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ADIPOSE AND MUSCLE TISSUE METABOLISM IN CANCER CACHEXIA

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Tileinkað eiginkonu minni, Völu og börnum mínum, Kristínu Ísold and Ágústi Atla.

ABSTRACT

Background: Loss of adipose and muscle tissue mass is a key feature of cancer cachexia. Weight loss, glucose intolerance, and insulin resistance are seen in patients with pancreatic ductal adenocarcinoma (PDAC). The mechanism behind the loss of adipose tissue is unknown but has been attributed to increased adipocyte lipolysis, systemic inflammation, apoptosis or reduced lipogenesis. The volume of adipose and muscle tissue in cancer cachexia can be determined by computed tomography (CT).
Aims: The aim of this research was to: 1) Investigate the action of insulin on glucose metabolism and the content of energy metabolites in the muscle tissue of patients with PDAC. 2) Determine whether alterations in fat cell numbers, lipolysis and lipogenesis could account for some of the functional changes observed in adipose tissue in cancer cachexia. 3) Investigate if inflammation is involved in the loss of adipose tissue in cancer cachexia. 4) CT determined tissue volume, could give information about the distribution of wasting of muscle and adipose tissue in patients with recently diagnosed cancer cachexia.
Material and Methods: Muscle biopsies from patients with PDAC and three control groups were assessed for glycogen, adenosine triphosphate and phosphocreatine content. Also measured were glucose incorporation into glycogen, glucose transport in human muscle and rat muscle cell conditioned by PDAC cells. In cancer cachexia patients (CC) and two cancer control patient groups, weight stable (WS,) and gastric obstruction (GO), blood, subcutaneous adipocytes and differentiated preadipocytes were investigated for: 1) Expression of genes regulating inflammation and measurement of systemic and local secretion of inflammation markers (interleukin 6 (IL-6)). 2) *In vivo* lipolytic activity, lipolysis, lipogenesis and expression of genes regulating lipolysis. 3) Measurements of number and volume of adipocytes. The volume of muscle and adipose tissue was measured with CT and body composition.
Results: Patients in the CC group lost 10% of their habitual weight, patients in GO group lost 17% and patients in WS group lost 3%. Glucose transport, muscle glycogen, and adenosine triphosphate contents were decreased in patients with PDAC compared with control patients, and insulin stimulation did not significantly increase glucose incorporation into glycogen *in vitro* in patients with PDAC. Media conditioned with PDAC cells did not affect glucose transport in rat muscle cell. Circulating levels of IL-6 and *in vivo* lipolytic activity were increased in patients in the CC group compared to control patients. In patients in the CC group, there was increased lipolytic effect of catecholamines and natriuretic peptide, and the expression levels of Hormone Sensitive Lipase (HSL) mRNA and protein were increased compared to those in the control patients. The antilipolytic effect of insulin in mature adipocytes and the stimulated lipolytic effect in differentiated preadipocytes were unaltered in cancer cachexia. Patients in the GO group had no change in adipocyte lipolysis. There were no differences in mRNA expression of IL-6 or secretion in adipose tissue and lipogenesis. Adipocytes were decreased in size but their numbers were normal in patients in the CC group compared with those in the WS control group. Adipose tissue was reduced in patients in the CC and GO groups, both according to CT and body composition. CT showed that patients in the CC group displayed a selective decrease in visceral adipose tissue.
Conclusion: Wasting of adipose tissue is a prominent part of the cancer cachexia syndrome and commences before the wasting of muscle tissue. The insulin resistance for active glucose transport in the skeletal muscle of pancreatic cancer patients is not directly related to factors from pancreatic cancer. It is lipolysis, not inflammation, increased apoptosis or decreased lipogenesis, which is involved in the loss of adipose tissue in patients with cancer cachexia. There is increased expression and activity of HSL, which gives rise to an increased rate of lipolysis in patients with cancer cachexia. Although cancer patients with gastrointestinal obstruction, at the time of diagnosis, have lost almost twice the amount of body weight compared to patients with cancer cachexia, the latter group displays more loss of visceral adipose tissue.

List of Publications

I. T. Agustsson, M. D'souza, G. Nowak, B. Isaksson. Mechanisms for skeletal muscle insulin resistance in patients with pancreatic ductal adenocarcinoma. Nutrition (2010), doi:10.1016/j.nut.2010.08.022.

II. T. Agustsson, M. Ryden, J. Hoffstedt, V. van Harmelen, A. Dicker, J. Laurencikiene, B. Isaksson, J. Permert and P. Arner. Mechanism of Increased Lipolysis in Cancer Cachexia. Cancer Res 2007;67(11):5531–7.

III. M. Ryden, T.Agustsson, J. Laurencikiene, T. Britton, E. Sjolin, B. Isaksson, J. Permert, P. Arner. Lipolysis—Not Inflammation, Cell Death, or Lipogenesis—Is Involved in Adipose Tissue Loss in Cancer Cachexia. Cancer 2008;113:1695-704

IV. T Agustsson, P. Wikrantz, M. Ryden, T. Brismar and B. Isaksson. Adipose tissue volume is diminished in recently diagnosed cancer patients with cachexia. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

2-DOG	2- DeOxyGlucose
8-Br-cAMP	8-Bromocyclic AMP
ANOVA	Analysis of variance
APP	Acute Phase Protein
APR	Acute Phase Response
ATGL	Adipose TriGlyceride Lipase
ATP	Adenosine TriPhosphate
BAY	4- isopropyl-3- methyl-2-[1-(3-(S)-methyl-piperidin-1-yl)-methanoyl]-2H-isoxazol- 5-one
BB	Langvarandi BrisBólga
BK	Krabbamein í Brisi
BMI	Body Mass Index
BMR	Basal Metabolic Rate
cAMP	cyclic Adenosine 3,5-MonoPhosphate
CC	Cancer Cachexia
CD	Cluster of Differentiation
CK	Cancer Kakexia
cGMP	cyclic Guanosine 3,5-MonoPhosphate
CP	Chronic Pancreatitis
CRP	C-Reactive Protein
CT	Computed Tomography
DEXA	Dual-x-ray Absorbmetry
DMEM	Dulbecco's Modified Eagle's Medium
DT	DatorTomografi
EC_{50}	Half-maximum Effective Concentration
EE	Energy Expenditure
EPA	Eicosapentaenoic Acid
FCS	Fetal Calf Serum
FFA	Free Fatty Acid
GS	Góðkynja Sjúkdómur
GLUT	GLUcose Transporter type
GO	Gastric Obstruction in Cancer
HDL	High Density Lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMB	Hydroxy-β-Methylbutyrate
HSL	Hormone Sensitive Lipase
Hu	Hounsfield units
IFN-γ	Interferon Gamma
IGF-I	Insulin like Growth Factor I
IL	Interleukin
KHB	Krebs Henseleit bicarbonate Buffer
KK	Hópur með Krabbameins Kakexíu

mRNA	Messenger Ribonucleic Acid
NF-kβ	Nuclear Factor Kappa Beta
NSAIDs	NonSteroidal Anti Inflammatory Agents
OGTT	Oral Glucose Tolerance Test
PC	Pancreatic Cancer / Pankreas Cancer
PCR	Polymerase Chain Reaction
PDAC	Pancreatic Ductal AdenoCarcinoma
PEST	PEnicillin and STreptomycin
PG-SGA	Patient Generated Subjective Global Assessment
REE	Resting Energy Expenditure
RNA	Ribonucleic Acid
SD	Standard Deviation
SEM	Standard Error of the Mean
SM	Hópur með stíflu í meltingarvegi / Stopp i Mag- tarmkanalen
TNF-α	Tumour Necrosis Factor Alpha
TPN	Total Parenteral Nutrition
VEGF-B	Vascular Endothelial Growth Factor B
VLDL	Very Low Density Lipoprotein
VS	Vikt Stabil
WS	Weight Stable Cancer
ZAG	Zinc-α-2-Glycoprotein
ÞS	Hópur með Stöðuga Þyngd

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

The word cachexia, made from the words kakos and hexis, originates from the Greek language and is a description of a bad condition.

The idea that a tumour substance can give rise to cancer cachexia was published for the first time in 1962. Before that time it was believed that the body destroyed fat and muscle tissue in order to "feed" the tumour [1].

For decades has it been difficult to define the cachexia syndrome. In advanced cachexia, though not in early, the most dramatic symptoms are weight loss and anorexia, as well as early satiety, anaemia, and oedema [2]. In the early stages of cachexia, these features may occur to a variable degree but the severity of symptoms may change during the course of the illness. The complex, multifactorial origin of cachexia does not follow a uniform pathophysiological profile and this has created obstacles to conducting clinical studies both at a mechanical level and in order to target therapeutic interventions [2]. The cancer cachexia syndrome is common in patients with gastrointestinal cancer [3, 4]. In these patients cancer cachexia is a collective term for all the signs and symptoms they may develop, which are not related to or caused by the mechanical compression or obstruction of the cancer or by the treatment of the cancer such as chemotherapy, radiotherapy or surgery [5]. Cachexia induced by the treatment of the cancer is likely to be caused by different pathophysiological mechanisms from those resulting in cancer cachexia [5], as the body composition following anorexia induced weight loss is more similar to what is observed in starvation than in cancer cachexia [2]. The cancer cachexia syndrome includes weight loss, loss of muscle and adipose tissue, anaemia and alterations in lipid, protein and carbohydrate metabolism [6]. The loss of muscle and adipose tissue may precede any reduction in food intake, which can be normal or even increased [1, 7], however anorexia is commonly present in patients with cancer cachexia [7]. Non muscle protein compartments are relatively preserved in cancer cachexia, thus distinguishing cancer cachexia from simple starvation [2]. Even with intensive nutritional support, including total parenteral nutrition, it is difficult to reverse the process [8]. Although several theories regarding the pathophysiological mechanisms have been proposed there is no universally accepted explanation for the cause of cancer cachexia [9-11]. Cancer patients with cachexia, have shorter median survival, more psychological problem and worse quality of life than cancer patients without cachexia [12]. Loss of respiratory muscle in cancer cachexia patients has been found to contribute to up to 50% of deaths in cancer patients [13]. Weight loss approaching 30% is incompatible with life [2].

1.2 DEFINITIONS

1.2.1 Cachexia

1.2.1.1 General

Until 2006 there was no official definition of cachexia. Due to there being many different types of cachexia it is very important when discussing cachexia to know what definition is being used. For example today's treatment of patients with heart-, lung- and renal failure is so advanced that it has converted groups of dying patients to living patients left with chronic diseases. All these patients, along with patients suffering from chronic infections (tuberculosis or HIV), rheumatic disease and cancer, can develop a condition called cachexia. To make this more complicated, since 2006, several different definitions of cachexia have been published.

Three of these definitions are:

1. European Palliative Care Research Collaborative organization: *''Cancer cachexia is a multifactorial syndrome defined by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism. A key defining feature is ongoing loss of skeletal muscle mass which cannot be fully reversed by conventional nutritional support, leading to progressive functional impairment''* [14].

2. Special Interest Group on Cachexia and Anorexia:
   ''Cachexia is a multifactorial syndrome characterized by severe body weight loss, muscle and fat loss and increased protein catabolism due to underlying disease(s). Cachexia is clinically relevant since it increases patients' morbidity and mortality. Contributory factors to the onset of cachexia are anorexia and metabolic alterations, i.e., increased inflammatory status, increased muscle proteolysis, impaired carbohydrate, protein and lipid metabolism. Considering the wide range of clinical manifestations of cachexia, the staging of this syndrome is warranted'' [15].

3. Society for Cachexia and Wasting Disorders:
   ''Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with wasting disease. Wasting disease is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity'' [16].

At the 5th Cachexia Conference, in Barcelona Spain, December 2009, a consensus was reached between representatives of these three groups. All three different definitions were accepted and it was decided that they are in the most part alike [17]. In table 1 the three definitions are compared.

Table 1. Terms included in the different definitions (J Am Med Dir Assoc, 2010, vol. 10, Page 229-30).			
Condition	European Palliative Care Research Collaborative organization	Special Interest Group on Cachexia and Anorexia	Society for Cachexia and Wasting Disorders
Weight loss	+	+	+
Muscle loss	+	+	+
Fat loss	-	+	-/+
Anorexia	+	+	+
Inflammation	-/+	+	+
Insulin resistance	-	+	+
Muscle protein breakdown	-	+	+
Fatigue	-	-	+
Morbidity	+	+	+
Sarcopenia	+	+	+
Starvation	-	-	+
Reversion by nutritional support	+	-	-

During this conference the need to work on more disease-specific definitions was pointed out and also the necessity to include the term "pre-cachexia" as a condition with no or very small weight loss (< 5% of habitual body weight in 6 months). Pre-cachexia is a condition that is associated with chronic disease in conjunction with anorexia, inflammation and/or metabolic alterations [17].

1.2.1.2 Related to the thesis

When preparations were commenced in 2003 for the research leading to the published studies included in this thesis, including recruitment of the first patients in the spring of 2004, there was no definition for cachexia or cancer cachexia available. The following definition was therefore constructed.

> CANCER CACHEXIA:
> Unintentional weight loss with no evidence of gastrointestinal obstruction, with > 5% of habitual weight lost during the last three months or > 10% weight loss during the last six months.

This definition was based on findings from a literature search regarding what is considered to be normal weight loss over a given period of time. The finding from this literature search was that any weight loss of > 5% of habitual weight during a period of six months [18] was abnormal. However a more constricted definition was used in this research to minimise the risk of false positive diagnosis of cancer cachexia. Consequently there was an increased risk of false negative results in weight stable cancer patients. It was decided that this was acceptable because there would be few patients included in the study, and the outcome should have such magnitude (true positive), that borderline changes (false negative) would not be relevant.

The definition of cancer cachexia used in this thesis is based on weight loss only. According to more recent definitions [14-16] of cachexia and cancer cachexia, weight loss alone is not sufficient to diagnose cachexia. Most of the other parameters required are parameters that were investigated in this thesis.

1.2.2 Anorexia

The definition of Anorexia is the loss of the desire to eat. It should not be confused with Anorexia Nervosa [15], a psychiatric disease, which is much less common than anorexia but more widely publicised in the media. Multifactorial pathology lies behind anorexia and may be related to a disturbance in the function of the hypothalamus, where energy intake is controlled. Hypotheses exist that chronic systemic inflammation alters the function of the hypothalamus, resulting in a change in its function that renders the hypothalamus unable to recognize and respond appropriately to persistent peripheral hunger signals [19]. Anorexia is often associated with cancer cachexia but not always; there are cancer cachexia patients who show no signs of anorexia [5].

1.2.3 Sarcopeni

Sarcopeni, made from the words sarx and penia, comes from the Greek language and describes the poverty of flesh. The term sarcopenia is commonly used to describe the loss of skeletal muscle mass and strength that occurs as part of the normal aging process. By the seventh and eighth decade of life, maximum voluntary contractile strength has decreased by 20-40% [20]. Most of the loss of strength can be accounted for by decreased muscle mass [21]. Loss of skeletal muscle fibres secondary to decreased numbers of motor-neurons appears to be a major contributing factor, but other factors, including decreased physical activity, altered hormonal status, decreased total caloric and protein intake, inflammatory mediators and factors leading to altered protein synthesis must also be considered [15, 22].

1.3 WHO GETS CANCER CACHEXIA?

The typical cancer cachexia patient is a patient with pancreatic ductal adenocarcinoma (PDAC). These patients have the highest chances of all cancer patients of developing cancer cachexia with 85% of them becoming cachectic. It is not known why a few pancreatic cancer patients do not develop cancer cachexia but it is believed that this is due to variations in the tumour phenotype, or the host genotype or both, and that these factors determine the development of cancer cachexia [5]. Cancer cachexia is common in all patients with gastrointestinal cancer, while patients with non-Hodgkin's lymphoma, breast cancer, acute lymphocytic leukaemia, and sarcomas have the lowest frequency of cancer cachexia [5].

1.4 SIGNS AND SYMPTOMS OF CANCER CACHEXIA

1.4.1 Weight Loss

1.4.1.1 Adipose Tissue

Wasting of adipose tissue occurs early in cancer cachexia [23]. Loss of adipose tissue stores cannot be explained by reduced appetite alone, as cancer cachexia often precedes the onset of anorexia. Weight loss is more severe in animal models of cachexia than of

food restriction [24]. Increased plasma glycerol levels [25] indicate increased lipolysis activity in cancer cachexia. Increased production of lipolytic factors from adipose tissue such as interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) and an increase in tumour-derived lipolytic factors such as Zinc-α-2-glycoprotein (ZAG) could explain the increased lipolysis in cancer cachexia [26, 27]. Altered action of the major lipolysis-regulating hormones may additionally be of importance [26]. These hormones regulate lipolysis by separate mechanisms, all of which converge at the final rate-limiting step in lipolysis activation, with activation of hormone-sensitive lipase (HSL) [28]. Subcutaneous adipose tissue in cancer cachexia is characterised by atrophy and reduction in adipocyte cell size, and is accompanied by an increase in tissue matrix fibrosis [29].

1.4.1.1.1 Adipocyte Lipase Activity

In basal (not stimulated) lipolysis, adipose triglyceride lipase (ATGL) is more active than HSL. Both ATGL (highly active) and HSL (minimally active) hydrolyse triglycerides to diacylglycerol. In basal lipolysis (minimal HSL activity), diacylglycerol is re-esterified back to triglycerides or is used to build glycerolphospholipids. But when HSL is stimulated (via catecholamine or natriuretic peptides) HSL hydrolyses diacylglycerol to monoacylglycerol and monoglyceride lipase hydrolyses monoglyceride to glycerol. When HSL is extremely active or over-expressed, adipocytes cannot accumulate triglycerides [30, 31].

