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New insights into adipose tissue atrophy in cancer cachexia

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Profound loss of adipose and other tissues is a hallmark of cancer cachexia, a debilitating condition associated with increased morbidity and mortality. Fat loss cannot be attributable to reduced appetite alone as it precedes the onset of anorexia and is much more severe in experimental models of cachexia than in food restriction. Morphological examination has shown marked remodelling of adipose tissue in cancer cachexia. It is characterised by the tissue containing shrunken adipocytes with a major reduction in cell size and increased fibrosis in the tissue matrix. The ultrastructure of 'slimmed' adipocytes has revealed severe delipidation and modifications in cell membrane conformation. Although the molecular mechanisms remain to be established, evidence suggests that altered adipocyte metabolism may lead to adipose atrophy in cancer cachexia. Increased lipolysis appears to be a key factor underlying fat loss, while inhibition of adipocyte development and lipid deposition may also contribute. Both tumour and host-derived factors are implicated in adipose atrophy. Zinc-α2-glycoprotein (ZAG), which is overexpressed by certain malignant tumours, has been identified as a novel adipokine. ZAG transcripts and protein expression in adipose tissue are up regulated in cancer cachexia but reduced with adipose tissue expansion in obesity. Studies *in vitro* demonstrate that recombinant ZAG stimulates lipolysis. ZAG may therefore act locally, as well as systemically, to promote lipid mobilisation in cancer cachexia. Further elucidation of ZAG function in adipose tissue may lead to novel targets for preventing adipose atrophy in malignancy.

Adipose atrophy: Cancer cachexia: Zinc-α2-glycoprotein

Cancer cachexia

Cachexia is derived from the Greek words 'kakos' and 'hexis', meaning 'bad condition'. It is a complex metabolic syndrome that comprises weight loss with reductions in skeletal muscle and adipose tissue mass, anorexia and weakness[1]. It usually occurs in chronic diseases such as cancer, chronic obstructive pulmonary disease, chronic heart failure and end-stage renal failure[1]. Cachexia not only markedly impairs the quality of life but is associated with increased morbidity and mortality.

Most patients with cancer develop cachexia at some point during the course of their disease and approximately half all patients with cancer have weight loss at diagnosis[2]. Clinically, cachexia should be suspected if involuntary weight loss of >5% premorbid weight occurs within a 6-month period[1]. The frequency of weight loss varies with the type of malignancy, being more common and severe in patients with cancers of the gastrointestinal tract, prostate and lung[3]. Cachexia has a detrimental effect on cancer treatment as a result of, for example, poor responses to chemotherapy[4]. Weight loss is also a prognostic indicator of decreased survival in patients with cancer[5,6]. It is considered that ≤ 20% of all cancer deaths are directly attributable to cachexia[2,7].

Evidence is accumulating that cancer cachexia arises from multiple metabolic alterations, such as reduced appetite, increased energy expenditure and tissue breakdown. The main tissues that are affected during the development of cachexia are skeletal muscle and white adipose tissue. The mechanisms of muscle wasting have been the focus of intensive research, with the demonstration of reduced protein synthesis and enhanced proteolysis in experimental models of cachexia and in patients

Abbreviations: UCP, uncoupling protein; ZAG, zinc-α2-glycoprotein.

