

efflux by 2.5 to 5.0-fold suggesting protein catabolism. In the rat a single intravenous injection of TNF resulted in a significant increase in UCP2 and UCP3 gene expression in skeletal muscle (51), which may play a role in the increase of energy expenditure associated with cytokine treatment.

For some time it was thought that the effect of TNF on protein degradation *in vivo* was an indirect effect, since it was not possible to directly stimulate muscle catabolism by TNF *in vitro* using either tyrosine or 3-methylhistidine as a measure of the proteolytic rate. However, two recent reports indicate a direct effect of TNF on muscle catabolism. Incubation of isolated rat soleus muscle with TNF for 180 minutes resulted in an increase in ubiquitin gene expression, but produced no change in expression of the C8 proteasome subunit (52). Although this change was suggested to be evidence for an upregulation of the ubiquitin-dependent proteolytic system care must be taken in the interpretation of rises in mRNA for ubiquitin, since this may also signal an increased cell death through apoptosis (53), rather than an overall increase in muscle proteolysis. Using myotubes derived from the C₂C₁₂ muscle cell line Li *et al.* (54) found a reduced protein content and loss of myosin heavy chain after incubation with TNF for 72 hours. Total cellular ubiquitin conjugated muscle proteins were increased, but no measurements were made on proteasome activation. TNF activated binding of nuclear factor-kappaB (NF-kappaB) to its targeted DNA sequence, and stimulated the degradation of NF-kappaB-inhibitory protein (I-kappaB), and this effect was determined to be due to reactive oxygen species. Thus the mechanism of protein degradation induced by TNF *in vitro* was similar to that previously found *in vivo* (44).

Indirect evidence that TNF may activate the proteasome system is provided from rats bearing the Yoshida sarcoma (55). Expression of MSS1, an ATPase subunit of the 19S complex was found to be increased in wasting muscle from tumour-bearing rats and this was normalized when the animals were given pentoxifylline, which reduces the expression of TNFmRNA. However, when 35 patients with lung, gastrointestinal and other tumours and weight loss and anorexia were administered pentoxifylline no beneficial effect was observed (56). This suggests that TNF may not be a major contributor to muscle wasting in human cancer cachexia. Indeed a number of studies (57-59) have failed to find elevated concentrations of TNF in the serum of cachectic cancer patients, although raised concentrations have been found in cachectic patients with parasitic infections such as leishmaniasis and malaria (60), septicemia (61) and AIDS (62). It has been suggested that increases of TNF in the serum of cancer patients may be transient, and thus unmeasurable, but this would not explain why elevated levels are found in other weight losing conditions. These results have lead to the search for

other cytokines which may initiate muscle wasting in cancer.

2.5. IL-6

Most of the studies linking IL-6 to cancer cachexia have come from mice bearing murine colon-26 adenocarcinoma. In this model cachexia was associated with increasing serum levels of IL-6 and administration of anti-IL-6 immunoglobulin significantly suppressed the progression of cachexia (63). IL-6 may play a central role in muscle wasting in this model, since antibodies to the IL-6 receptor reduced the loss of gastrocnemius muscle, but did not affect overall loss of body weight or adipose tissue (64). This suggests that there may be other factors in addition to IL-6 involved in the overall process of cachexia in this model. This view is substantiated from studies with two variants of the colon-26 tumour, clone 20, which is cachexia-inducing and clone 5, which does not induce cachexia in mice. Although IL-6 mRNA was detected only at the tumour site of the clone 20 variant (65) serum levels of IL-6 were found to be elevated in mice transplanted with either clone, although levels were found to be 35% lower in animals bearing the clone 5 variant (66). Also infusion of IL-6 into mice bearing clone 5 tumours did not induce weight loss (67). IL-6 also failed to cause weight loss in normal mice, despite being associated with an increased APP production (65). In a separate study (68) murine IL-6 was repeatedly administered to healthy mice over a 7 day period at a dose of 250 micro grams kilogram body weight⁻¹ day⁻¹, and, although it produced an hepatic APP response, there was no effect on body weight or food intake. However, another member of the IL-6 superfamily, ciliary neurotrophic factor (CNTF) when administered at the same dose level as IL-6 did produce anorexia and lean tissue wasting with a 218% increase in carcass protein breakdown rate compared with freely fed controls, and it also increased APP production. These results suggest that APP production alone was not sufficient to induce cachexia. The effect of CNTF on muscle protein catabolism appears to be indirect, since it was unable to enhance myofibrillar protein degradation when incubated directly with EDL muscles *in vitro*. Mice implanted with C6 glioma cells genetically modified to secrete CNTF showed rapid breakdown of both adipose tissue and skeletal muscle, together with depressed levels of glucose and triglyceride culminating in death after a period of 7-10 days (69). Thus CNTF exhibits a number of extra-neural effects.