1.4.1.2 Muscle Tissue

Wasting of muscle tissue in cancer cachexia occurs later than the wasting of adipose tissue [23]. Most studies in cancer patients with a weight loss of 10% or more have suggested an increased activity and expression of the ubiquitin-proteasome pathway in skeletal muscle [32] resulting in breakdown of the muscle. It has been suggested that depression of protein synthesis may be more important when the weight loss increases to more than 20%. The mechanism for the muscle wasting at weight losses greater than 20% requires further investigation, the ubiquitin-proteasome pathway alone cannot account for the high levels of protein breakdown observed [33].

1.4.2 Body Composition

It is important to realize that the changes in body composition reflect a process that changes with time. In a group of cancer patients who had lost 10% of their habitual weight, adipose tissue diminished preferentially from the chest and abdomen, followed by leg and arm adipose tissue, respectively. Lean body mass diminished from the arm tissue compartment, whereas in the central body areas and leg tissue compartments it increased [23]. In another group of cancer patients who had lost 30% of their habitual weight, an 85% decrease in total body adipose tissue and a 75% decrease in skeletal muscle protein mass were the most dramatic changes observed in body composition. Interestingly, the visceral protein compartment was relatively preserved and this is one of the main features that differentiates cancer cachexia from simple starvation [2]. The body composition alterations in cancer cachexia differ from that seen in anorexia, where most of the weight loss is from adipose tissue, and only a small proportion from muscle, whereas in cancer cachexia there is an equal loss of adipose and muscle tissue (late stages of cancer cachexia). Additionally in Anorexia Nervosa, the loss of visceral (non muscle) protein mass occurs in proportion to the

loss of muscle mass, while in cancer cachexia visceral protein mass is conserved, and may even increase [5].

1.4.3 Energy Expenditure

Increased energy expenditure may contribute to the wasting process seen in cancer cachexia. About 70% of the total energy expenditure in healthy individuals at rest arises from the resting energy expenditure (REE). The REE in cancer patients is strongly determined by the type of tumour present. REE has been found to be elevated in patients with both lung [34] and pancreatic cancer [35], whereas no increase in REE is found in patients with gastric and colorectal cancer [34]. These observations may however depend on where in the cancer cachexia process the patient is, newly diagnosed or near death [5].

1.4.4 Acute Phase Response

In patients with pancreatic cancer, REE is significantly higher in those patients who demonstrate an elevated acute phase response (APR) [35]. The APR constitutes a series of changes in liver protein synthesis, with a shift from production of albumin to acute phase proteins (APP), such as CRP, fibrinogen, serum amyloid A, 2-macroglobulin, and α-1 antitrypsin. These changes are also observed as a response to tissue injury, infection, or inflammation [5].

1.4.5 Systemic Inflammation

Animal studies initially suggested that there was a role for pro-inflammatory cytokines in cancer cachexia. IL-1, IL-6, TNF-α and IFN-γ are associated with anorexia and weight loss in rodent tumour models [36-38]. These pro-inflammatory cytokines are inducers of the APR. In cancer patients the circulating concentration of IL-6 correlates well with markers of systemic inflammation such as C-Reactive Protein (CRP) [37]. CRP is the prototypical positive acute phase response and is measured routinely in the clinical setting. The presence of such an acute phase reaction can then be interpreted as an indirect marker of pro-inflammatory cytokine activity (IL-1, IL-6 and TNF-α), and serum CRP concentration has been positively correlated with weight loss [39, 40]. REE in patients with pancreatic cancer is increased compared with controls, and those patients demonstrating an APR have a higher REE when compared with cancer patients without Acute Phase Response [35]. This explains why inflammation is associated with cancer cachexia [37]. Long exposure to inflammatory agents in adipose tissue increases lipolysis [41].

1.4.6 Glucose Intolerance

Skeletal muscle and adipose tissue are important factors in maintaining normal glucose concentrations, and this is regulated by insulin. Glucose intolerance occurs in the majority of patients with pancreatic cancer [42] and is the result of increased peripheral insulin resistance [43]. This peripheral insulin resistance is partially relieved after the pancreatic tumour is removed [44]. Insulin resistance in cancer cachexia is associated with increased expression of mRNA for TNF-α and reduced mRNA expression of GLUT4, an active insulin dependent glucose transporter. TNF-α reduces insulin

signalling by inhibiting both tyrosine kinase activity at the insulin receptor site and reducing phosphatidylinositol-3-kinase activity [38].

1.5 TREATMENT OF CANCER CACHEXIA

There is no optimum treatment available for cancer cachexia and treatment until now has been focused on treating the signs and symptoms. Presently however the treatments that are being designed are attempting to target the pathophysiological pathways that cause cancer cachexia as these become better understood. The mainstay of present treatment is dietary consultation and emotional support [16]. All other forms of treatment are still experimental and should only be used in clinical trials.

1.5.1 Total Parenteral Nutrition

There is no role for total parenteral nutrition in catabolic patients with cancer cachexia. These patients are not starving and they have normal gastrointestinal function. All measures should be taken to support targeted food quantity and quality. In this clinical situation the supply of additional calories is not effective and only causes side effects [16]. Questions have now been raised about the above statement regarding the ineffectiveness of total parenteral nutrition as an addition to chemo- or radiotherapy in patients with cancer cachexia. There is a lack of up to date research in this field [16]. TPN is a life-saving method of nutritional support in patients who have gastrointestinal obstruction, pain or other "mechanical reason" for being unable to eat [45, 46].

1.5.2 Agents Affecting Appetite

1.5.2.1 Ghrelin receptor agonist

Ghrelin is produced in the upper part of the stomach and has a potent stimulating effect on stomach motility. It is released from the gastric mucosa in the upper part of the stomach, as a response to starvation and thereby stimulates food intake [47, 48]. It is additionally a potent stimulator of appetite and is used in clinical trials for the treatment of post-operative ileus and diabetic gastro pares. In a study of 60 cancer cachexia patients, ghrelin administration increased muscle strength and lean body mass but had no effect on body weight or the quality of life. The disadvantages are that ghrelin has to be administered as a continuous intravenous infusion [5, 38].

1.5.2.2 Corticosteroids

Because of their catabolic effect on skeletal muscle [49], corticosteroids such as dexamethasone, prednisolone, and methylprednisolone are used clinically to enhance appetite, the sensation of well-being and of performance, usually at the end-stages of cancer. Despite improvements observed in the quality of life, they have however, no beneficial effect on body weight [5, 16].

1.5.2.3 Megestrol Acetate

Megestrol Acetate is a synthetic progestin, that stimulates appetite through the hypothalamus [50] and/or by down regulating the synthesis and release of pro-inflammatory cytokines[51]. Following its administration an increase in appetite,

quality of life and body weight is observed. Analysis of body composition shows that there is an increase in adipose tissue mass but not in lean body mass [52]. Side effects include an increased risk of thromboembolism, an increase in oedema, reduced response to chemotherapy and a shorter survival trend is observed [5, 16, 38].

1.5.2.4 Cannabinoids

Cannabinoids stimulate appetite and mood and increase the quality of life but do not increase body weight. The negative effect seen on gastrointestinal motility can cause problems and outweigh its benefits [5, 16, 38].

1.5.3 Agents Affecting Mediators or Signalling Pathways

1.5.3.1 Anabolic steroids

Anabolic agents, such as the oral agent oxandrolone, are associated with an increase in body weight and lean body mass, improved nitrogen balance and quality of life in cachexia associated with infectious [53] or pulmonary diseases. Ongoing studies are currently investigating its role in the treatment of cancer cachexia [38, 54].

1.5.3.2 ACE inhibitor

Renin from the kidneys converts Angiotensin I to Angiotensin II. Angiotensin II is a potent arteriolar constrictor and increases blood pressure. Angiotensin II induces muscle catabolism, promotes anorexia and insulin resistance, and increases tumour angiogenesis [55]. In theory, Angiotensin II (ACE) inhibitors could protect against cancer cachexia. Published studies have shown some positive results and a phase III study is under way with results expected late in 2011 [38].

1.5.3.3 Eicosapentaenoic acid (EPA)

EPA is found in fish oil and it inhibits the expression and activity of the ubiquitin-proteasome pathway and blocks NF-kβ activation. EPA reverses muscle atrophy and stimulates appetite, but to a much lesser extent than megestrol acetate. A combination of EPA and megestrol acetate does not have greater effect in stimulating appetite than megestrol acetate alone [56]. It requires to be investigated if this combination of therapy has fewer side effects than using megestrol acetate alone. EPA preserves adipose tissue [57] and this may be due to the down regulating effects on ZAG [5, 57].

1.5.3.4 β-Hydroxy-β-Methylbutyrate (HMB)

HMB is similar to EPA, inhibits the expression and activity of the ubiquitin-proteasome pathway [58] and blocks NF-kβ activation. In advanced cancer cachexia, treatment with HMB increases body weight and lean body mass, with no changes in adipose tissue mass [59].

1.5.3.5 Megestrol Acetate

There is evidence available that progestin can inhibit tumour growth by interference in the G1 phase of cell division (unknown mechanism) [60].

1.5.3.6 Thalidomide

Thalidomide has various actions including the inhibition of TNF-α production, angiogenesis, down regulation of the ubiquitin-proteasome pathway and it blocks NF-kβ activation [61]. It delays the loss of body weight and lean body mass in cancer cachexia [62], but does not increase the quality of life. Side effects include increased risk of thromboembolism, neuropathy and it has a negative effect on gastrointestinal motility. The severity of the side effects, with only relatively minor positive treatment success, gives it a limited therapeutic potential in cancer cachexia [5, 38, 63].

1.5.3.7 Nonsteroidal Anti-Inflammatory Agents (NSAIDs)

NSAIDs alone or in combination with megestrol acetate, reduce REE and serum CRP, increase body weight, improve the quality of life and prolong mean survival time in patients with cancer cachexia [64].

1.5.4 Surgery

There are no prospective studies available involving patients who have well defined cancer cachexia before surgical operation and appropriate confirmation of cancer cachexia by assessment of body composition performed pre and post-operatively. There are published studies on pancreatic cancer patients showing that peripheral insulin resistance is partially relieved post-operatively [44]. Patients with cancer cachexia have a mean survival of 451 days after operation as compared to weight stable patients who have a mean survival of 654 days ($p = 0{,}001$) [65].

1.6 INTRODUCTION RELATED TO THE THESIS

The presence of insulin resistance in skeletal muscle has been suggested in experiments on patients with PDAC [43, 66-68]. In addition, impaired insulin action on phosphatidylinositol-3-kinase activity, glucose transport, and glycogen synthesis activity has been shown [68, 69]. Insulin function is known to be dependent on the energy levels of the target cells [70]. Insulin-stimulated glucose transport into muscle cells and glucose non-oxidative metabolism to glycogen are energy-requiring processes that are impaired in patients with PDAC [68, 69]. This could imply that skeletal muscle insulin resistance in these patients is associated with a decreased muscle content of energy metabolites. Insulin resistance and weight loss are coupled to increased energy expenditure in patients with PDAC [35]. Decreased insulin action on skeletal muscle glucose transport and further metabolism of glucose into glycogen could therefore be influenced by a decreased muscle content of adenosine triphosphate (ATP) and phosphocreatine. PDAC cells could influence active glucose transport in skeletal muscle.

Several studies regarding the relationship between body fat distribution and lipid metabolism show that visceral adipose tissue plays an important role in the pathophysiological alterations in lipid metabolism and insulin sensitivity [35, 69, 71]. Through elevated lipolysis, visceral adipose tissue releases increased levels of free fatty acids to the liver through the portal vein. Increased intrahepatic lipid concentration disturbs and deteriorates glucose and insulin homeostasis leading to peripheral insulin resistance [72].

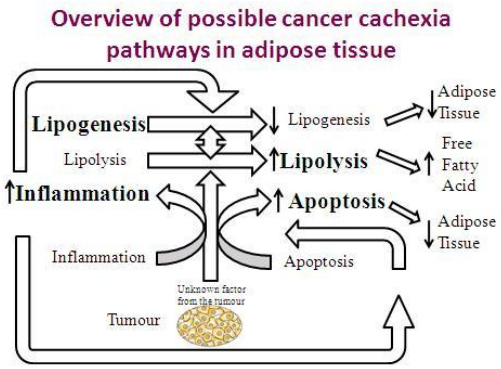


Fig. 1. Flowchart showing the possible pathways involved in wasting of adipose

Figure 1 shows an overview of possible pathways which could lead to wasting of adipose tissue and increased plasma level of free fatty acids. Increased production of lipolytic factors from adipose tissue such as IL-6 and TNF-α or tumour-derived lipolytic factors such as ZAG could explain the increased lipolysis in cancer cachexia [26, 27]. Furthermore, altered action of the major lipolysis-regulating hormones, catecholamines (stimulatory), natriuretic peptides (stimulatory), and insulin (inhibitory), may be of importance [26]. These hormones regulate lipolysis by separate mechanisms, all of which converge at the final rate-limiting step in lipolysis activation i.e. hormone-sensitive lipase (HSL)) [28]. The recently described natriuretic peptide system is mediated by the cyclic guanosine 3,5-monophosphate (cGMP) pathway, which stimulates protein kinase G activating HSL [73]. Hormonal regulation of lipolysis in adipose tissue cells by insulin, catecholamines, and natriuretic peptides may be altered in cancer cachexia.

White adipose tissue is a highly active tissue that, apart from its metabolic function, releases an array of secreted products that have both local and systemic effects on lipid and glucose metabolism [74]. Increased adipose tissue inflammation is an important factor in the development of obesity-related complications, such as insulin resistance [75, 76]. The infiltration of inflammatory cells, primarily macrophages, into the adipose tissue promotes local production of inflammatory mediators, such as cytokines, both from leukocytes and adipocytes, which in turn initiate a negative set of effects on adipocyte function [77] and insulin responsiveness and this may induce adipose tissue cell death. For instance, it is well established that IL-6 and TNF-α promote adipocyte lipolysis and attenuate insulin signalling through several mechanisms [78]. Furthermore TNF-α can induce apoptosis in human adipocytes [79]. Metabolic alterations, including increased insulin resistance, are observed when adipose tissue mass is reduced considerably [80]. The remaining adipose tissue in patients with cancer cachexia may display an altered inflammatory reaction that could promote significant changes in adipose tissue cell function, including increased lipolysis and possibly reduced lipogenesis.

The volume of muscle and adipose tissue mass can be determined by computed tomography (CT). This is often assessed in clinical weight reduction trials in obese subjects with or without diabetes [81, 82]. The impact of cancer cachexia on the volume of muscle, subcutaneous and visceral adipose tissue in patients with recently diagnosed gastrointestinal cancer, is an interesting subject to consider investigating. Volume determined by CT could give information about distribution or wasting of muscle, subcutaneous and visceral adipose tissue in patients with recently diagnosed cancer cachexia [83].

2 AIMS

2.1 STUDY I

To investigate if in pancreatic cancer:

- Decreased insulin action on skeletal muscle glucose transport is influenced by decreased ATP levels in muscle cells
- Pancreatic cancer cells change glucose metabolism in muscle

2.2 STUDY II

To investigate in cancer cachexia patients:

- The rate of lipolysis
- The factors involved in lipolysis

2.3 STUDY III

To investigate if in cancer cachexia patients:

- Lipogenesis in adipose tissue is decreased
- Inflammation in adipose tissue is increased
- Apoptosis in adipose tissue is increased

2.4 STUDY IV

To investigate if in cancer cachexia patients:

- There is a difference in the volume of adipose- and muscle compartments between cancer cachexia patients and benign disease control patients
- Adipose tissue wasting is an early phenomenon in the cancer cachexia syndrome

3 MATERIALS AND METHODS

3.1 STUDIES DESIGN

In Study I, there were four groups of patients:

a) Pancreatic Ductal Adenocarcinoma (PDAC)
b) Other Cancers
c) Chronic Pancreatitis (CP)
d) Benign Controls

The study patients were recruited consecutively at the surgical outpatient clinic or from those who were already scheduled for surgical treatment. Patients with known diabetes were excluded. Postoperatively, the correct diagnosis was confirmed in all patients by histopathological examination.

In studies II-IV, patients with newly diagnosed gastrointestinal cancer who attended the surgical outpatient clinic between January 2004 and December 2008 were evaluated for inclusion in the study. Patients who had not received previous cancer treatment, and who were willing to participate, were included.

Based on clinical examination by the same surgeon, patients were allocated to:

a) Cancer cachexia group (CC): Defined as those patients with unintentional weight loss of > 5% of habitual weight during the last three months **or** > 10% during the last six months and with no evidence of gastrointestinal obstruction

b) Weight stable group (WS): Defined as those patients with unintentional weight loss of < 5% of habitual weight during the last three months **and** < 10% during the last six months and with no evidence of gastrointestinal obstruction

c) Gastric obstruction (GO) group: Defined as those patients who reported weight loss as defined for the CC group above, in combination with evidence of obstructive gastrointestinal cancer, resulting in malnutrition.

The researchers responsible for lipolysis and lipogenesis studies, gene and protein expression and secretions studies, preadipocyte and radiological studies were blinded regarding which groups the patients were in. Tumour staging was based on histopathological examination in those patients who had surgery and on a combination of radiological findings and case notes review in those patients who did not have surgery [84]. None of the patients had jaundice.