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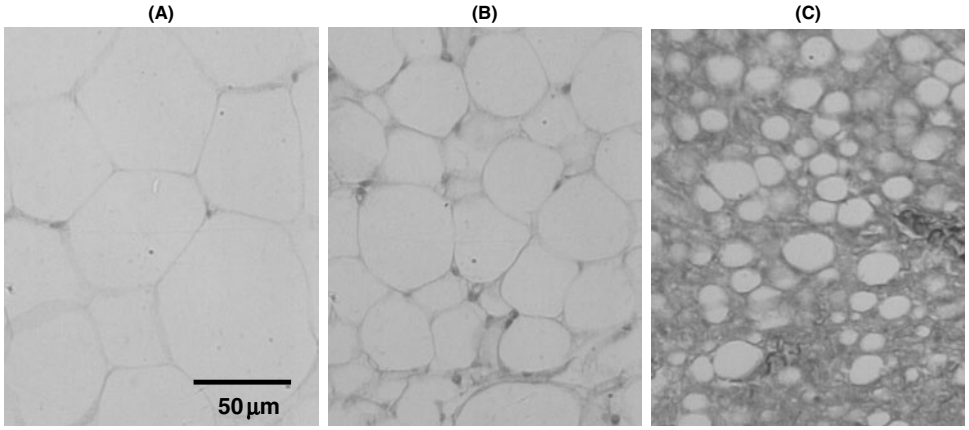

Fig. 1. Light microscopy of Sirius Red-stained sections of epididymal adipose tissue from control (A), pair-fed (B) and MAC16 tumour-bearing (C) mice at day 18 after tumour inoculation. (Adapted from Bing *et al.* 2006[26].)

with cancer cachexia[3,8]. Although profound loss of adipose tissue is a hallmark of cancer cachexia, much less is known of the underlying cellular and molecular mechanisms. A better understanding of fat depletion in malignancy is crucial for the development of effective treatments for the syndrome. The present article reviews studies on the mechanisms and potential mediators of adipose atrophy in cancer cachexia.

Adipose tissue in metabolic health

It is well documented that adipose tissue plays an important metabolic role by storing TAG in periods when energy input exceeds expenditure and releasing NEFA during energy deprivation[9]. As the largest energy reserve in the body, adipose tissue has a major impact on energy flux, plasma lipid levels and glucose uptake. There is compelling evidence that alterations in adipose tissue mass and metabolism have a major impact on whole-body energy homeostasis[10]. It has been shown that too little fat in lipodystrophy, as with too much fat in obesity, is a major risk for insulin resistance, dyslipidaemia and vascular diseases[11].

In addition to its primary role as a fuel reservoir, white adipose tissue has been affirmed as a major endocrine organ, since the tissue synthesises and secretes an array of hormones and proteins, termed adipokines[12]. Adipose tissue has extensive cross talk with other organs including the brain, liver and skeletal muscle through these adipokines. Over the last decade a growing number of adipokines have been identified, such as leptin, adiponectin, TNFα, visfatin and chemerin, which act locally in an autocrine and/or paracrine manner and/or as endocrine signals to modulate appetite, nutrient metabolism, insulin sensitivity, inflammation and adipose tissue development[13–17].

Adipose atrophy in cachexia

Extensive loss of adipose tissue is a prominent feature of cancer cachexia. Although it is not clear whether there are site differences in fat loss, a study using computed tomography scanning has revealed that patients with cancer cachexia who have gastrointestinal carcinoma have a smaller visceral adipose tissue area than control subjects[18]. In a follow-up study of patients with cancer the analysis of body composition (by dual-energy X-ray absorptiometry) has shown that in progressive cancer cachexia the loss of body fat is more rapid than that of lean mass and occurs preferentially from the trunk followed by leg and arm adipose tissue[19]. A recent study using retrospective computed tomography scan images of patients with advanced colo-rectal cancer has shown that the most rapid loss of adipose tissues ($\leq 41\%$) occurs within 3 months of death[20]. Marked falls of $\leq 85\%$ body fat have been observed in patients with lung cancer, which may lead to hyperlipidaemia and insulin resistance as well as complicating anti-tumour therapies[21,22]. With the current escalation in obesity, the paradox of higher BMI as a long-term risk factor but having better survival has been observed in several wasting diseases, including chronic obstructive pulmonary disease, chronic heart failure, end-stage renal failure and cancer[23]. A recent study has reported that obesity estimated by an elevated BMI appears to have a protective effect against prostate cancer-specific mortality[24].