In contrast with these results CHO cells transfected with the IL-6 gene produce weight loss and cachexia in nude mice (70). Loss of muscle mass is associated with increased mRNA levels for cathepsins B and L, as well as both poly- and mono-ubiquitin, but the levels of MSS1 and S4, two ATPase subunits of the 19S complex and the C2 and C8 subunits of the proteasome did not change (71). The muscle atrophy was completely blocked by anti-mouse IL-6 receptor antibody (71). In mice bearing the colon-26 adenocarcinoma weight loss was also associated with increased mRNA levels for cathepsins B and L as well as enzyme activity, poly-ubiquitin and proteasome subunits in muscle (64). In this case anti-mouse IL-6 receptor antibody reduced the activity of

cathepsins B and L as well as mRNA levels of cathepsin-L and poly-ubiquitin, but had no effect on the mRNA levels of proteasome subunits, although it did reduce the loss of the gastrocnemius muscle weight to 84% of that in control mice. Using C₂C₁₂ myotubes *in vitro* Ebisui *et al.* (72) found that IL-6 shortened the half-life of long-lived proteins and increased the activity of the 26S proteasome and cathepsins B and L, suggesting activation of both non-lysosomal (proteasomes) and lysosomal (cathepsin) proteolytic pathways. Interestingly in the same system TNF prolonged the half-life of long-lived proteins, while reducing the protease activities of the 20S proteasome and cathepsins B and L. These results suggest a role for IL-6 in the regulation of muscle proteolysis.

However, these results have been questioned from a study of sepsis in IL-6 knockout mice (73). Thus although IL-6 was not detected in the plasma of IL-6 knockout mice compared with their wild-type counterparts, total and myofibrillar protein breakdown rates were the same in both groups of animals as were ubiquitin mRNA levels. In addition treatment of normal mice or of cultured IL-6 myotubes with IL-6 did not influence protein breakdown rates. Also mice bearing the colon 26 adenocarcinoma clone 20 transfected with the IL-10 gene were found not to develop cachexia, despite the fact that serum IL-6 levels were still elevated (74). These results suggest that other factors in addition to IL-6 may be involved in muscle catabolism in cachexia, despite the report that weight losing patients with nonsmall cell lung cancer have been shown to have significant increases in serum IL-6 and C-reactive protein as a measure of APP production, compared with patients with the same tumour, but without weight loss (75).

2.6. IL-1

Intratumoral injections of IL-1 receptor antagonist into mice bearing the colon 26 adenocarcinoma caused a significant reduction in cachexia (76), while administration to rats bearing the Yoshida ascites hepatoma was found to be ineffective in preventing tissue depletion and protein hypercatabolism (77). IL-1, when isolated from adherent human monocytes was found to stimulate muscle protein degradation in intact muscles by a process sensitive to inhibition of lysosomal thiol proteases (78). However, recombinant human IL-1-beta was not able to reproduce this effect, suggesting that it was due to another factor (79). In addition infusion of IL-1-beta together with TNF into rats bearing the Yoshida sarcoma reduced the tumour fractional protein synthetic rate, but had no effect on muscle protein catabolism, and transfection of a cachexia-inducing tumour (colon-26) with the gene for the IL-1 receptor antagonist failed to inhibit the production of cachexia (77). These results cast doubt on whether IL-1 has any role in protein catabolism in cancer cachexia.

2.7. IFN-gamma

Severe cachexia has been shown to develop rapidly in nude mice inoculated with CHO cells constitutively producing mouse IFN-gamma (80). Passive immunization against IFN prior to tumour cell inoculation prevented the cachexia, but both IFN release and the

presence of tumour cells were found to be required for activity. This implies that although IFN has a role in the pathogenesis of cachexia other factors are also involved. In mice bearing the Lewis lung tumour weight loss was associated with production of IFN (81). Antibodies to this cytokine attenuated the loss of body fat, but had no effect on total body protein. While intravenous administration of IL-6 or leukaemia inhibitory factor (LIF) produced no change in ubiquitin gene expression in rat skeletal muscle, TNF, IL-1 and IFN all caused an increased expression of both the 1.2 and 2.4 kilobase transcripts (82). IFN also induces expression of proteasome subunits of the beta-type, but these are not found in muscle. There have been no direct studies linking IFN to muscle protein catabolism.