The patients in studies II and IV were divided into three groups:

a) Cancer cachexia
b) Weight stable
c) Gastric obstruction

The patients in study III were divided into three groups:

a) Cancer cachexia
b) Weight stable
c) Patients with benign disease

3.1.1 Study I

The patients were divided into four groups: PDAC, other cancers, CP and benign controls. Venous blood samples were taken at the patient clinical laboratory and OGTT performed. Muscle biopsies were taken in the operating theatre and were frozen immediately or transported fresh directly to the research laboratory and analyzed for glycogen, adenosine triphosphate and phosphocreatine content. Glucose transport was monitored in muscle strips and myotubes (Fig. 2).

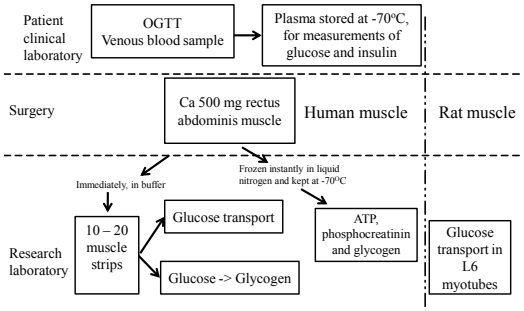


Fig. 2. Flowchart for study I Patient's examinations and muscle biopsy research.

3.1.2 Study II

The patients were divided into three groups: cancer cachexia, weight stable and gastric obstruction. Adipose tissue biopsies were obtained under local anaesthesia, blood samples were taken and energy expenditure and body composition measured at the patient clinical laboratory. Adipose tissue biopsies were transported fresh to the research laboratory for measurement of lipolysis, protein and gene expression, and isolation of preadipocytes (Fig. 3).

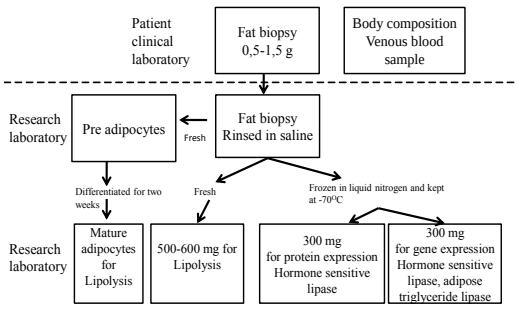


Fig. 3. Flowchart for study II, Patient's examinations and adipose tissue biopsy research.

3.1.3 Study III

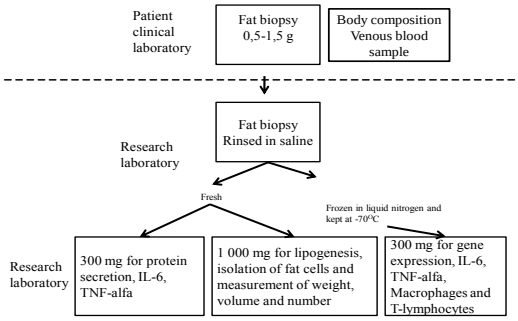


Fig. 4. Flowchart for study III, Patient's examinations and adipose tissue biopsy research.

The patients were divided into three groups, cancer cachexia, weight stable and no cancer. The no cancer group consisted of patients who were thought, before surgery, to have cancer, but who, after histopathological examination, were found not to have cancer. These patients were then reclassified. Adipose tissue biopsies were obtained under local anaesthesia and blood samples were taken at the patient clinical laboratory. Adipose tissue biopsies were transported fresh to the research laboratory for isolation of adipocytes and measurements of their weight, volume and number. Lipogenesis, gene expression and cytokine secretion was measured (Fig. 4).

3.1.4 Study IV

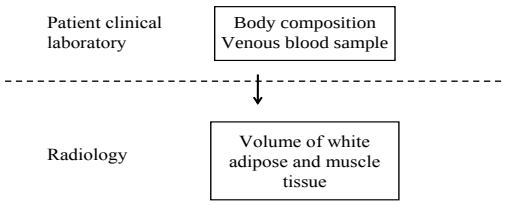


Fig. 5. Flowchart for study IV, Patient's examinations and adipose tissue biopsy research.

The patients were divided into three groups, cancer cachexia, weight stable and gastric obstruction. Blood samples were taken and energy expenditure and body composition measured at the patient clinical laboratory. Adipose and muscle tissue volume was measured with computed tomography (CT) (Fig. 5). Patients with benign disease, based on post-operative reclassification according to histopathological examination, were excluded.

3.2 HUMAN CLINICAL STUDIES

3.2.1 Clinical Examination

In Study I, body weight was measured at the surgical outpatient clinic and OGTT was performed following an overnight fast. In studies II-IV, the patients attended the patient clinical laboratory after an overnight fast. Height, weight, hip and waist measurements were taken and body composition determined, with bioimpedance using Bodystat Quad Scand 4000 (Bodystat Ltd.). Basal Metabolic Rate (BMR) was calculated with a well-established formula [85] and Energy Expenditure (EE) was measured by indirect calometry. The nutritional status was assessed by using a standardized questionnaire for oncology; patient-generated-subjective global assessment (PG-SGA) [86].

3.2.2 Adipose Tissue Biopsies

Abdominal subcutaneous WAT samples (0.5–1.5 g) were obtained by needle biopsy as described by others [87]. The tissue samples (≈5 mg each) were rapidly rinsed in 0.9% saline and submitted to lipolysis investigation. Subject to the volume available, one portion of the collected adipose tissue sample was frozen in liquid nitrogen and stored at -70^{o}C for later gene expression studies. Another portion of the collected adipose tissue was used for cytokine secretion, and finally a portion of the collected adipose tissue was used for isolation of fat cells and lipogenesis studies. It has previously been shown that adipose tissue samples removed and frozen in this way are free from damaged cells and blood [88]. It should be pointed out that the adipose tissue samples were obtained pre-operatively, by needle biopsy under local anaesthesia and not during surgery, as it has previously been demonstrated that general anaesthesia influences adipose tissue function [30].

3.2.3 Plasma Analysis

A venous blood sample was obtained for the determination of haemoglobin, transferrin, glucose, triglycerides, high density lipoprotein (HDL), glycerol, free fatty acids, albumin, C-Reactive Protein (CRP), insulin-like growth factor I (IGF-I), leptin, noradrenalin, adrenaline and insulin by the hospital's accredited routine chemistry laboratories. Additionally atrial natriuretic peptide was measured using a RIA kit (Phoenix Peptides). Plasma glycerol and free fatty acid (FFA) levels were presented as a ratio of total body fat. TNF-α and IL-6 levels were determined as described by others [89].

3.3 LABORATORY STUDIES

3.3.1 Muscle Biopsy Procedure and Strip Preparation

Following induction of general anaesthesia, skeletal muscle biopsies (ca 500 mg) were surgically excised from the rectus abdominalis muscle. The samples were transported immediately to the laboratory in Krebs-Henseleit bicarbonate buffer (KHB) solution containing 5mM HEPES, 0.1% bovine serum albumin, 5mM glucose and 15mM mannitol. In the laboratory, 10 to 20 muscle strips were prepared from the sample and mounted on Plexiglas clamps [90]. For equilibrium, these strips were incubated in KHB in sealed vials for 15 minutes.

3.3.2 Glucose Incorporation into Glycogen

Following the equilibrium period, the skeletal muscle strips were incubated with or without insulin (1000mU/mL) for 2 hours in 2 mL of KHB solution containing 5 mM glucose and [U-14C] glucose (0.3mCi/mL). After incubation, the muscle strips were rapidly removed and frozen in liquid nitrogen at -70^{o}C. The frozen skeletal muscle strips were processed as described by Cuendet [91], glycogen was precipitated as done by Stauffacher [92], and [U-14C] glucose was used to estimate the rate of glucose conversion into glycogen. Glucose conversion into glycogen is expressed as nanomoles per gram per hour and fold increase over basal glucose conversion into glycogen.

3.3.3 ATP, Phosphocreatine, and Glycogen Content

ATP and phosphocreatine were extracted from 5 mg of ground freeze-dried muscle and measured fluorometrically by enzyme methods described by Harris [93]. The glycogen content of the muscle was determined using hexokinase and glucose-6-phosphate dehydrogenase, measuring the production of reduced nicotinamide adenine dinucleotide by spectrophotometry, after alkaline destruction of free glucose and hydrolysis of glycogen with amyloglucosidase, described by Lust [94]. The glycogen content was expressed as millimoles of glucosyl (a glycosyl radical $C_{16}H_{11}O_5$ derived from glucose per kilogram of dry weight [95]).

3.3.4 Glucose Transport

3.3.4.1 Glucose Transport in Isolated Muscle Strips

The muscle strips were incubated in KHB containing 0 or 1000 mU/mL of insulin for 30 minutes. Following that [3H] 3-O-methylglucose (5 mM, 2.5mCi/mmol) and [14C] mannitol (15 mM, 26.3 mCi/mmol; ICN Biomedical, Costa Mesa, CA, USA) were added to the buffers. The vials were subsequently incubated for 20 minutes. All these serial incubations were maintained at 35^{o}C with 95% O_2/5% CO_2.

Glucose transport was assessed in skeletal muscle using the glucose analogue [3H] 3-O- methyl glucose, as previously described for rat epitrochlearis muscle [96], with modifications for human skeletal muscle as described by Zierath [97]. The 3-O-methylglucose enters the muscle cells using the same transport carrier protein as glucose. This glucose analogue cannot be further metabolized, thus allowing direct assessment of glucose transport activity without influencing the glucose metabolism. The incubated muscle samples were processed as previously described [96] and sample aliquots were counted in a liquid scintillation counter with the channels preset for simultaneous measurement of [3H] (glucose) and [14C] (mannitol) . The amount of each isotope present in the sample was determined and used to calculate the extracellular space (mannitol) and the intracellular concentration of 3-O-methyl glucose. The intracellular water content of the muscle sample was determined by subtracting the measured extracellular water space from the total muscle water. The extracellular space was estimated using [14C] mannitol. Mannitol cannot pass through the muscle cell membrane and was therefore used as an indirect measurement of muscle cell viability [98]. The basal rate of 3-O-methylglucose transport was expressed per millilitre of intracellular water. Insulin-stimulated glucose transport was expressed as fold increase over basal.

3.3.4.2 Glucose Transport in L6 Myotubes

Rat L6 myoblasts, human PDAC-cells (MiaPaca 2) and fibroblasts (HFF1) were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Human osteosarcoma cells (MG-63) were obtained from a fellow laboratory at the Karolinska Institutet. Rat L6 myoblasts were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 22.5 Mm glucose, 10% (v/v) fetal calf serum (FCS), 3% (v/v) 100 U/mL of penicillin, and 3% (v/v) 100 mg/mL of streptomycin (PEST). The cells were seeded in 12-well plates. Differentiation of the myoblasts was commenced at 80% confluence, by microscope, using DMEM supplemented with 5.6

mM glucose, 2% (v/v) FCS, and 3% (v/v) PEST for 5 days with a medium change on day 2 and day 4. The differentiation of myoblasts to myotubes was monitored by microscopic observation. Starvation was started on day 6, for 21 hours, using either DMEM supplemented with 5.6 mM glucose, 0% (v/v) FCS, 3% (v/v) PEST, and 6% (v/v) of 1 mg/mL of bovine serum albumin (unconditioned media) or DMEM supplemented with 5.6 mM glucose, 0% (v/v) FCS, 3% (v/v) PEST, and 6% (v/v) of 1 mg/mL of bovine serum albumin conditioned media. Conditioned media was made by using DMEM supplemented with 5.6 mM glucose, 0% (v/v) FCS, 3% (v/v) PEST, and 6% (v/v) of 1 mg/mL of bovine serum albumin, incubated with fibroblasts, osteoblasts, or pancreatic carcinoma cells for 24 hours. After those 24 hours in preparation of conditioned media, the glucose concentration, was measured and increased up to 5.6 mM if required. The myotubes were incubated with 0.4 mM 2- deoxyglucose (2-DOG) with or without insulin (2000 nM Actrapid) for 60 minutes and thereafter with radioactive 2-DOG for 10 minutes. Glucose transport was terminated by ice-cold phosphate buffered saline. NaOH was then added for 90 minutes to lyse the cells. Protein concentrations were determined by a Bio-Rad colorimetric assay (Bio-Rad, Hercules, CA, USA). The radioactivity in the lysate was determined by liquid scintillation counting. The 2- DOG uptake was expressed as nanomoles of 2-DOG per milligram of protein per minute. MiaPaca 2, HFF1, and MG-63 were grown in media according to instructions from the ATCC and the fellow laboratory. The cells had undergone 4 to 10 passages from the time they were defrosted, until they were used to make the conditioned media.

3.3.5 Isolation of Adipocytes from Adipose Tissue

The adipocytes were isolated from the adipose tissue according to the collagenase procedure described by Rodbell [99]. In summary, the tissue was cut into 20-mg pieces and incubated (1 g tissue/mL medium) in Krebs-Ringer phosphate (KRP) buffer (pH 7.4) containing 4% bovine serum albumin (BSA) and 0.5 mg/mL collagenase Type I for 60 minutes at 37^{o}C in a shaking water bath. The isolated adipose cells were collected on a nylon mesh filter and washed 4 to 5 times with 0.1% KRP-BSA buffer. The purity of the isolation procedure was estimated by investigating 200 cells from each sample under a light microscope. The number of isolated cells that did not resemble adipose tissue cells or cell material that was stuck to an adipose cell was always 0 to 2 per 200 counted adipocytes.

3.3.6 Measurement of Adipose Cell Volume and Number

Mean adipose cell volume and weight was determined in isolated adipose tissue cells as follows: The greatest dimension of an adipose tissue cell was determined with direct microscopy, and the greatest mean dimension of 100 cells from each patient was determined. The mean adipose tissue cell volume and weight was calculated by using the formulas developed by Hirsch and Gallian [100]. The coefficient of variation for this method is 2% to 3%. The mean value is essentially the same as that determined from adipose tissue cell sizing of intact samples of human adipose tissue [100]. The total number of adipose tissue cells in the incubated sample was calculated as the lipid weight of the incubated adipose tissue cells divided by the mean adipose tissue cell weight. The total number of adipose tissue cells in the body was calculated as the amount of body fat divided by the mean adipose tissue cell weight. It is well recognized

that mean adipose tissue cell volume and weight differs between various adipose tissue regions in humans. However, the differences are small and introduce only a marginal error when a single source is used for the calculation of total adipose tissue cell numbers, as discussed previously [101]. In the whole cohort, total adipose tissue cell numbers (mean ± standard error) were $45 \pm 4 \times 10^9$, which is in the same range as found in several other studies [100, 101]. In a separate methodological study, the bioimpedance method was used to determine body fat by comparing it with dual-x-ray absorbmetry (DEXA) in 7 men and 14 women with a body mass index (BMI) from 17 kg/m^2 to 46 kg/m^2 and age range of 22 to 79 years. There was an excellent correlation between the two measures ($r = 0.92$; linear regression) with slope and intercept near 1.0 and zero, respectively. The coefficient of variation for the 2 measures was 3.6% in non-obese patients and 3.9% in obese patients (the group was divided at a BMI of 30 kg/m2) (not published data). Taken together, these methodological data strongly suggest that the determination of the total number of adipose tissue cells in the body is accurate.

3.3.7 Lipogenesis

Lipogenesis assessment in isolated adipose tissue cells was performed as described in detail previously [102]. In brief, adipose tissue cells from adipose tissue were isolated and incubated for 2 hours at 37°C in a buffer containing [3H]-glucose without (basal) and with different concentrations of insulin. After incubation, lipids were extracted, and glucose radioactive [3H] uptake into the total lipid mass was used as an index of lipogenesis. The ability of insulin to stimulate basal lipogenesis was calculated. In addition, the half-maximum effective concentration (EC_{50}) of insulin for each individual concentration response curve was determined.

3.3.8 Lipolysis

Isolated adipose tissue cells were prepared by collagenase treatment; adipose tissue cell size was determined and lipolysis experiments were performed on isolated adipose tissue cells as described [103]. In summary, diluted adipose tissue cell suspensions (2%, vol/vol) were incubated in an albumin buffer (pH 7.4) supplemented with glucose, ascorbic acid, and, for the insulin experiments, adenosine deaminase and 10^{-3} mol/L of 8-bromocyclic AMP (8-Br-cAMP). The cells were incubated at 37°C for 2 hours without (basal) or with increasing concentrations (10^{-16}–10^{-5} mol/L, depending on the hormone used) of noradrenalin, atrial natriuretic peptide, insulin together with 10^{-2} mol/L 8-Br-cAMP, 8-Br-cAMP (10^{-2} mol/L) alone, or with 10^{-2} mol/L 8-bromo-cGMP (8-Br-cGMP). Thereafter, glycerol release into the incubation medium (lipolysis index) was determined. In further lipolysis experiments, the buffer containing 8-Br-cAMP plus adenosine deaminase was also supplemented with a maximum effective concentration (maximal number receptors) (10^{-5} mol/L) of a highly selective inhibitor of hormone-sensitive lipase, 4- isopropyl-3- methyl-2-[1-(3-(S)-methyl-piperidin-1-yl)-methanoyl]-2H-isoxazol- 5-one, termed BAY [104].
The lipolysis data were expressed in two ways. The first was glycerol release per gram of lipids in the studies of mature adipose tissue cells or per gram of protein in the preadipocyte studies. The second was as a function of basal (non–hormone stimulated) glycerol release. The latter was defined as insulin-induced values divided by 8-Br-cAMP–induced values in the anti-lipolytic experiments and noradrenaline- or atrial

natriuretic peptide induced values divided by basal values in the lipolytic experiments. Maximum effect was defined as the value obtained at maximum effective hormone concentration. Priority was given to expression of lipolysis as a function of basal rate because others have shown a strong correlation between this way of expressing lipolysis and HSL gene or protein expression in human subcutaneous adipose cells [105].