Fat loss cannot be explained by reduced appetite alone, as it often precedes the onset of anorexia and is much more severe in animal models of cachexia than in food restriction[25]. This pattern is also observed at the tissue level, as morphological examination has shown marked changes in adipose tissue plasticity in mice with cancer cachexia compared with *ad libitum*-fed and pair-fed controls[26]. Adipose tissue from tumour-bearing mice contains shrunken adipocytes of various sizes with a dilated interstitial space. Further morphometric analysis has revealed that the adipocyte size is dramatically reduced. In the tissue matrix increased fibrosis is evident with strong collagen-fibril staining (Fig. 1). Moreover, the ultrastructural features of 'slimmed' adipocytes are characterised by severe delipidation and alterations in cell membrane conformation, with irregular cytoplamic projections and increased

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mitochondria that are electron dense. Taken together, these changes illustrate adipose remodelling in cancer cachexia. A recent study of adipose tissue from patients with cancer has also shown tissue atrophy in subjects with cachexia but not in those without cachexia (C Bing and NA Stephens, unpublished results).

Mechanisms of adipose atrophy

Although the molecular basis of adipose atrophy is poorly understood, evidence suggests that fat loss may arise from an enhanced catabolic response and disrupted anabolic processes. Increased lipolysis appears to be a key factor[27,28]. In patients with cancer cachexia there is an increase in glycerol and fatty acid turnover compared with patients with cancer without cachexia[29]. It has also been shown that whole-body lipolysis, measured by the rate of appearance of glycerol, is higher in patients with cancer who are losing weight than in healthy subjects[27]. Studies have demonstrated that lipolytic activity (fasting plasma glycerol or fatty acids) is increased in patients with cancer cachexia[28,30]. Increased expression and activity of hormone-sensitive lipase, a rate-limiting enzyme of the lipolytic pathway, is thought to promote lipolysis. Hormone-sensitive lipase mRNA and protein levels have been shown to be increased in adipose tissue of patients with cancer cachexia[28,31]. It is therefore proposed that inhibition of hormone-sensitive lipase may prevent or reverse cachexia-associated fat loss. Furthermore, in mature adipocytes isolated from subcutaneous fat of patients with gastrointestinal adenocarcinoma the lipolytic effects of catecholamines and natriuretic peptide are increased by >2-fold in patients with cachexia, although the basal lipolysis is unchanged[28]. However, gene expression of adipose TAG lipase is not affected in patients with cachexia[28]. It is also postulated that the fatty acids liberated by lipolysis may serve as substrates for oxidation[32], which might be mediated by the adipocyte-specific gene cell death-inducing DNA fragmentation factor-α-like effector A. Cell death-inducing DNA fragmentation factor-α-like effector A mRNA levels are increased in patients with cancer cachexia and its overexpression *in vitro* stimulates adipocyte fatty acid oxidation while decreasing glucose oxidation through inactivation of the pyruvate dehydrogenase complex[32].

In addition to increased lipolysis, fat loss may be attributable to a decrease in lipid deposition. Circulating insulin, the hormone that promotes fat deposition and glucose transport in adipose tissue, is reduced in the tumour-bearing state[33–35]. A fall in lipoprotein lipase activity in white fat has been reported in tumour-bearing mice[36]. This outcome may lead to reduced cleavage of TAG from plasma lipoproteins into glycerol and NEFA for storage, resulting in an increased net flux of lipid into the circulation. There is also evidence that fat diminution in cachexia could be the result of impairment in the formation and development of adipose tissue. A recent study has shown that the expression of the genes encoding several key adipogenic transcription factors, including CCAAT/enhancer-binding protein-α and -β, PPARγ and sterol regulatory element-binding protein-1c, is markedly reduced in white fat of mice with cancer cachexia[26]. mRNA levels of sterol regulatory element-binding protein-1c targets, genes encoding lipogenic enzymes fatty acid synthase, acetyl-CoA carboxylase, stearoyl-CoA desaturase 1 and glycerol-3-phosphate acyl transferase, also fall markedly[26,37]. Finally, glucose, which serves as a substrate for lipid synthesis, is transported into the adipocyte via the insulin-responsive facilitative glucose transporter GLUT-4. However, there is a decrease in GLUT-4 mRNA in white fat of mice with cancer cachexia[26], which could be a downstream effect of inhibited CCAAT/enhancer binding protein α since its deficiency is associated with abnormal subcellular localisation of GLUT-4[38].