2.8. PIF

Evidence that muscle catabolism in cachexia may be due to a circulatory proteolysis-inducing factor came from studies in mice (83) and man (84). Thus serum from mice bearing the cachexia-inducing MAC16 tumour was found to produce an increase in protein catabolism in isolated gastrocnemius muscle as measured by tyrosine release (83), and a similar effect was seen using serum samples of cancer patients with weight loss greater than 10% (84). PIF was subsequently purified from both the MAC16 tumour and from the urine of cachectic cancer patients by affinity chromatography using an antibody derived from the serum of mice bearing the MAC16 tumour (85). Both the human and murine forms of PIF appeared to be chemically and immunologically identical comprising a sulphated glycoprotein of Mr 24kDa. PIF was capable of initiating muscle protein degradation directly *in vivo*, an effect mediated through the N- and O-linked sulphated oligosaccharide chains (86). Administration of the purified PIF to normal mice produced rapid weight loss (about 10% in 24 hours) with specific depletion of the non-fat carcass mass, which was reversed by antibody treatment (87). Weight loss was associated with a significant decrease in the weight of the spleen and soleus and gastrocnemius muscles, but with no effect on the weight of the heart or kidney and an increase in weight of the liver (88). The decrease in lean body mass was accounted for by an increase (by 50%) in protein degradation and a decrease (by 50%) in protein synthesis in gastrocnemius muscle (89). PIF induces an accumulation of ubiquitin-protein conjugates in gastrocnemius muscles in weight-losing mice, and the ATP-ubiquitin-dependent proteolytic pathway probably plays a major role in PIF-induced protein catabolism (88).

Unlike the cytokines PIF induces protein catabolism directly *in vitro* using isolated gastrocnemius (86) or soleus muscles (89) and the C₂C₁₂ mouse myoblast cell line derived from the satellite cell population of the thigh muscle of a two-month-old mouse (90). Protein degradation induced in both soleus muscle (89) and C₂C₁₂ myoblasts (90) by PIF followed a bell-shaped dose-response curve, with high concentrations of PIF being inhibitory to further protein catabolism. A similar situation has been observed *in vivo* in a rat model of cachexia (91). Thus the rate of muscle catabolism, as measured by phenylalanine release, was highest at small tumour burdens

and decreased as the tumour grew larger, which appeared to be caused by the loss of the capacity of the tumour to further breakdown muscle. The effect is similar to hormone-induced desensitization of lipolysis in adipocytes, and may result from down-regulation of receptors.

PIF-induced protein catabolism in C₂C₁₂ myoblasts was accompanied by an increased release of arachidonic acid with a dose-response curve parallel to that of protein degradation (90). The arachidonic acid was rapidly metabolized to prostaglandins (PG) E₂ and F_{2α} and to 5-, 12-, and 15-hydroxyeicosatetraenoic acids (HETES). Several studies have implicated PGE₂ production with protein degradation in cancer cachexia, and PGE₂ has been shown to directly increase protein degradation in diaphragm and soleus muscles (92). Thus both of the non-steroidal anti-inflammatory drugs naproxin (93) and acetylsalicylic acid (94) have been shown to inhibit the elevated muscle catabolism in rats implanted with the Yoshida ascites hepatoma, while ibuprofen has been shown to reverse weight loss and improve quality of life in patients with advanced gastrointestinal cancer (95). However, other studies (90,96) have failed to confirm an increased protein degradation when PGE₂ was incubated with C₂C₁₂ cells or intact muscles, while inhibition of PGE₂ production in muscles from septic rats by indomethacin did not lower proteolytic rate (97). Our own studies (90) suggest that of the metabolites of arachidonic acid formed 15-HETE produced a significant increase in protein degradation in C₂C₁₂ myoblasts, with a bell-shaped dose-response curve similar to that produced by PIF. This suggests that PIF may induce protein degradation as a result of an increased synthesis of 15-HETE.