3.3.9 Preadipocytes

In five cancer cachexia patients and eight weight-stable control patients, a sufficient amount of adipose tissue stroma was obtained for preadipocyte isolation. These cells were differentiated exactly as described by Langin [104], for 2 weeks, and lipolysis experiments without (basal) or with 10^{-5} mol/L of noradrenaline were performed as described by Lofgren [106].

3.3.10 Gene Expression

Gene expression analyses were performed as described previously [89]. Total RNA was extracted from WAT tissue using the RNeasy mini kit (Qiagen GmbH). The Agilent 2100 Bioanalyzer (Agilent Technologies) was used to confirm the integrity of the RNA. One microgram of total RNA from each sample was reverse transcribed to cDNA using the Omniscript RT kit (Qiagen) and random hexamer primers (Invitrogen). cDNA synthesis was performed simultaneously for all patients using the same mix of primers and RT kit. In the final volume of 25 µL, 5 ng of cDNA were mixed with 2x SYBR green polymerase chain reaction (PCR) master mix (Bio-Rad Laboratories, Inc.) and primers (Invitrogen). The primer pairs were selected to yield a single amplicon based on dissociation curves and analysis by agarose gel electrophoresis.
The primers used were 5-GTGTCAGACGGCGAGAATG-3 (sense) and 5-TGGAGGGAGGGAGGGATG-3 (antisense) for adipose triglyceride lipase, 5-GGAAGTGCTATCGTCTCTGG-3 (sense) and 5-GGCAGTCAGTGGCATCTC-3 (antisense) for HSL,
To determine if there were local signs of increased inflammation in adipose tissue, subcutaneous WAT was assessed for mRNA expression. Messenger RNA (mRNA) was analyzed for IL-6 (NM_000600.2), TNF-α (NM_000594.2), CD68 (NM_001251.2), and CD3D (NM_000732.4).
The control used was 5-TGACTCAACACGGGAAACC- 3 (sense) and 5-TCGCTCCACCAACTAAGAAC-3 (antisense) for 18S.
Quantitative real-time PCR was performed in an iCycler IQ (Bio-Rad Laboratories Inc.) and was quantified using TaqMan kits (Applied Biosystem, Foster City, Calif) for Hs00174131_m1 (IL-6), Hs00174128_m1 (TNF-α), Hs00154355_m1 (CD68), and Hs00174158_m1 (CD3D).
Expression of mRNA was normalized to the 18S internal control by using a comparative Ct method. The patient with the highest Ct value was used as a reference point from which all other Ct values for the target gene and the reference gene was subtracted. The Ct values were then normalized to ribosomal RNA for 18S using the formula 2ΔCt-target gene / 2ΔCt-reference gene. Previous studies have demonstrated that these markers correlate well with infiltration of macrophages (CD68) and T-lymphocytes (CD3) [107, 108].

3.3.11 Protein Expression

Approximately 300 mg of white adipose tissue was crushed and lysed in a protein buffer [1% Triton-X 100, Tris-HCl (pH 7.6), and 150 mmol/L NaCl, 4jC], supplemented with protease inhibitors [1 mmol/L phenylmethylsulfonyl fluoride and Complete (Boehringer Mannheim)], and homogenized. The homogenate was centrifuged and the infranatant was collected and saved. The protein content was assayed using BCA Protein Assay Reagent Kit (Pierce). One hundred micrograms of total cellular protein was loaded on polyacrylamide gels and separated by standard 12% SDS-PAGE. The gels were transferred to polyvinylidine fluoride membranes (Amersham Pharmacia). The blots were blocked for 1 hour at room temperature in Tris-buffered saline with 0.1% Tween 20 and 5% non fat dried milk. This was followed by an overnight incubation at 4^{o}C in the presence of antibodies directed against HSL [109] and the control protein β-actin (Sigma). Secondary a-rabbit antibodies conjugated to horseradish peroxides were provided by Sigma. Antigen-antibody complexes were detected by chemiluminescence using a detection kit (SuperSignal) from Pierce and specific bands of complexes were detected and quantified using a Chemidoc XRS system and the Quantity One Software from Bio-Rad, and expressed as the HSL/β-actin ratio.

3.3.12 Cytokine Secretion

TNF-α and IL-6 secretion in the adipose tissue medium, described above, was measured exactly as described previously [89]. In summary, a portion of adipose tissue was incubated in an albumin-containing buffer for 2 hours at 37^{o}C. After incubation, an aliquot of the medium was removed and stored at -70^{o}C for later analysis of TNF-α and IL-6. The total amount of protein secreted was found to be related to the wet weight, lipid weight, and the number of adipose tissue cells in the incubated samples.

3.4 RADIOLOGICAL STUDIES

Computed Tomography (CT) was performed as a staging procedure and assessed by the multidisciplinary cancer team. All CT examinations were performed within 1 – 2 weeks following the diagnosis of cancer or hepatic metastases.
Later, these images were used to obtain measurements of the total adipose tissue, visceral adipose tissue, abdominal and para-spinal muscle areas, using segmentation of a 1 cm thick slice through the centre of the third lumbar vertebra. The total WAT area was then defined as all pixels with a density within the range of -150 and -40 Hounsfield units (Hu). The total adipose tissue area that was measured from the CT was in cm^2 and when multiplied by 1 cm (CT segmentation thickness) gave the volume in cm^3. The visceral adipose tissue volume was obtained by a rough manual delineation of the abdominal cavity (Fig. 6A). The subcutaneous adipose tissue volume was calculated by subtraction of the visceral volume from the total volume. The total muscle area was defined as all pixels with a density within the range of 1 to 100 Hu. Para-spinal muscle and abdominal muscle were manually delineated (Fig. 6B and 6C) carefully avoiding the intestine.

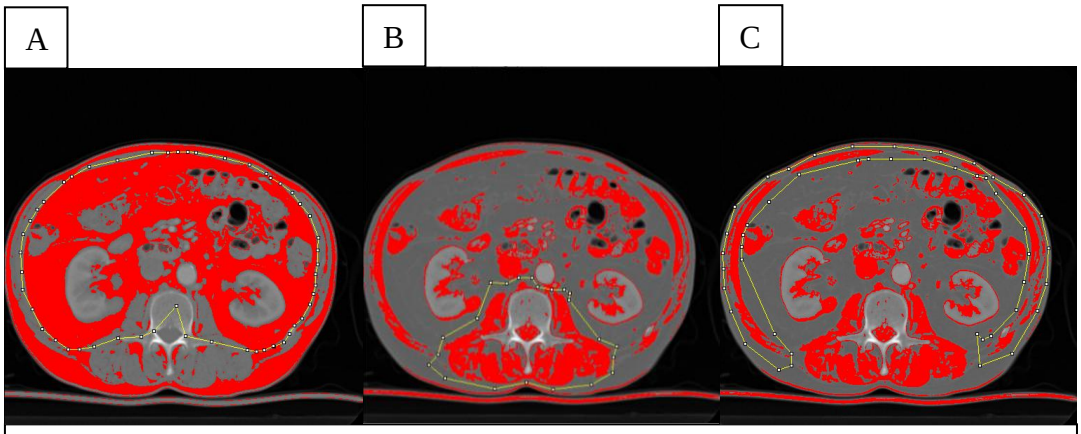


Fig. 6. CT image of the abdomen where: (A) the volume of visceral white adipose tissue (WAT); (B) the volume of para-spinal muscle; (C) the volume of abdominal muscle, was obtained by a rough manual delineation of the abdominal cavity, para-spinal and abdominal muscles. The volume is expressed in cm^3 adipose tissue or muscle.

3.5 STATISTICAL METHODS

In all the studies the values were reported as: a) mean ± SD / SEM; b) median with range. The results were compared using ANOVA and appropriate post hoc tests, Student's unpaired or paired t test, or linear regression by the method of least squares. Kruskal-Wallis and Mann-Whitney tests were used to compare SGA and tumour score values. Correlation calculations were made according to Pearson.

In study II, a power calculation was made before patient recruitment and was based on the previously known distribution of maximum noradrenalin induced glycerol release divided by basal glycerol release [103]. The recruitment of weight-stable patients was expected to be easier and anticipated the relative proportion between the three groups (CC, WS, GO) to be 2:3:2. Based on these estimations and mean and SD of noradrenalin/basal lipolysis, it was calculated that 7 cancer cachexia patients, 11 weight-stable control patients, and 7 weight-losing control patients had to be recruited to detect a 70% difference between cancer cachexia and the two control groups at $P < 0.05$ (ANOVA) with 80% power using Sample Power (SPSS, Inc.).

In study III, the patients were recruited in a consecutive manner as 2 groups; weight-stable cancer and cancer cachexia. A power calculation was made before patient recruitment using Sample Power (SPSS Inc., Chicago, Ill). It was based on the three major measures in adipose tissue and in blood circulation in the current study (i.e., gene expression, protein secretion, and circulating levels of TNF-α and IL-6). As far as is known, there have been no reports on the adipose tissue values in cancer patients, except for circulating TNF-α and IL-6 levels, which were determined with other methods than those used in our study. Therefore, we used previously published data obtained in individuals without cancer for the parameters detailed above [89]. These values were generated in our own laboratory using the same methods that we used in the current study. Lg-values for means and standard deviations were used to obtain a normalized distribution. We conducted a two-group analysis, setting the weight-stable group values as the historic mean and standard deviation values and the cancer cachexia values as 1.3 times the historic values, thus setting the effect size at 30%.This

effect size was chosen for two reasons. First, a smaller effect size would necessitate recruitment of a very large number of patients. Second, a dietary challenge caused a 25% to 30% significant change in the measured TNF-α and IL-6 parameters in the previous study in individuals without cancer [89]. According to our calculations, it would be anticipated that a 30% increase (or decrease) in the cancer cachexia group would be found compared to the other groups at a $P = 0.05$ or better (two-tailed Student t test) for mRNA expression, protein secretion, and serum levels of TNF-α and IL-6 if we recruited 14 patients in each group with a power ranging from 94% to 99% (mean power, 97%). It was initially assumed that the distribution of patients should be equal, allocating 14 patients to each group by recruiting 28 patients in a consecutive fashion. In fact after recruitment, there were 13 cachectic patients and 15 non cachectic patients available for inclusion in the study. However, this had no important effect on the power calculation when the power analysis was repeated on the actual number of patients or when the two groups were divided into three groups, after histopathological examination.

3.6 ETHICAL APPROVAL

The ethics committee for human studies at the Karolinska Institutet approved the study protocols and informed consent was obtained from each patient before participation.

4 RESULTS

4.1 NUMBERS OF PATIENTS AND THEIR DIAGNOSES

4.1.1 Study I

There were thirteen patients with PDAC and eight patients in each of the other three groups. There were patients with chronic pancreatitis, patients with cancers other than PDAC, and control patients with benign diseases. Table 2 shows the diagnoses of the patients in the cancer and benign groups.

Table 2. Diagnoses of patients in the cancer and benign groups in study I.

Cancer patients		**Patients with benign disease**	
Cholangiocarcinoma	2	Pancreatic cystadenoma	5
Gallbladder cancer	2	Benign liver cyst	1
Papilla of Vater cancer	1	Idiopathic thrombocytopenic purpura	1
Duodenal cancer	1	Adenoma of the papilla of Vater	1
Gastric cancer	1		
Colon cancer	1		

4.1.2 Study II

There were seven cancer patients in the CC group, eleven in the WS group and eight in the GO group. Table 3 shows diagnoses of the patients.

Table 3. Diagnoses of patients in study II.

Cancer Cachexia (CC)		**Weight-stable cancer (WS)**		**Gastro-intestinal obstruction in cancer (GO)**	
Pancreatic Ductal Adenocarcinoma (PDAC)	5	Pancreatic Ductal Adenocarcinoma (PDAC)	3	Pancreatic Ductal Adenocarcinoma (PDAC)	1
Liver metastasis from Colorectal cancer	1	Liver metastasis from Colorectal cancer	4	Oesophageal cancer	5
Gastric cancer	1	Gastric cancer	1	Chronic cholecystitis	1*
		Oesophageal cancer	1	Chronic pancreatitis	1*
		Chronic pancreatitis	2*		

* Four patients did not have cancer, two in WS and two in GO groups.

4.1.3 Study III

In the CC group there were thirteen patients, in the WS group there were ten patients and in the group with benign disease there were five patients. Table 4 shows the diagnoses of the patients in these groups.

Table 4. Diagnoses of patients in study III.

Cancer Cachexia (CC)		**Weight Stable (WS)**		**Patients with benign disease**	
Pancreatic Ductal Adenocarcinoma (PDAC)	6	Pancreatic Ductal Adenocarcinoma (PDAC)	7	Chronic cholecystitis	2
Liver metastasis from Colorectal cancer	1	Liver metastasis from Colorectal cancer	1	Chronic pancreatitis	3
Gastric cancer	1	Gastric cancer	1		
Oesophageal cancer	5	Oesophageal cancer	1		

4.1.4 Study IV

There were thirteen patients in the CC group, seventeen in the WS group and ten in the GO group. Table 5 shows the distribution of cancer types in the different groups.

Table 5. Diagnoses of patients in study IV.

Cancer Cachexia (CC)		**Weight-stable cancer (WS)**		**Gastro-intestinal obstruction in cancer (GO)**	
Pancreatic Ductal Adenocarcinoma (PDAC)	9	Pancreatic Ductal Adenocarcinoma (PDAC)	4	Oesophageal cancer	9
Liver metastasis from Colorectal cancer	1	Liver metastasis from Colorectal cancer	5	Gastric cancer	1
Gastric cancer	3	Gastric cancer	3		
		Oesophageal cancer	3		
		Cholangiocarcinoma	2		

4.2 HUMAN CLINICAL STUDIES

4.2.1 Demographics and Nutritional Status

Tables 6 - 8 provide lists of the characteristics of the patients in studies I - IV. Patients with PDAC in study I were older than patients in the benign control group (Table 6).

Table 6. Characteristics of the patients in study I.					
Measure	**Pancreatic Ductal Adenocarcinoma (PDAC)**	**Cancer**	**Chronic pancreatitis**	**Patients with benign disease**	**p**
BMI, Kg/m^2	23,6 ± 1,3	23,0 ± 2	21,3 ± 0,7	25,7 ± 1	N.S.
Age, years	70 ± 2	68 ± 3[1]	52 ± 3	53 ± 4	< 0,05[1]
Weight loss, % of habitual weight*	6 ± 3	3 ± 3	6 ± 3	2 ± 2	N.S.

NOTE: Values are mean ± SEM.

Abbreviations: BMI, Body Mass Index

*: During previous three months

1: PDAC vs. benign controls

In studies II and IV patients in the CC group had a lower waist perimeter measurement than patients in the WS group, patients in the weight losing groups had lower BMI and hip perimeter measurements than the WS group, and there were differences in weight loss and PG-SGA between all the groups (Table 7). There were no differences in the tumour stage between the groups.

Table 7. Characteristics of the patients in studies II and IV				
Measure	**Cancer Cachexia (CC)**	**Weight-stable cancer (WS)**	**Gastro-intestinal obstruction in cancer (GO)**	**p**
BMI, Kg/m^2 (n=40)	21,9 ± 0,6	25,1 ± 0,9	20,8 ± 1,3	< 0,01[1,3]
Age, year (n=40)	64,9 ± 1,9	62,5 ± 2,5	62,7 ± 1,9	N.S.
Weight loss, % of habitual weight (n=36)*	10,0 ± 1,8	3,1 ± 1,6	17,1 ± 3,2	< 0,001[1,2,3]
Waist perimeter, cm (n=36)	85,1 ± 3,3	95,4 ± 2,2	86,8 ± 3,8	< 0,05[1]
Hip perimeter, cm (n=36)	94,2 ± 1,5	99,5 ± 1,4	92,3 ± 2,4	< 0,05[1,3]
Waist / Hip ratio (n=36)	0,90 ± 0,09	0,96 ± 0,05	0,94 ± 0,06	N.S.
PG-SGA, points (n=36)	8 (1-17)	3 (1-13)	12 (3-19)	< 0,001[1,2,3]
Tumour stage (n=40)	3 (1-4)	3 (1-4)	3 (3-4)	N.S.

NOTE: Values are mean ± SEM or median (range).

Abbreviations: N.S. Non Significance; PG-SGA, patient-generated-subjective global assessment; BMI, Body Mass Index

*: During previous six months

1: Cachexia vs. Weight- stable cancer, 2: Cachexia vs. Cancer with obstruction

3: Weight-stable cancer vs. Cancer with obstruction

In study III patients in the CC group had lost more weight, had lower BMI and higher PG-SGA scores than the other groups (Table 8). There were no differences in tumour stage between the groups.

Table 8. Characteristics of the patients in study III.

Measure	Cancer Cachexia (CC)	Weight Stable (WS)	Patients with benign disease	p
BMI, Kg/m^2 (n=28)	22,2 ± 0,6	26,5 ± 1,0	28,0 ± 6,2	< 0,01[1,2]
Age, year (n=28)	65 ± 2	63 ± 2	59 ± 3	N.S.
Weight loss, % of habitual weight (n=28)*	11,7 ± 1,8	1,0 ± 1,2	3,0 ± 1,8	< 0,01[1,2]
PG-SGA, points (n=28)	11 (5-18)	3 (1-3)	4 (1-10)	< 0,001[1,2]
Tumour stage (n=28)	3 (1-4)	3 (1-4)	0	N.S.

NOTE: Values are mean ± SEM or median (range).