Potential mediators of adipose atrophy

Several factors produced by tumours and host tissues in the presence of a tumour burden are suggested to be able to mediate fat loss in cachexia. These factors include pro-inflammatory cytokines such as TNFα, IL-1β and IL-6 and the lipid-mobilising factor zinc-α2-glycoprotein (ZAG; also known as AZGP1), each of which can be derived from the tumour and also from the host tissues. Their potential involvement in cachexia-associated fat depletion will be discussed.

Cytokines

TNFα, also termed cachectin, was first identified as the cachexia-inducing factor in chronic diseases such as cancer and persistent infection[39]. Recent data have shown that TNFα infusion can induce systemic lipolysis in human subjects[40]. Treatment with TNFα *in vitro* increases glycerol release from rodent and human adipocytes, probably by inhibiting lipoprotein lipase activity[41] and down regulating the expression of perilipin, which then enables hormone-sensitive lipase to access the surface of lipid droplets[42]. TNFα-induced adipocyte lipolysis is the outcome of activation of the TNFα receptor 1-dependent pathway[43,44], which involves the stimulation of extracellular signal-regulated kinase 1 and 2, mitogen-activated protein kinase, c-Jun N-terminal kinase and protein kinase A[45,46]. TNFα also has an inhibitory effect on adipocyte differentiation via the Wnt-signalling pathway[47,48]. In addition, both TNFα and IL-1β are able to inhibit glucose transport in murine and human adipocytes[49] and consequently decrease the availability of substrates for lipogenesis. Some studies suggest that TNFα also increases lipid deployment, probably via up-regulation of uncoupling protein (UCP) 2 and UCP3 expression in skeletal muscle[50,51], offering a mechanism to remove NEFA resulting from lipolysis. Although these studies indicate a role for TNFα in reducing fat mass, its importance in cancer-related adipose atrophy is still debatable. It is largely a result of the observations that circulating TNFα levels are unchanged or undetectable[52,53], as well as elevated[54,55], in patients with cancer cachexia.

IL-6 has been shown to moderately increase lipolysis in human adipose tissue *in vitro*[56]. Treatment with CNTO-328, a monoclonal antibody to IL-6, is able to reverse

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tumour-induced cachexia in nude mice[57]. Recent work has shown that IL-6 is necessary for the onset of adipose and skeletal muscle wasting in the Apc(Min/+) mouse[58]. Despite serum IL-6 levels being elevated in patients with cancer[59,60], it is still unclear whether circulating IL-6 correlates with the extent of cachexia[53,59–61]. Since TNFα and IL-6 are also produced by adipose tissue, although probably mostly from non-fat cells[62], their autocrine and/or paracrine effects may be important in cachexia. However, studies in mice with cancer cachexia have shown that TNFα and IL-6 mRNA levels in white fat are unaffected by the tumour burden[26,63]. In a recent study of patients with gastrointestinal cancer no alterations were found in gene expression of TNFα and IL-6 and their protein release by adipose tissue under cachectic states[64]. In addition, there is no apparent infiltration of macrophages and lymphocytes in adipose tissue of mice with cachexia[26] and patients with cancer cachexia[64].