Protein degradation induced by PIF in C₂C₁₂ myoblasts, together with arachidonate release and 15-HETE production were totally inhibited by the polyunsaturated fatty acid, eicosapentaenoic acid (EPA). Administration of pure EPA to weight losing mice bearing the MAC16 adenocarcinoma completely prevented weight loss (98). This effect was specific to EPA, since it was not seen with other related fatty acids such as docosahexaenoic acid (98) or gamma-linolenic acid (99). Preservation of lean body mass in animals receiving EPA was attributed to a significant reduction in the enhanced protein degradation (99). Pretreatment of mice with EPA (0.5grams kilogram⁻¹) also completely prevented PIF-induced loss of body weight (100). Clinical studies support the ability of EPA to attenuate the development of cachexia in patients with unresectable pancreatic cancer (101). Daily supplementation with 12grams of fish oil resulted in patients who had a median weight loss of 2.9kilograms month⁻¹ prior to supplementation becoming weight stable, with a median weight gain of 0.3kilogram month⁻¹. Measurements of mid-arm muscle circumference and triceps skinfold thickness also showed no significant worsening from pre-study levels during supplementation. When EPA was combined with a conventional oral nutritional supplement patients showed significant weight gain at both 3 (median 1kilogram) and 7 weeks (median 2kilograms) after supplementation (102). Both performance status and appetite were significantly

improved at 3 weeks and the resting energy expenditure fell significantly. In contrast with the studies on nutritional supplementation alone there was a significant rise in lean body mass, but no change in fat mass or lean body mass and this may have contributed to an observed rise in Karnofsky performance status. This is the first study to report a beneficial effect on lean body mass in cachectic subjects by any form of treatment. Overall survival in this study was at the upper end of that seen in chemotherapy trials, but without the side effects associated with chemotherapy. In a recent study 60 patients with generalized solid tumours were randomized to receive either fish oil (3.06grams EPA and 2.07grams DHA day⁻¹) or placebo until death (103). The mean survival was significantly higher for the patients receiving the fish oil supplement, confirming that effective treatment of loss of lean body mass will benefit cancer patients, not only in an improved quality of life, but also with an extended survival time.

2.9. Other factors

A new member of the transforming growth factor-beta (TGF-beta) superfamily known as growth/differentiating factor-8 (GDF-8) has recently been described (104). GDF-8 is expressed specifically in developing and adult muscle and appears to function specifically as a negative regulator of skeletal muscle growth. It is not known if this contributes to cancer cachexia, but if it does molecules that inhibit GDF-8 signalling could have a therapeutic role.

Imbalanced plasma amino acid levels have been seen in cachectic cancer patients and this can produce anorexia and weight loss in animal models. Blockage of type 3 serotonergic receptors (SHT₃) can overcome this in animal models and so the SHT₃ receptor antagonist ondansetron was tested in patients with metastatic cancer and greater than 5% loss of body weight (105). However the patients continued to lose weight, although a significant improvement in food enjoyment was reported.

3. PERSPECTIVE

Although anorexia frequently accompanies cachexia it is unlikely that it plays a significant role in the overall loss of lean body mass. Thus nutritional supplementation alone is ineffective in the treatment of cachexia. Instead cachexia appears to be initiated by tumour or host products found in the circulation. While cytokines undoubtedly play a role in the loss of muscle mass in infectious cachexia there is less evidence that they function in this way in cancer cachexia. Instead tumour catabolic products such as PIF are probably involved, although the effect could be synergistic with cytokines. While the lysosomal and calcium-dependent proteolytic pathways play some role in the loss of skeletal muscle in cancer most attention has been directed towards the ATP-ubiquitin-dependent proteolytic pathway. Factors controlling this multi-enzyme pathway are just beginning to be understood, but until we have found the off-switch it will not be possible to restore lean body mass to cancer patients. Fortunately when this pathway is activated all the enzymes are switched on in a co-ordinate manner,

suggesting the possibility of a common trigger. Knowledge of the control points in this pathway are important not just for the treatment of cancer cachexia, but also other catabolic conditions such as starvation, acidosis, burns and sepsis. One of the most promising agents to emerge for the treatment of muscle wasting in cachexia is EPA, but further controlled studies are required to confirm its efficacy. However, preliminary clinical studies to date provide support for the concept that knowledge of the biochemical mechanisms of skeletal muscle protein catabolism in cancer cachexia will benefit patients not only in an improved quality of life, but also in an extended survival time.

4. REFERENCES

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