Abbreviations: N.S. Non Significance; PG-SGA, patient-generated-subjective global assessment; BMI, Body Mass Index

*: During previous six months. 1: Cachexia vs. Weight- stable cancer 2: Cachexia vs. No cancer

4.2.2 Energy Expenditure

There were no differences in the increase in EE from the calculated BMRs between the groups, as shown in Table 9. But within the groups, it was only patients in the CC group who's EE had significantly increased from the calculated BMR, results not shown.

4.2.3 Body Composition

Tables 9 and 10 show the patients' body composition in studies II to IV. In studies II and IV patients in the weight losing groups had less adipose tissue mass than patients in the WS group. In other respects there was no statistically significant difference in body composition between the groups.

Table 9. Body composition of the patients in studies II and IV.

Measure	Cancer Cachexia (CC)	Weight-stable cancer (WS)	Gastro-intestinal obstruction in cancer (GO)	p
Body fat, % (n=36)	24,4 ± 1,8	27,4 ± 1,2	22,7 ± 0,4	N.S.
Fat mass, kg (n=34)	16,5 ± 1,1	21,1 ± 1,0	16,4 ± 2,0	< 0,05[1,2]
Lean body mass, kg (n=34)	57,7 ± 4,2	58,5 ± 3,5	54,9 ± 4,4	N.S.
Total body water, kg (n=34)	42,5 ± 2,7	43,0 ± 2,1	43,3 ± 2,6	N.S.
Increase of EE from BMR, % (n=34)	13,0 ± 2,6	11,2 ± 2,9	14,1 ± 3,7	N.S.

NOTE: Values are mean ± SEM.

Abbreviations: EE, Energy Expedience; BMR, Basal Metabolic Rate

1: Cachexia vs. Weight- stable cancer; 2: Weight-stable cancer vs. Cancer with obstruction

In study III there were differences in the patients´ adipose tissue mass between all the groups but no statistically significant differences in lean body mass (Table 10).

Table 10. Shows the body composition of the patients in study III.

Measure	Cancer Cachexia (CC)	Weight-stable cancer (WS)	Patients with benign disease	p
Fat mass, kg (n=28)	16,8 ± 1,0	24,0 ± 1,0	31,0 ± 5,4	< 0,001[1,2,3]
Lean body mass, kg (n=28)	54,0 ± 3,9	58,4 ± 5,3	49,4 ± 4,8	N.S.

NOTE: Values are mean ± SEM.

1: Cachexia vs. Weight- stable cancer, 2: Cachexia vs. No cancer, 3: Weight-stable vs. No cancer

4.2.4 Plasma Glucose, Insulin Concentrations, OGTT

Glucose tolerance was normal in all of the control patients, whereas 11 of 13 patients with PDAC were diabetic or glucose intolerant. Fasting plasma glucose concentrations were significantly increased in patients with PDAC compared with control patients and patients with other cancers. Fasting plasma insulin concentrations were not statistically different across groups, although insulin levels tended to be increased in patients in the cancer and PDAC groups and decreased in patients in the CP group compared with control patients. Results are presented in Table 11.

Table 11. Glucose and insulin concentrations and OGTT in study I.

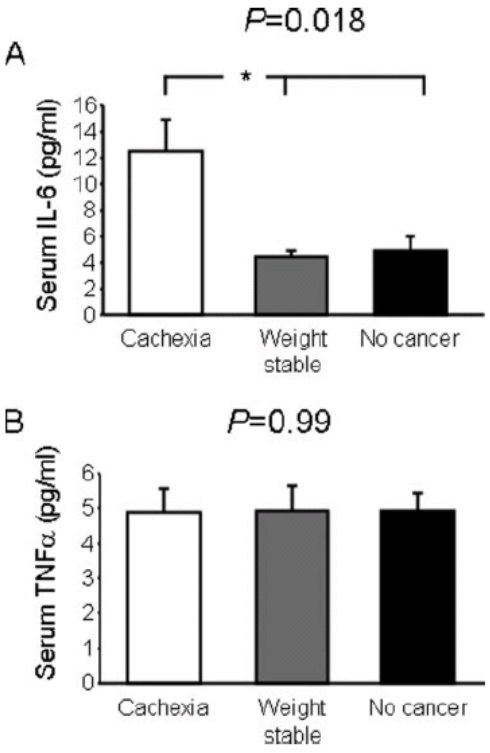
Measure	Pancreatic Ductal Adenocarcinoma (PDAC)	Cancer	Chronic pancreatitis	Patients with benign disease	p
OGTT (normal / impaired / diabetes)	2 / 3 / 8	5 / 0 / 3	3 / 1 / 4	8 / 0 / 0	< 0,05[1,2,3]
Fasting plasma glucose, mM	8,1 ± 0,6	5,3 ± 0,3	7,0 ± 1,0	5,0 ± 0,1	< 0,05[1,2]
Fasting plasma insulin, pM	50 ± 9	59 ± 6	30 ± 8	40 ± 4	N.S.
FFAs, mM	0,86 ± 0,05	1 ,19 ± 0,08	1,50 ± 0,48	0,72 ± 0,06	N.S.

NOTE: Values are mean ± SEM or absolute number.

Abbreviations: OGTT, Oral glucose tolerance test; FFAs, Free Fatty Acids

1: PDAC vs. benign controls, 2: PDAC vs. cancer, 3: Chronic pancreatitis vs. Benign controls

4.2.5 Serum Levels of IL-6 and TNF-α



Circulating levels of IL- 6 were increased in the cancer cachexia group ($p < 0,05$) (Fig. 7A) compared to the two other groups. Circulating levels of TNF-α were similar in all three groups (Fig. 7B).

Fig. 7. Tumour necrosis factor α (B) was similar in the three groups but there was an increased level of interleukin 6 (A) in the cancer cachexia group. Nutrition (2010), doi:10.1016/j.nut. 2010.08.022.

4.2.6 Other Plasma and Serum Measurements

Tables 12 and 13 show various plasma and serum measurements in studies II-IV. Table 12 shows that in studies II and IV, glycerol and free fatty acid levels were higher in patients in the CC group than in patients in the WS group. The weight losing groups had lower levels of triglyceride, cholesterol and leptin than patients in the WS group ($p < 0.01$-0.05).

Table 12. Plasma and serum measurements in studies II and IV.

Measure	Cancer Cachexia (CC)	Weight-stable cancer (WS)	Gastro-intestinal obstruction in cancer (GO)	p
P-Noradrenaline, nmol/L (n=26)	2,2 ± 0,4	3,0 ± 1,0	2,4 ± 0,2	N.S.
P-Adrenaline, nmol/L (n=26)	0,22 ± 0,14	0,20 ± 0,13	0,25 ± 0,24	N.S.
P-Atrial natriuretic peptid, ng/L (N=26)	13 ± 5	12 ± 4	14 ± 4	N.S.
P-Glucose, mmol/L (n=36)	6,7 ± 0,5	6,2 ± 0,4	5,5 ± 0,2	N.S.
P-Insulin, mU/L (n=33)	5,3 ± 1,0	10,4 ± 2,0	7,1 ± 1,9	N.S.
P-Triglyceride, mmol/L (n=36)	0,9 ± 0,1	1,4 ± 0,1	0,9 ± 0,1	< 0,01[1,2]
P-HDL, mmol/L (n=36)	1,6 ± 0,2	1,5 ± 0,1	1,7 ± 0,2	N.S.
P-Cholesterol, mmol/L (n=34)	4,2 ± 0,3	5,2 ± 0,3	4,1 ± 0,3	< 0,05[1,2]
P-Leptin, ng/mL (n=36)	4,5 ± 0,6	9,0 ± 1,5	4,3 ± 0,8	< 0,05[1,2]
S-IGF-I, µg/L (n=36)	109,8 ± 10,2	111,3 ± 13.8	95,7 ± 15,8	N.S.
S-Albumin, g/L (n=36)	35,1 ± 1,3	37,2 ± 0,9	34,7 ± 1,2	N.S.
S-C-Reactive Protein, µg/L (n=36)	26,9 ± 11,6	4,86 ± 2,2	14,1 ± 9,6	N.S.
P-Glycerol, µmol/L/kg body fat (n=33)	6,9 ± 1,3	3,9 ± 0,6	6,4 ± 0,6	< 0,05[1]
P-Free Fatty Acids, µmol/L/kg fat (n=33)	53,8 ± 8,6	32,5 ± 3,6	51,9 ± 7,3	< 0,05[1]

NOTE: Values are mean ± SEM. Abbreviations: P, Plasma; S, Serum.; N.S., Non Significant; HDL, High Density Lipoprotein; IGF-I, Insulin-like Growth Factor I;

1: Cachexia vs. Weight- stable cancer; 2: Weight-stable cancer vs. Cancer with obstruction

In study III glycerol and free fatty acid levels were higher and transferrin levels were lower in patients in the CC group compared to patients in the other groups ($p < 0,05$) (Table 13).

Table 13. Plasma and serum measurements in study III

Measure	Cancer Cachexia (CC)	Weight Stable (WS)	Patients with benign disease	p
P-Glucose, mmol/L (n=28)	6,1 ± 0,4	6,8 ± 0,6	7,3 ± 1,1	N.S.
P-Insulin, mU/L (n=28)	6,5 ± 0,9	12,8 ± 3,2	16,2 ± 5,2	N.S.
Haemoglobin, g/L (n=28)	131 ± 5,1	134 ± 5,3	123 ± 5,4	N.S.
S-Albumin, g/L (n=28)	34,3 ± 1,2	38,0 ± 0,9	38,8 ± 1,8	N.S.
S-Transferrin,g/L (n=28)	2,1 ± 0,1	2,6 ± 0,1	2,7 ± 0,2	< 0,05[1,2]
P-Glycerol, µmol/L/kg body fat (n=28)	9,8 ± 2,0	3,3 ± 0,3	3,2 ± 0,4	< 0,05[1,2]
P-Free Fatty Acids, µmol/L/kg fat (n=28)	87,0 ± 2,0	30,0 ± 0,4	34, 0 ± 1,0	< 0.05[1,2]

NOTE: Values are mean ± SEM. Abbreviations. P, Plasma; S, Serum; N.S., Non Significant

1: Cachexia vs. Weight- stable cancer; 2: Cachexia vs. no cancer

4.3 LABORATORY STUDIES

4.3.1 Glycogen

Glycogen content was significantly lower in patients in the PDAC group than in patients in the control group (119 ± 17 versus 213 ± 24 mmol of glycosyl units/kg of dry weight, $p < 0.05$; Fig. 8A). Glycogen content in patients in the CP and cancer groups was not different from patients in the control group. There were no statistically significant differences between patients in the PDAC and cancer groups. In all patients, glycogen content was significantly correlated with ATP content ($R^2 = 0.74$, $p < 0.05$). In the basal state, glucose incorporation into glycogen was not significantly different across groups, although patients in the CP, cancer, and PDAC groups tended to have lower rates (Fig. 8B). Insulin stimulation resulted in a 1.3-fold increase of glucose incorporation into glycogen ($p < 0,05$) in

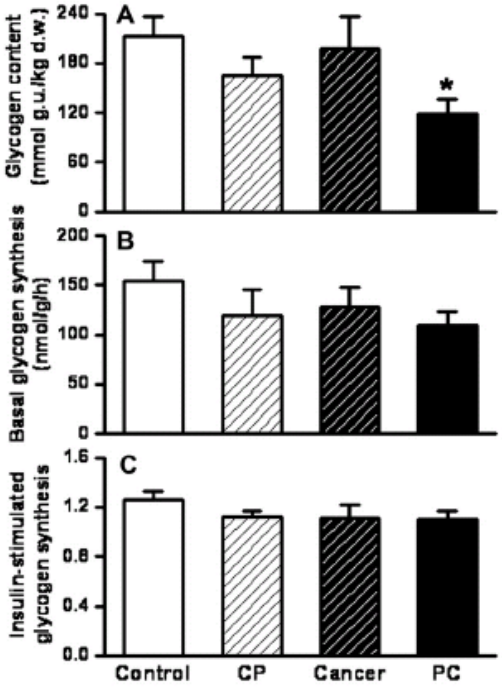
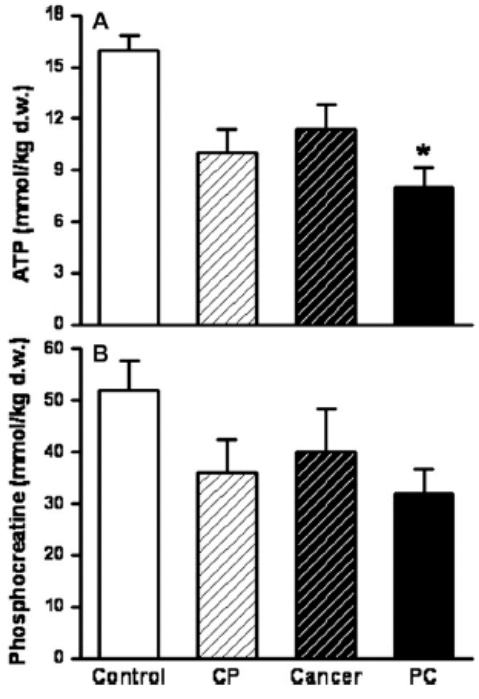


Fig. 8. Glycogen content (A) and glucose incorporation into glycogen in the absence (B) or presence (C) of insulin in skeletal muscle, incubated in glucose. Data presented as mean ± SEM. * $P < 0.05$ compared with control. CP, chronic pancreatitis; PC, Pancreatic Ductal Nutrition (2010), doi:10.1016/j.nut.2010.08.022.

patients in the control group (Fig. 8C). In patients in the PDAC, CP and cancer groups, insulin stimulation did not result in a significant increase in glucose incorporation into glycogen.

4.3.2 ATP and Phosphocreatine Content



The ATP content was significantly lower in patients in the PDAC group than in the control group (8.4 ± 1.2 versus 15.8 ± 0.8 mmol/kg of dry weight, $P < 0.05$). There were no statistically significant differences between patients in the PDAC and cancer groups. Patients in the CP and cancer groups also had decreased muscle ATP content, but these did not reach statistical significance compared with patients in the control group (Fig. 9A). The trend was similar for phosphocreatine content, although the differences between means across the groups were not statistically significant (Fig. 9B).

Fig. 9. Content of ATP (A) and phosphocreatine (B) in skeletal muscle. Data are presented as mean ± SEM. * $P < 0.05$ compared with control. ATP, adenosine triphosphate; CP, chronic pancreatitis; PC, Pancreatic Ductal Adenocarcinoma. Nutrition (2010), doi:10.1016/j.nut.2010.08.022.

4.3.3 Glucose Transport

4.3.3.1 Activity in Isolated Muscle Tissue

Basal 3-O-methylglucose transport was not significantly different among patient groups (Fig. 10A). Insulin stimulation resulted in a significant fold increase in glucose transport in all patient groups, except those with PDAC (data not shown). Insulin-stimulated glucose transport was significantly decreased in patients with PDAC compared with the control group patients (Fig. 10B). Insulin-stimulated glucose transport in patients in the CP and cancer groups was not different from that in

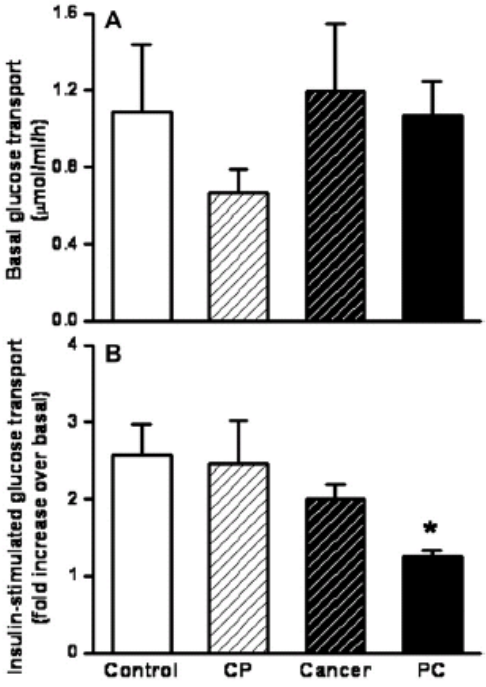
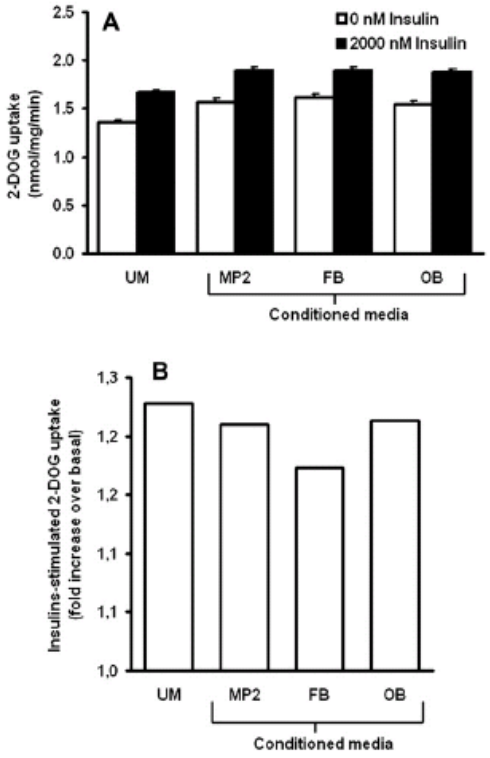


Fig. 10. The 3-O-methylglucose transport in isolated muscle strips, incubated in glucose in the absence (A) or presence (B) of insulin. Data are presented as mean ± SEM. * $P < 0.05$ compared with control. CP, chronic pancreatitis; PC, pancreatic cancer. Nutrition (2010), doi:10.1016/j.nut.2010.08.022.

patients in the control group, although there was a tendency for decreased glucose transport in patients in the cancer group. In all patients, there was no correlation between insulin-stimulated glucose transport and muscle ATP content (data not shown).