ZAG, a lipid-mobilising factor

ZAG is a 41 kDa soluble protein first isolated from human plasma[65] and subsequently identified in secretory epithelial cells, including those of liver, breast, prostate and the gastrointestinal tract[66]. The crystal structure of ZAG reveals that it belongs to the class I MHC family. There is a non-peptidic ligand in the ZAG counterpart of the MHC peptide-binding groove, which may relate to its signalling function[67]. ZAG is overexpressed by several types of malignant tumour, such as breast, prostate and bladder cancers[37,68,69], and ZAG levels are elevated in serum and seminal fluid of patients with prostate cancer[37,70]. The biological functions of ZAG were largely unknown until a lipid-mobilising factor, purified from the urine of patients with cancer cachexia, was shown to be identical to ZAG in electrophoretic mobility, immunoreactivity and amino acid sequence[71]. ZAG has also been purified from a murine adenocarcinoma (MAC16) that induces profound cachexia[72]. Amino acid sequence analysis has revealed that murine and human ZAG display an overall homology of 59%[73], but share up to 100% identity in specific regions thought to be important in lipid metabolism[67].

Treatment with purified ZAG can cause weight loss in genetically-obese *ob/ob* mice[72] and normal mice[74,75], and body composition analysis indicates that ZAG-induced weight loss is a result of selective reduction in body fat but not lean mass. ZAG has been shown *in vitro* to stimulate glycerol release from isolated murine adipocytes in a dose-dependent manner[72,75]. The lipolytic effect of ZAG has been postulated to be mediated by β3-adrenoceptors and the activation of the intracellular cAMP pathway. ZAG has been shown to be able to produce a comparable increase in cAMP levels to that obtained with isoprenaline and ZAG-induced lipolysis can be attenuated by the specific β3-adrenoceptor antagonist SR59230 in adipocytes[72,75,76].

In addition to lipid mobilisation there is also evidence that ZAG promotes lipid utilisation in brown adipose tissue and skeletal muscle. ZAG administration *in vivo* in mice leads to an up-regulation of UCP1 mRNA and protein expression in brown adipose tissue and of skeletal muscle UCP2 and UCP3 mRNA[74]. ZAG induces expression of UCP1 protein and O_2 uptake *in vitro* in primary cultures of brown adipose tissue[72,77]. Hence, by up regulating UCP in brown adipose tissue and muscle, ZAG may provide a mechanism for the disposal of excess fatty acids liberated from enhanced lipolysis, which could lead to increased energy expenditure during cachexia.

Adipose-derived ZAG in cancer cachexia

The secretory function of adipose tissue and the potent fat-mobilising effect of ZAG have led to the postulation that this protein could also be produced by adipose tissue, thereby modulating adipocyte metabolism[78]. Work from the authors' group has demonstrated that the *ZAG* gene and protein are expressed by the major white fat depots (epididymal, perirenal, subcutaneous, mammary gland) and the interscapular brown fat of mice[78]. Further detection using immunocytochemistry has shown the presence of ZAG protein in the cytoplasm of adipocytes in adipose tissue[78]. In human subjects ZAG mRNA and protein have been shown to be expressed in both visceral and subcutaneous fat depots[78]. Futhermore, ZAG mRNA and protein are detected in differentiated human Simpson-Golabi-Behmel syndrome adipocytes. Most importantly, ZAG, which contains a secretory signal sequence[37], has been shown to be secreted into the culture medium by differentiated Simpson-Golabi-Behmel syndrome adipocytes[79]. Subsequent quantification of ZAG secretion levels in the culture medium has revealed its concentration to be in the range of ng/ml per 24 h, close to that of adiponectin[80]. Taken together, these findings indicate that ZAG is indeed a novel adipokine produced abundantly by adipocytes and the protein may have a major action locally in the regulation of fat mass.

Adipose-derived ZAG appears to be inversely associated with body fat mass. ZAG mRNA and protein levels are markedly increased in adipose tissue of mice with cancer cachexia and, furthermore, the increase in ZAG protein content is related to the extent of weight loss in these animals[78]. In contrast, as a reference adipokine, leptin mRNA and circulating leptin levels are strikingly repressed in tumour-bearing mice[26,35,78] (Fig. 2). Very recently, it has been shown that ZAG mRNA and protein expression are also up regulated in adipose tissue in patients with cancer cachexia (T Mracek, NA Stephens, X Xiao and C Bing, unpublished results). Contrarily, studies of obese subjects have shown that *ZAG* gene expression is down regulated in subcutaneous adipose tissue of obese women[81] and men[82]. Furthermore, recent work has demonstrated that ZAG mRNA levels are negatively correlated with total fat mass in human subjects with a wide range of BMI[80].

Although the role of ZAG in adipose tissue remains to be established, its effect on fat loss has been further supported by a recent study that shows that ZAG-knock-out mice are vulnerable to weight gain when fed a high-fat diet and this outcome appears to be the result of decreased lipolytic response to several stimuli, such as isoprenaline, CL316243, foskolin and isobutylmethylxanthine, in adipocytes[83]. Recent work has demonstrated that recombinant ZAG stimulates lipolysis in human adipocytes (T Mracek,

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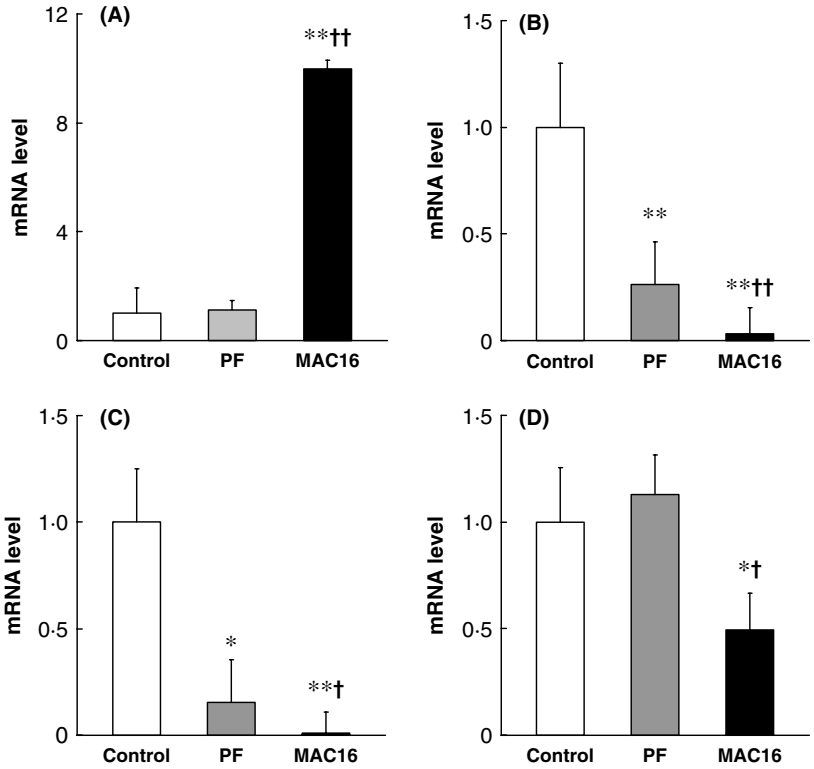

Fig. 2. mRNA levels of zinc-α2-glycoprotein (A), leptin (B), CCAAT/enhancer-binding protein α (C) and sterol regulatory element-binding protein-1c (D) in epididymal adipose tissue of control (□), pair-fed (PF; ▩) and MAC16 tumour-bearing (■) mice, quantified by real-time PCR and normalised to β-actin. Values presented as fold changes relative to controls are means with their standard errors represented by vertical bars for eight mice per group. Mean values were significantly different from those for the control group: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$. Mean values were significantly different from those for the PF group: †$P<0{\cdot}05$, ††$P<0{\cdot}01$. (Adapted from Bing *et al.* 2006[26].)