4.3.3.2 Activity in L6 Myotubes



Transport of 2-DOG was determined with and without the presence of 2000 nM insulin. Basal glucose uptake was similar across the groups (Fig. 11A), and exposure to insulin resulted in a significant increase in glucose transport in all patient groups (Fig. 11B). There were no significant differences in basal or insulin stimulated glucose transport in myotubes without or with exposure to medium conditioned by PDAC cells, fibroblasts or osteoblasts.

Fig. 11. (A) Basal and insulin-stimulated 2-DOG transport and (B) insulin stimulated fold increase over basal, L6 myotubes in conditioned media. 2-DOG, 2-deoxyglucose; FB, fibroblasts; MP2, MiaPaca 2; OB, osteosarcoma cells; UM, unconditioned medium. Nutrition (2010), doi:10.1016/ j.nut.2010.08.022.

4.3.4 Lipogenesis

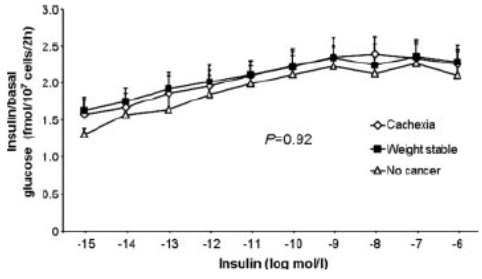


Fig. 12. There was no difference between the groups in insulin-stimulated lipogenesis. P value given is for the overall difference and was determined by repeated-measures analysis of variance. CANCER 2008; 113(7), 1695-1704.

Figure 12 shows that the concentration response curves in the three patient groups were superimposed almost completely. The maximum insulin effect and EC_{50} were calculated for each patient. The maximum insulin effect in the three groups varied between 2.3- and 2.6-fold stimulation (p = 0.51). EC_{50} for insulin varied between 0.02 and 0.04 nmol/L in the three groups (p = 0.92). Therefore, insulin-stimulated lipogenesis was almost identical in the three studied patient groups. Basal lipogenesis (expressed per number of isolated fat cells) did not differ between patient groups (p = 0.35).

4.3.5 Lipolysis

Insulin inhibited lipolysis in a similar concentration-dependent manner in the three patient groups. Maximum inhibition of lipolysis varied from 55% to 60% in these groups. Noradrenaline and atrial natriuretic peptide stimulation of basal lipolysis rate was much more efficient (P < 0.001, ANOVA repeated measure) in CC patients as

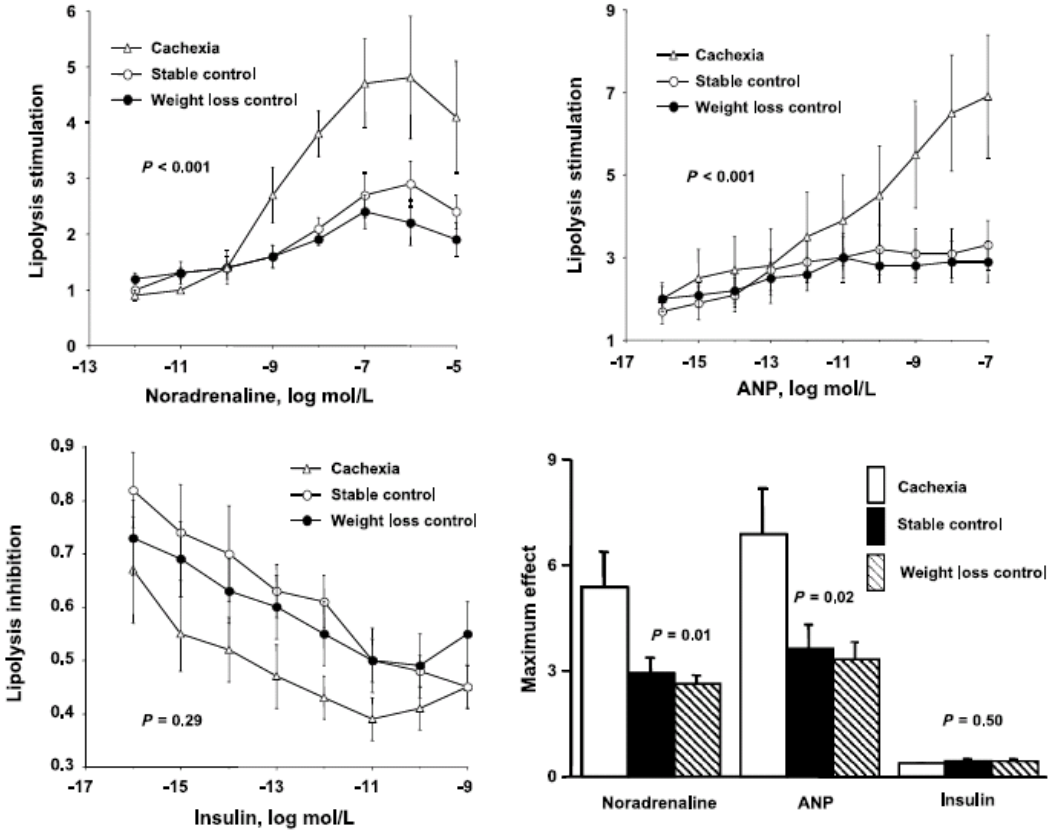


Figure 13. Lipolysis in mature fat cells of cachexia, weight stable and gastro-intestinal obstruction patients. ANP, atrial natriuretic peptide. Cancer Res 2007;67(11):5531–7

compared with the control groups, who did not differ between each other. At the maximum effective concentration, noradrenaline stimulated lipolysis 5-, 3-, and 2.5-fold in the CC, WS, and GO patient groups respectively (p = 0.01). Corresponding values for natriuretic peptide were 7-, 4-, and 3-fold (p = 0.02) (Fig.13).
When lipolysis was expressed as absolute values of glycerol release (µmol/g of lipid/2 h), as shown in Table 13, this mode of expression gave similar results to those above; however, some of them did not reach a level of statistical significance (P = 0.06–0.07). Furthermore, basal (nonhormonal) lipolysis did not differ between patient groups (Table 14).

Table 14. Shows lipolysis expressed as absolute values of glycerol release (µmol/g of lipid/2 h)				
Condition	**Cancer Cachexia (CC)**	**Weight-stable cancer (WS)**	**Gastro-intestinal obstruction in cancer (GO)**	**p**
Basal	0,98 ± 0,7	1,16 ±0,67	1,49 ± 0,67	0,44
Noradrenaline	3,1 ± 1,7	1,5 ± 1,0	1,49 ± 0,67	0,06
Atrial natriuretic peptide	4,0 ± 1,5	2,1 ± 1,9	2,6 ± 1,2	0,07
8-Br-cAMP	5,1 ±2,3	2,7 ± 2,7	3,1 ± 1,6	0.02
8-Br-cAMP + insulin	1,9 ± 1,0	1,3 ± 0,7	1,9 ± 0,9	0,31

NOTE: Values are mean ± SD of glycerol release (µmol/g of lipid/2h).

The cAMP analogue 8-Br-cAMP caused a 7-fold stimulation of lipolysis in the CC patient group as compared with a 3-fold stimulation in patients in the WS and GO groups (Fig. 14). The selective HSL inhibitor BAY decreased the lipolytic effect of the cAMP analogue by >90%, with no differences between the patient groups (Fig. 14). Finally, 8-BrcGMP stimulated lipolysis 10 ± 6-fold in patients with cancer cachexia and 3.0 ± 1.6-fold and 2.7 ± 0.5-fold, respectively, in patients in the two control groups (p = 0.03).

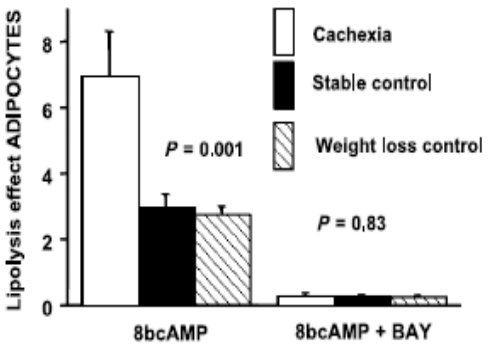


Fig. 14. Lipolytic effect of a cAMP analogue (8-bcAMP) with or without HSL inhibitor BAY. Cancer Res 2007;67(11):5531–7

4.3.6 Preadipocytes

In preadipocytes (Fig. 15) differentiated to adipocytes, the lipolytic effect of noradrenaline was the same in patients with cancer cachexia and in weight-stable control patients (≈8-fold stimulation of basal rate). Similar results were obtained when preadipocyte lipolysis was expressed as a function of cellular protein content (values not shown).

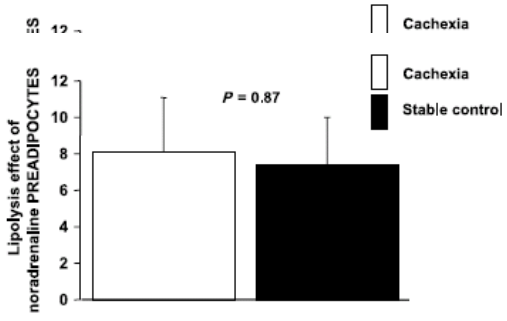


Fig. 15. Lipolysis in preadipocytes differentiated into adipocytes. Cancer Res 2007;67(11):5531–7

4.3.7 Gene Expression

4.3.7.1 HSL and ATGL

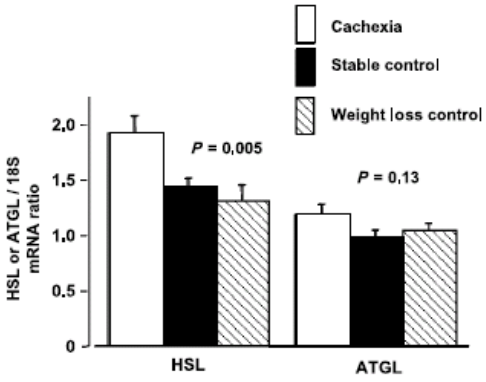


Fig. 16. Expression of mRNA for hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL). Cancer Res 2007;67(11):5531–7

In adipose tissue of CC patients, HSL mRNA expression was >50% higher compared with patients in the two control groups, which did not differ between each other, but there was no difference between the three patient groups in adipose triglyceride lipase expression (Fig. 16). As regards maximum lipolytic effect of noradrenaline or atrial natriuretic peptide, both hormones showed a strong positive correlation with HSL gene expression ($r = 0.58$; $p = 0.005$ for noradrenaline and $r = 0.50$; $p = 0.02$ for atrial natriuretic peptide; graphs not shown). No such correlation was observed for adipose triglyceride lipase mRNA ($r \approx 0.15$).

4.3.7.2 Inflammations Markers

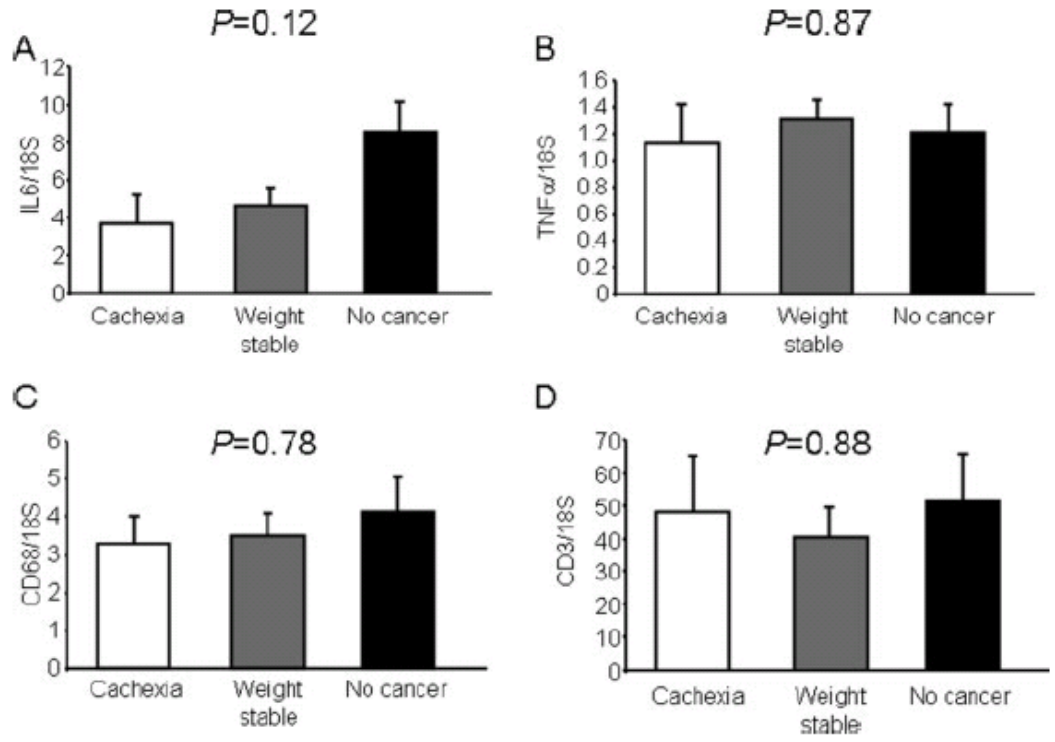


Fig. 17. There was no difference in mRNA expression for either (A) interleukin6 (IL-6), (B) tumor necrosis factor α (TNF-α), (C) macrophage (CD68), or(D) T-lymphocyte (CD3). 18S indicates internal control. CANCER 2008; 113(7), 1695-1704.

In contrast to the elevated serum IL-6 levels, expression of IL-6 mRNA in adipose tissue did not differ between the patient groups (Fig. 17A) ($p = 0.12$). If anything, IL-6 expression tended to be lower in the CC patient group. Similarly, no differences in

TNF-α expression in adipose tissue were observed (Fig. 17B) (p=0.87). To assess whether there were signs of increased leukocyte infiltration in adipose tissue, mRNA expression for CD68 (a macrophage marker) and CD3 (a T-lymphocyte marker) were measured. Similar to the findings with cytokine mRNA, the levels of CD68 (Fig. 17C) (p=0.78) and CD3 (Fig. 17D) (p=0.88) did not differ between groups.

4.3.8 Protein Expression

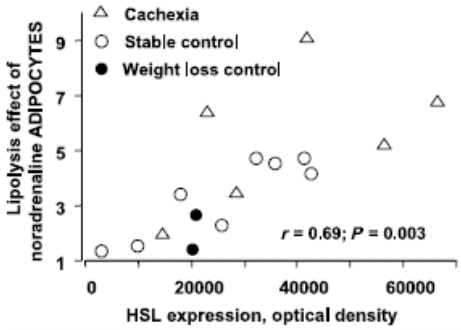


Fig. 18. Relationship between HSL protein expression (ratio of actin) and lipolytic effect of noradrenaline. Cancer Res 2007;67(11):5531–7

The number of subjects investigated was too small for an analysis of the three separate patient groups. However, HSL protein expression showed 2-fold increase in patients with cancer cachexia as compared with the two control groups (0.23 ± 0.57 versus 0.69 ± 0.34; p = 0.032). When all protein data were compiled, there was a strong positive correlation between HSL protein expression and noradrenaline-stimulated lipolysis (Fig. 18). As much as 50% of the variation in lipolysis (adjusted r^2) could be explained by variations in HSL. Similar correlations were obtained with atrial natriuretic peptide (r = 0.67; p = 0.005) or 8-Br-cAMP (r = 0.85; p < 0.001). The results were not altered in a significant way when absolute values for HSL expression were used instead of HSL/actin ratio (r = 0.67–0.85; graphs not shown). h-Actin expression was not correlated with lipolysis (r = 0.03–0.07). The mRNA and protein expression of HSL showed a very strong correlation (r = 0.81; p=0.001).

4.3.9 Adipocytes Number and Volume

The adipocytes in patients in the CC group were smaller than the adipocytes in patients in the WS or no cancer groups. There were no differences between patients in the CC and GO groups (Table 15 and 16). There were no differences in the number of adipocytes between the patient groups (Table 16).

Table 15. Adipocyte volume in study II.

Measure	Cancer Cachexia (CC)	Weight-stable cancer (WS)	Gastro-intestinal obstruction in cancer (GO)	p
Adipocyte volume, pL (n=26)	299 ± 85	653 ± 176	395 ± 102	< 0,001[1]

NOTE: Values are mean ± SEM. 1: Cachexia vs. Weight- stable cancer

Table 16. Adipocyte volume and number in study IV.

Measure	Cancer Cachexia (CC)	Weight Stable (WS)	Patients with benign disease	p
Adipocyte volume, pL (n=26)	339 ± 30	574 ± 40	678 ± 113	< 0,001[1,2]
Adipocyte number, x 10^9	42 ± 2	45 ± 2	49 ± 10	NS

NOTE: Values are mean ± SEM. 1: Cachexia vs. Weight- stable cancer; 2: Cachexia vs. No cancer

4.3.10 Cytokine Secretion

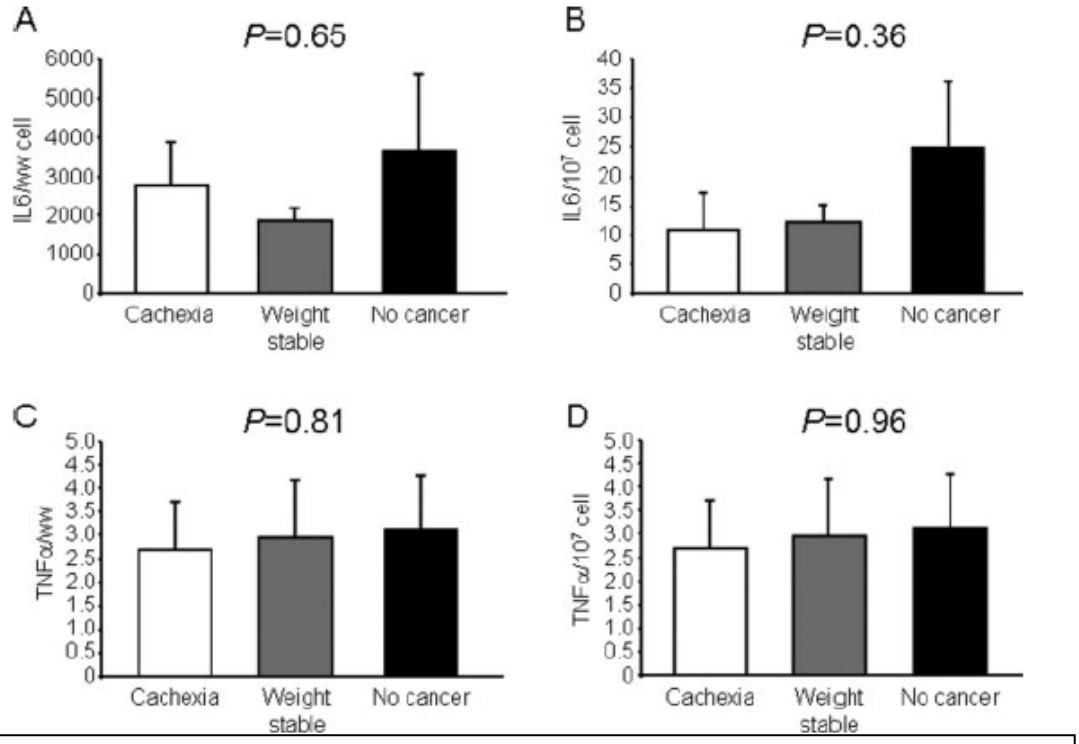


Fig. 19. In adipose tissue, no difference was noted in (A and B) IL-6 secretion or (C and D) TNF-α secretion, regardless of whether secretion was expressed per wet weight (ww) (A and C) or per x 10^7 fat cells (B and D). CANCER 2008; 113(7), 1695-1704.

To determine whether the secretion of IL-6 and TNF-α from adipose tissue corresponded to the findings at the mRNA level, in vitro incubations of adipose tissue specimens were analyzed. The secretion rate of IL-6 did not differ significantly between specimens from the three patient groups irrespective of whether the rate was expressed per gram of wet weight fat tissue (Fig. 19A) ($p = 0.65$) or per 10^7 fat cells (Fig. 19B) ($p = 0.36$). Similarly, there was no significant difference in TNF-α secretion expressed in either of the 2 ways (Fig. 19C,D) ($p = 0.81$ and $p = 0.96$, respectively). There was also no between-group difference when protein secretion was expressed per lipid weight of adipose tissue (values not shown).

4.4 CORRELATIONS BETWEEN SELECTED VARIABLES

In patients in the CC group, percentage weight loss correlated with both glycerol ($r^2=0.66$, $p<0.0005$) and circulating fatty acids ($r^2=0.65$, $p< 0.0005$). There was also a correlation between the volume of visceral adipose tissue and waist perimeter ($r^2=0.7$, $p< 0.005$). In patients in the WS group, waist perimeter correlated with both visceral adipose tissue ($r^2=0.85$, $p<0.001$) and fat mass ($r^2=0.62$, $p<0.0005$). In patients in the GO group, the volume of visceral adipose tissue correlated to both fat mass ($r^2=0.63$, $p<0.05$) and waist perimeter ($r^2=0.65$, $p<0.01$). The amount of visceral adipose tissue was negatively correlated to both glycerol ($r^2=0.76$, $p<0.05$) and free fatty acids ($r^2=0.79$, $p<0.01$). There was a correlation between the volume of subcutaneous adipose tissue with both fat mass ($r^2=0.72$, $p<0.05$) and perimeter of waist ($r^2=0.85$, $p<0.001$). There was a negative correlation between the percentage weight loss and fat mass ($r^2=0.61$, $p<0.05$).

There was a correlation between waist perimeter and volume of visceral adipose tissue in patients in all groups (CC (r^2=0.70, p<0.005), WS (r^2=0.85, p<0.001), GO (r^2=0.65, p<0.01). Neither CRP nor basal metabolic rate correlated with any other metabolic variable.

4.5 RADIOLOGICAL STUDIES

There was less visceral adipose tissue in patients in the CC group compared to patients in the WS group ($p < 0.05$). In contrast, no statistically significant difference was observed in visceral adipose tissue between patients in the GO and WS groups. Both weight-losing patient groups had less subcutaneous and visceral adipose tissue ($p < 0.001$) compared to patients in the WS group. There were no differences in the volumes of vertebral or abdominal muscle across the patient groups (Table 17).

Table 17. Volume of muscle and adipose tissue in cm^3 in study IV.

Measure	Cancer Cachexia (CC)	Weight-stable cancer (WS)	Gastro-intestinal obstruction in cancer (GO)	p
Para-vertebral muscle, cm^3 (n=40)	72.8 ± 4.6	78.7 ± 4.3	70.4 ± 3.4	N.S.
Abdominal muscle, cm^3 (n=40)	52.1 ± 3.3	60.5 ± 5.3	51.9 ± 4.0	N.S.
Total adipose tissue, cm^3 (n=40)	195.4 ± 3.5	336.4 ± 3.1	196.6 ± 4.0	$< 0.01^{1,2}$
Subcutaneous adipose tissue, cm^3 (n=40)	98.8 ± 7.5	159.7 ± 13.1	86.8 ± 21.0	$< 0.001^{1,2}$
Visceral adipose tissue, cm^3 (n=40)	96.6 ± 20.2	176.6 ± 22.0	109.8 ± 35.1	$< 0.05^{1}$

NOTE: Values are mean ± SEM.

1: Cachexia vs. Weight- stable cancer, 2: Weight-stable cancer vs. Cancer with obstruction

5 DISCUSSION

5.1 DEMOGRAPHICS AND NUTRITIONAL STATUS

Patients with CC (studies II-IV) and PDAC (study I) lost a greater percentage of their weight than patients in the WS group, 10% vs. 3%, in study I (6% vs. 2%) (Tables 6, 7 and 8). These were patients who had no evidence of a mechanical reason for impaired food intake and were therefore demonstrating true cancer cachexia [5]. Their PG-SGA score was 8 which was higher than for patients in the WS group. Patients in the GO group had lost 17% of their habitual weight in the previous 6 months, more than three times what would be regarded as normal weight loss over a 6 month period [18]. Their PG-SGA score was 12, which was significantly higher than the scores of patients in the CC group. PG-SGA score 0-8 means patients are regarded as "Not at nutritional risk" where patients who score ≥ 9, are considered to be "At nutritional risk" [110]. Patients in the GO group were classified as being moderately to severely malnourished, at the time their cancer was diagnosed [110]. BMI and hip perimeter measurements were smaller in patients in the weight losing groups compared to patients in the WS group. Waist perimeter measurement was smaller in patients in the CC group compared to those in the WS group, and was not significantly different between patients in the GO group and WS group. There was good and statistically significant correlation between waist perimeter and volume of visceral adipose tissue in patients in all the groups. It is difficult to discuss the importance of this finding, no-one having noted this in the literature before and it is not possible to say if this is related to diminished volume of visceral adipose tissue. Tumour staging was similar across all patients groups, which indicates that the severity of the cancer disease did not explain the differences observed in this study. The self reported information about weight loss is a possible source for recall bias, but the bias would be similar for all patient groups and other researchers have shown that this is a relatively minor bias [111].

5.2 ENERGY EXPENDITURE

Table 9 showed that there were no differences between the patient groups regarding increased REE from the calculated BMR. But within the groups, patients in the CC groups had a significantly increased REE (results not shown). When taking into account that all the patients were newly diagnosed with cancer, it could be speculated that there would be a difference between the patient groups in the future, when the disease advanced [5] and the finding that there is significant increase in REE in patients in the CC group supports that.

5.3 BODY COMPOSITION AND RADIOLOGICAL STUDIES

5.3.1 Adipose Tissue

Body composition showed that there was less adipose tissue mass in patients in the weight losing groups compared to those in the WS group but no difference in percentage of body fat, lean body mass or total body water (Tables 9 and10). However, only one of the patients was extremely lean (lowest BMI was 14.1 kg/m^2 and the next

18.6 kg/m^2), indicating that patients who were studied may have been in an early cancer cachexia phase, when there is predominantly loss of adipose tissue. There was less volume of total adipose tissue and subcutaneous adipose tissue in patients in the weight losing groups compared to patients in the WS group (Table 17). This result supports other reports, showing that wasting of adipose tissue is the predominant cause of weight loss in both newly diagnosed cancer patients with cancer cachexia and in early starvation [23, 112, 113]. The most interesting finding was that patients in the CC group lost more visceral adipose tissue than patients in the WS group (Fig. 20). Ogiwara et al have previously shown that terminally ill cancer patients with cachexia, who all died within 3 months from the time of the CT scan, had less visceral adipose tissue than healthy non-obese, non-cancer control patients [114]. Freedman has shown that visceral adipose tissue increased during and after treatment for breast cancer [115]. Figure 20 shows that wasting of adipose tissue in patients in the CC group was more extensive than could be expected when comparing volume of adipose tissue (Fig. 20 B and C) to percentage weight loss (Fig. 20A).This thesis has shown that there was less volume of visceral adipose tissue in patients in the CC group compared to patients in the WS cancer group even at the time of diagnosis. The patients' visceral adipocytes were smaller but had higher metabolic activity than the subcutaneous adipocytes [116-119].

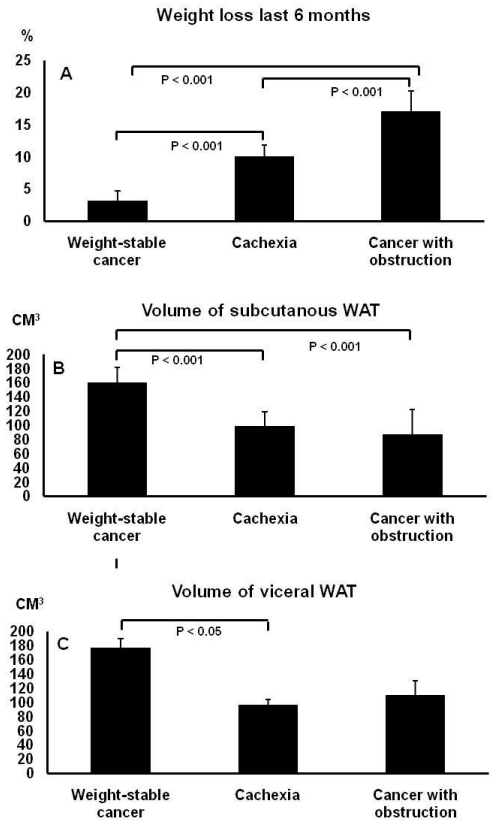


Fig 20. (A) Body weight loss, in last 6 months, (B) volume of subcutaneous white adipose tissue (WAT) and (C) volume of visceral WAT in the three different study groups, cancer cachexia (CC) patients, weight-stable cancer patients (WS) and cancer patients with gastrointestinal obstruction (GO).

5.3.2 Muscle Tissue

There were no differences in the lean body mass, total body water (Tables 9 and 10) or in the volume of muscle (Table 17) between the patients. Why there is no difference in volume of muscle between patients in the weight losing groups and those in the WS group is interesting. A study of cancer patients with weight loss of about 3% of their habitual weight showed no increase in mRNA expression and activity of the ubiquitin-proteasome pathway [120]. Bossola has shown that there is an increase in mRNA expression of the ubiquitin-proteasome pathway in cancer patients with weight loss of about 5-6% of their habitual weight [121] and many studies of muscle wasting in cancer patients who had lost 10% of their habitual weight, show increased mRNA

expression and activity of the ubiquitin-proteasome pathway [32, 122]. These findings could indicate that increased mRNA expression of the ubiquitin-proteasome pathway starts with 5% weight loss and increased activation of the ubiquitin-proteasome pathway starts with 10% weight loss. But the results of this thesis do not confirm that suggestion. Patients in the CC group had lost 10 % of their habitual weight and patients in the GO group had lost 17%, which indicates that there is a more complicated relationship between weight loss and activation of the ubiquitin-proteasome pathway. In this thesis, we have two methods, body composition and CT, that indicate non significant wasting of muscle tissue in patients in the GO group compared to the others groups. A possible explanation is that they were compared to patients in a WS group, who had lost 3% of their habitual weight and not compared with a population without cancer. But as stated earlier, the activation of the ubiquitin-proteasome pathway had not started at weight loss of 3% [120]. Another explanation could be the short period that the patients had had the cancer disease.

5.4 SYSTEMIC- AND ADIPOSE TISSUE INFLAMMATION

In healthy individuals, subcutaneous adipose tissue contributes significantly to *in vivo* circulating IL-6 levels in humans [123]. Furthermore, diet-induced reductions in circulating IL-6 occur in parallel with reduced adipose tissue secretion of the cytokine [89]. Figure 7 showed that there was increased systemic concentration of IL-6 in patients in the CC group ($p < 0,05$) compared to patients in the other groups but no difference in concentration of TNF-α, between the patient groups. There was a trend for a higher systemic level of CRP in patients in the CC group but it was not statistically significant (Table 12). In adipose tissue, there was no difference between the patient groups regarding expression of mRNA for IL-6 or TNF-α (Fig. 17).
Increased serum concentration of IL-6 in weight losing cancer patients has been reported [124] and increasing concentration of IL-6 correlates to the development of cancer cachexia in mice [125] but there is some debate on the role of serum concentration of TNF-α in weight losing cancer patients [5]. Controversially there is considerable evidence that TNF-α has a role in the wasting of adipose tissue [5, 126] and it has been suggested that IL-6 alone could not be responsible for the production of cancer cachexia [5]. There was no increased mRNA expression for CD68 (a macrophage marker) and CD3 (a T-lymphocyte marker) in the adipose tissue of patients in the weight losing group compared to patients in the other groups, which indicates that there was no increased inflammation in adipose tissue.
Also there was no increased production of IL-6 and TNF-α from adipose tissue in patients in the weight losing group (Fig. 19). Because IL-6 secretion was not increased in adipose tissue from patients in the CC group, despite elevated circulating levels of the cytokine, it is apparent that adipose tissue contributes to the circulating IL-6 levels only under certain conditions. In cancer cachexia, other sources that were not examined in this study (e.g., the liver and pancreas) may play dominant roles in plasma IL-6, a hypothesis that is supported by previously reported findings [127, 128].
These results do not support the theory [75, 76, 78] that increased wasting of adipose tissue in newly diagnosed cancer patients is stimulated by inflammation in the adipose tissue.
A limit of these inflammation studies is that the weight losing group had both patients with true cancer cachexia and cancer patients with gastrointestinal obstruction who were malnourished because of starvation.

5.5 PLASMA AND SERUM MEASUREMENTS

All patients with known diabetes at the time of recruitment were excluded from the study but 11 of the 13 PDAC patients had glucose intolerance or diabetes according to OGTT, previously unknown to them. Most patients with PDAC were diabetic or glucose intolerant without hypo-insulinemia, as has been previously reported [42, 68], indicating the presence of peripheral insulin resistance. Others have shown a relationship between diabetes or glucose intolerance with hypo-insulinemia and cancer [129]. Even there is evidence for a relationship between diabetes and pancreatic cancer, it is difficult to find any association between diabetes and cancer in general [130]. Fasting plasma glucose levels were higher in PDAC patient groups compared to patients with other-sites cancers and benign control patients. This indicates the presence of peripheral insulin resistance.

Patients in the two weight-losing groups had comparable values for triglyceride, cholesterol and leptin compared to patients in the WS group. Even if there was no difference in IGF-I concentration, there was a strong trend towards a lower concentration in patients in the GO group. Patients in the CC group had higher concentrations of glycerol and FFA than those in the WS group (Table 12). As noted earlier, patients in the GO group had much higher PG-SGA scores than those in the CC group and showed a trend towards low IGF-I. Both SGA and IGF-I are markers of malnutrition and this indicates the dangerous situation these patients were in [110, 131]. They had lost 17% of their habitual weight and it is known that when weight loss is approaching 30% it is incompatible with life [2]. When looking at PG-SGA and IGF-I in patients in the GO group and the high levels of glycerol and FFA in patients in the CC group, the data clearly suggest that the two weight-losing groups, at least in part, have different aetiologies underlying their reported weight loss.

5.6 GLYCOGEN, ATP AND PHOSPHOCREATINE CONTENT

The glycogen content in patients with PDAC was decreased compared with control patients (Fig. 8). This is unlikely to be due to only weight loss, because patients with CP had a similar weight loss without decreased muscle glycogen content (Fig. 8). Therefore, factors other than weight loss may have contributed to the decreased muscle glycogen content in PDAC. In contrast to the findings in control patients, there was no significant response to insulin in the rate of incorporation of glucose into glycogen in patients with PDAC, CP, or cancer, suggesting decreased insulin-stimulated non-oxidative glucose metabolism in all these patients compared with controls. There is a known association between decreased glycogen production and decreased glucose transport [132, 133].