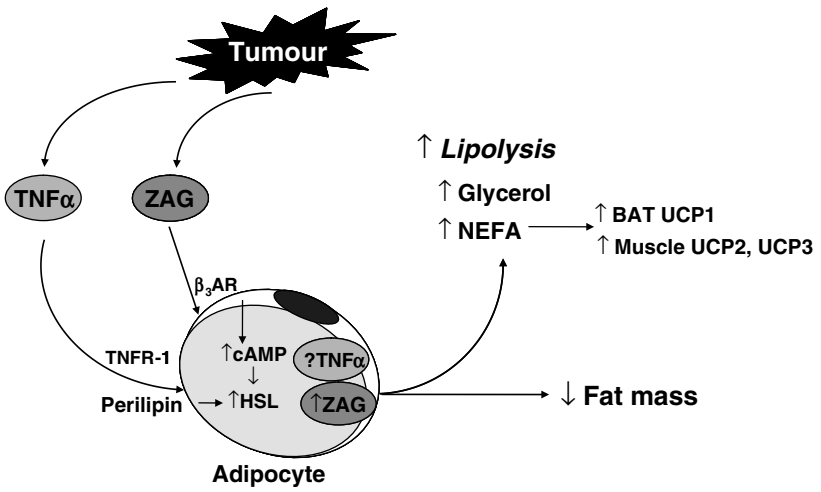

Fig. 3. A schematic diagram of lipid catabolism in cancer cachexia. Certain tumour and host tissue-produced factors, such as TNFα and zinc-α2-glycoprotein (ZAG), may act systemically and/or as autocrine and/or paracrine signals to stimulate adipocyte lipid metabolism. TNFα-induced lipolysis acts through a TNFα receptor 1 (TNFR-1) dependent pathway, which inhibits perilipin allowing hormone-sensitive lipase (HSL) to access the surface of lipid droplets. ZAG-stimulated lipolysis may be mediated by β3-adrenoceptors (β3AR) and the activation of the intracellular cAMP pathway. NEFA generated by enhanced lipolysis in cachexia may serve as substrates for lipid utilisation through uncoupling proteins (UCP) in brown adipose tissue (BAT) and skeletal muscle.↑, Increased; ↓, decreased.

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P Trayhurn and C Bing, unpublished results). Further studies are required to unravel the nature of the action of ZAG in human cancer cachexia. Overall, current data point to ZAG, as well as TNFα, as a potential candidate for mediating lipid catabolism in cancer cachexia (Fig. 3). Other factors may also be involved in fat loss in malignancy, e.g. macrophage inhibitory cytokine-1, which causes cachexia and a reduction in fat mass via its central effects on appetite[84]. Interestingly, recent work has found that macrophage inhibitory cytokine-1 is also produced by adipocytes and this factor may have autocrine and/or paracrine effects in adipose tissue[85].

Conclusion

Cancer cachexia, manifested by progressive weight loss, is a metabolic disorder associated with increased morbidity and mortality. Extensive loss of adipose tissue is a prominent feature of cachexia. The marked alterations in adipose morphology indicate tissue atrophy and this outcome cannot be attributable to reduced appetite alone. Evidence suggests that altered adipocyte metabolism may have an important role. Increased lipolysis appears to be a key factor, while impairment in lipid deposition and adipocyte development may also contribute. Adipose atrophy could be mediated by the tumour and/or host-derived factors. TNFα, as a potential mediator, has been linked with increased lipolysis. ZAG, a potent lipid-mobilising factor, has been identified as a novel adipokine and its expression in adipose tissue is up regulated in cancer cachexia. ZAG may therefore act locally, as well as systemically, to promote lipid breakdown. Further elucidation of the function of ZAG in adipose tissue may lead to novel targets for preventing adipose atrophy in malignancy.

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