When comparing glycogen content with muscle content of ATP and phosphocreatine (Fig. 8 and 9), the means of the different groups show the same reciprocal pattern, although only patients with PDAC had significantly lower contents of glycogen and ATP compared with control patients, possibly suggesting an association between non oxidative glucose metabolism and energy status of the muscle cells. This hypothesis is further supported by a significant correlation between glycogen and ATP content when analyzing all patients together. In contrast, there was no correlation between muscle glucose transport and energy content, suggesting that other mechanisms are likely to contribute to the decreased glucose transport seen in patients with PDAC.

5.7 GLUCOSE TRANSPORT

Insulin-stimulated glucose transport was markedly decreased in isolated skeletal muscle from patients with PDAC, confirming historical findings [68] and supporting the hypothesis that insulin resistance at the skeletal muscle cell level contributes to the high frequency of impaired glucose tolerance or diabetes in these patients [43]. Furthermore, 11 of 13 patients with PDAC were diabetic or glucose intolerant, indicating that this metabolic disturbance is strongly associated with PDAC. Peripheral insulin resistance and impaired glucose tolerance have been reported in other cancers [134, 135] and have been proposed to be a general malignant phenomenon. It has been suggested that this is due to induction of mRNA for TNF-α and down regulation of expression of mRNA for glucose transporter 4 (GLUT4) [134]. Three patients with cancers of other sites and four patients with chronic pancreatitis were diabetic according to their oral glucose tolerance test results. In these patients, insulin-stimulated glucose transport was not decreased compared with the controls, suggesting that mechanisms other than skeletal muscle insulin resistance, may contribute to their hyperglycaemia. Another possibility is that the skeletal muscle insulin resistance is caused by defects in intracellular glucose metabolism, such as decreased glycogen synthase activity and increased glycogen phosphorylase activity [69]. The decrease of insulin-stimulated glucose transport may be due to defective signalling steps proximal to glucose transport, such as decreased phosphatidylinositol- 3-kinase activity, as has previously been shown [68]. Increased lipid use in muscle has been proposed to cause insulin resistance in muscle [136] and increased free fatty acid uptake in muscle results in a decrease in phosphatidylinositol-3-kinase activity and glucose transport [137]. Another theory related to plasma lipid, is that wasting of adipose tissue causes increased plasma lipids concentration, which stimulates the activation of vascular endothelial growth factor B (VEGF-B) in muscle tissue, which causes up regulation of lipid receptors and down regulation of GLUT-4 receptors in muscle tissue and causes insulin resistances [138].

5.8 LIPOGENESIS

Lipogenesis is a process where three fatty acids are connected to glycerol, with ester bonding to build triglyceride [139]. Triglyceride is packaged into very low-density lipoprotein (VLDL) and secreted by the liver [140]. There is always *de novo* lipogenesis in humans [141, 142]. Figure 12 shows that lipogenesis is not diminished in patients in the CC group compared to the other groups ($p>0,05$); there is no difference in the concentration response curves between the three patient groups. This information supports the theory that it is increased expression and activity of HSL that increases lipolysis which is the cause of wasting of adipose tissue in cancer cachexia. But the observation that lipogenesis does not diminish is not always a positive thing. It is suggested that cancer cells can change their cell membrane structure, by activating *de novo* lipogenesis [143], and in that way change the saturation of their membranes. This can protect the cancer cells from mediators of the immune system and from cancer treatment. Cancer cells can also directly import lipid to their membrane from dietary fat, with their own lipoprotein lipase [143, 144].

5.9 LIPOLYSIS

5.9.1 Stimulation and Inhibition of Lipolysis

Lipolysis is the breakdown of lipids. It is the hydrolysis of triglycerides into three free fatty acids and glycerol, followed by further degradation, into acetyl units [30]. In our study, only patients in the CC group had significantly increased *in vivo* lipolysis although there was no evidence of increased sympathetic nervous activity or elevated levels of circulating natriuretic peptide (Table 12). As regards the cellular mechanism promoting this increase in lipolysis in human cancer cachexia, the antilipolytic effect of insulin was not significantly altered in patients in the CC group (Fig. 13), and it is unlikely that increased lipolysis in patients in the CC group is secondary to weight loss because patients in the GO group had the same adipocyte lipolytic activity as those in the WS group (Fig. 13). In contrast, the lipolytic effects of the two major lipolysis-regulating hormone systems in man (i.e., catecholamines and natriuretic peptides) were markedly increased in patients in the CC group as compared with those in the WS or GO patient groups (Fig. 13). Furthermore, stimulation of lipolysis with cAMP or cGMP analogues also showed increased effect in patients in the CC group (Fig. 14). Noradrenaline and natriuretic peptide stimulate adipocyte lipolysis through separate signal pathways (mediated by either cAMP or cGMP) that all converge at HSL, an enzyme that is also a target for cAMP and cGMP [73, 145]. The similar findings with the two cyclic nucleotides indicate that the cellular mechanism for increased lipolysis in cancer cachexia is due to enhanced lipolytic signalling of the hormone systems at the post-receptor level because of enhanced function of HSL. Indeed, this seems to be the case because lipolysis could be inhibited to the same extent in all three patient groups using the highly selective HSL inhibitor BAY (Fig 14). The novel lipase adipose triglyceride lipase may be important for determining adipose tissue cell lipolysis [30]. However in this study, there was no effect of cancer cachexia on adipose triglyceride lipase expression, and the expression of this enzyme did not correlate with lipolysis. Furthermore, there was <10% residual adipocyte lipolytic activity in all groups when HSL -induced lipolysis was inhibited, suggesting no, or a minor, role of adipose triglyceride lipase in the altered regulation of lipolysis in cancer cachexia (Fig.14).

5.9.2 Gene and Protein Expression

There was a 50% increase in mRNA expression of HSL in patients in the CC group and a two-fold increase in the amount of protein expression compared to the other patient groups (Figs. 16 and 18). This is in line with what others have found in adipose tissue [146]. HSL gene and protein expression correlated strongly with the lipolytic effect of noradrenaline or natriuretic peptide and could explain the increase in lipolytic effect. Furthermore, mRNA HSL and HSL protein levels were strongly interrelated and over-expression of HSL led to a marked increase in adipocyte lipase activity [30, 31].

5.10 PREADIPOCYTES

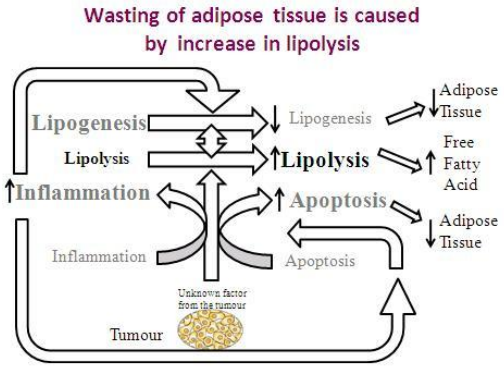
When adipocytes, which were differentiated from preadipocytes from patients in the CC group, were stimulated with catecholamines or natriuretic peptides, there was no increased lipolysis observed, opposite to what was seen in the original adipocytes from patients in the CC group. This indicates that the increase in the expression of HSL is caused by some unknown stimulant or stimulants and is not an epigenetic factor. The

influence of this unknown stimulant had been eliminated in the preadipocytes. These factors could be produced within adipose tissue or they could be circulating factors produced by certain tumours. One such lipolytic mediator, not investigated here, could be ZAG [26, 27]. It is quite possible that ZAG-induced lipolysis is increased in human cancer cachexia because the protein stimulates lipolysis through the same pathway as catecholamines [147, 148]. mRNA expression of ZAG and adipose tissue secretion of ZAG are increased in cancer cachexia, however the concentration of circulating ZAG is not increased in cancer cachexia [113].

5.11 ADIPOCYTES NUMBER AND VOLUME

Patients in the weight losing groups had significantly smaller adipocytes. Others have shown the same results, in cancer-bearing mice [29]. It has also been demonstrated in cancer cachexia patients with wasting of adipose tissue, that the small adipocytes are companied with adipose tissue atrophy and accompanied by an increase in tissue matrix fibrosis [29, 113]. This depletion of adipocytes volume is also an indicator of over expression and increased activity in HSL, the adipocytes cannot accumulate triglycerides [30, 31] and lose their volume. The same numbers of adipocytes in all three groups makes inflammation-stimulated apoptosis [79] an unlikely explanation for wasting of adipose tissue in cancer cachexia.

5.12 SUMMARY OF ADIPOSE TISSUE CHANGES IN CANCER CACHEXIA



In this thesis it has been shown that in cancer cachexia, there is increased mRNA expression and activation of HSL, in adipose tissue. This is the rate limiting factor in mobilising free fatty acids from lipid (lipolysis). There are no signs of increased lipogenesis or apoptosis and no signs of increased inflammation, which can also reduce lipogenesis, increase lipolysis and increase induced apoptosis (Fig. 21).

Fig. 21. Flowchart which shows that it is lipolysis, not inflammation, increased apoptosis or decreased lipogenesis, which is involved in loss of adipose tissue in cancer cachexia.

5.13 DEFINITION OF CACHEXIA

Many definitions exist for cachexia and cancer cachexia.

Bozzetti et al divided cancer cachexia into four classes [149] (Fig. 22). The authors used the database from an ongoing multicentre prospective investigation on the

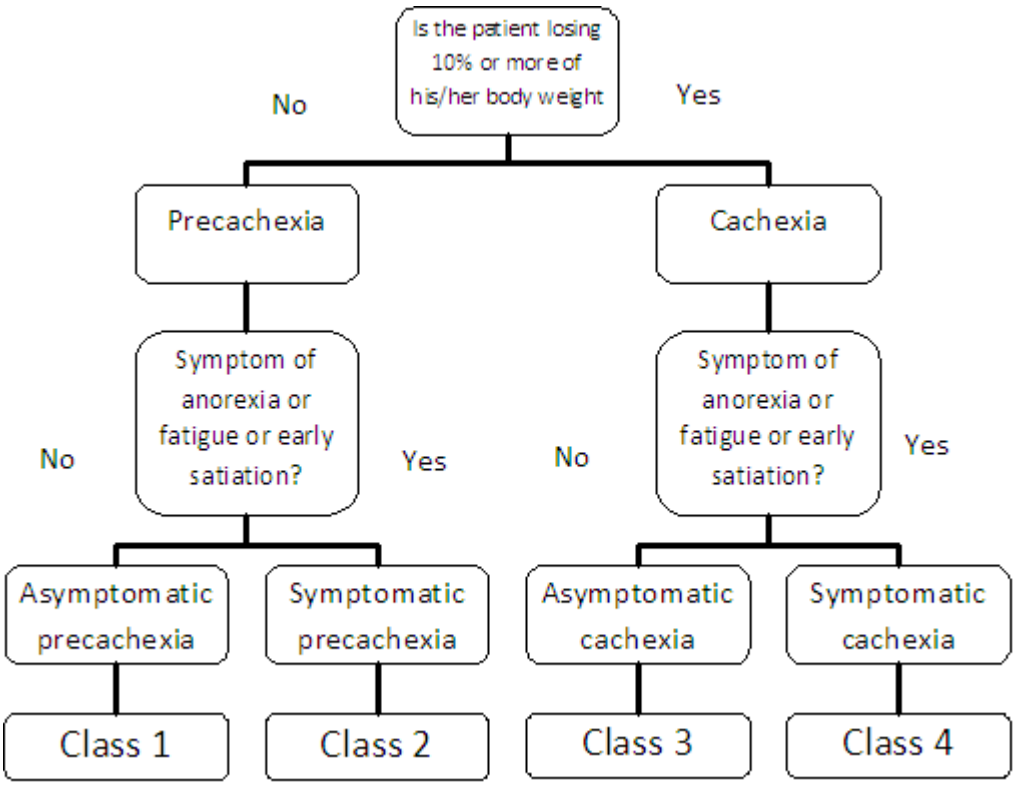


Fig. 22. Flowchart showing classification of cachexia and precachexia according to Bozetti et al. JPEN (2009);33(4),361-7.

screening of the nutrition risk in 1307 cancer out-patients from different university hospitals. The patients were divided into four classes based on combinations of body weight loss (<10%, pre-cachexia; ≥10%, cachexia) and the presence/absence of at least one another symptom of anorexia, fatigue, or early satiation. This was a combination of patients who were either newly diagnosed, waiting for therapy or palliative patients. There were no differences made between primary cancer cachexia and weight loss which was related to, or caused by, mechanical compression or obstruction by the cancer or by the treatment of the cancer such as chemotherapy, radiotherapy or surgery [5].

Fearon et al [150] published a three factor profile:

1. Weight loss (≥10%)
2. Low food intake (≤1500 kcal/d)
3. Systemic inflammation (C-reactive protein ≥ 10 mg/L)

They wanted to evaluate whether this might relate better to the adverse functional aspects of cancer cachexia and to a patient's overall prognosis than weight loss alone.

Their conclusion was that the three-factor profile gave a better prognostic value. This was based on 170 palliative pancreatic cancer patients, with median survival 130 days.

In our study many patients in the CC group had normal food intake and median PG-SGA score 8 (1-17). Score ≤ 8 is defined as "Not at nutritional risk" [110]. Five of 11 patients in CC group had CRP < 10 mg/L and CRP can also be high in starvation [149]. Seven of 13 patients in the CC group had weight loss less than 10% and would therefore not be classified as having cancer cachexia.

In many definitions there is a common focus on the loss of skeletal muscle mass [17] and the wasting of adipose tissue is not regarded as being essential in cancer cachexia [14, 17].

Even in the two latest definitions, one of which from Blum:

> *"Cachexia is a multi-factorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. The pathophysiology is characterized by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism"*[14]

The other from Fearon et al [151], loss of adipose tissue is not included as one of the foundations of cancer cachexia even if many have published its importance before [23, 112]. One explanation could be that many cancer patients are obese and have sarcopenia. When these patients get advanced cancer cachexia, they have enormous volumes of adipose tissue and muscle wasting has started [152, 153].

It seems, that all the definitions of cancer cachexia today are focused on patients who have very advanced cancer and are in need of, or soon going to need, palliative treatment in their home or in a palliative care institute.

According to these classifications of cancer cachexia, about 50% of the patients in the CC group would not been included as having cancer cachexia but all have it according to Fearon's latest definitions [151].

It is my belief that I have shown with this thesis that patients in CC group had cancer cachexia.

6 CONCLUSIONS

To investigate if in pancreatic cancer:

a) *Decreased insulin action on skeletal muscle glucose transport is influenced by decreased ATP levels in muscle cells*
b) *Pancreatic cancer cells change glucose metabolism in muscle*

The *in vitro* resistance for insulin stimulated glucose transport in the skeletal muscle of pancreatic cancer patients is not directly related to factors from pancreatic cancer, weight loss, decreased ATP concentration or plasma FFA levels.

To investigate if in cancer cachexia patients:

a) *Inflammation in adipose tissue is increased*
b) *Apoptosis in adipose tissue is increased*
c) *Lipogenesis in adipose tissue is decreased*

It is lipolysis, not inflammation, increased apoptosis or decreased lipogenesis, which is involved in loss of adipose tissue in cancer cachexia.

To investigate in cancer cachexia patients:

a) *The rate of lipolysis*
b) *The factors involved in lipolysis*

There is increased expression and activity of Hormone Sensitive Lipase (HSL), which leads to an increased rate of lipolysis in patients with Cancer Cachexia.

To investigate if in cancer cachexia patients:

a) *There is a difference in the volume of adipose- and muscle compartments between cancer cachexia patients and benign disease control patients*
b) *Adipose tissue wasting is an early phenomenon in the cancer cachexia syndrome*

Although cancer patients with gastrointestinal obstruction, at the time of diagnosis have lost almost twice the amount of their habitual body weight compared to patients with cancer cachexia, the latter group displays more loss of visceral adipose tissue. Wasting of adipose tissue is a prominent part of cancer cachexia syndrome and starts before the wasting of muscle tissue.

There is a need for a more specific definition for cancer cachexia in newly diagnosed cancer patients.

7 POPULÄRVETENSKAP

Introduktion: Ordet kakexi, kommer från orden kakos och hexis och härstammar från det grekiska språket och är en beskrivning av ett dåligt tillstånd.

Tanken att ett tumörämne kan ge upphov till cancer kakexi publicerades för första gången 1962. Innan dess trodde man att kroppen förstör fett och muskelvävnad för att "mata" tumören.

Kakexi är ett kliniskt syndrom som är svårt att definiera. De flesta relaterar det till viktminskning hos cancerpatienter. Avancerad kakexi präglas av kraftig viktminskning, anorexi, tidig mättnadskänsla, svaghet, anemi och ödem. I början av kakexi kan dessa drag förekomma i varierande grad men symptomen kan förändras i svårighetsgrad under sjukdomsgången. Den komplexa, mångfasetterade ursprungliga kakexin, följer inte någon enhetlig patofysiologisk profil och detta har skapat hinder för att behandla kakexi. Cancer kakexi syndromet är vanligt hos patienter med mag-, tarmcancer. Hos dessa patienter är cancer kakexi en synonym för alla tecken och symtom de kan utveckla, som inte har anknytning till eller orsakas av mekanisk kompression eller obstruktion av cancer eller av behandling av cancer, såsom kemoterapi, strålbehandling eller kirurgi. Även med intensivt nutritionsstöd, är det svårt att vända processen. Förlust av andningsmuskulatur hos cancerpatienter med kakexi har visat sig bidra till upp till 50 % av dödsfallen hos cancerpatienter. Viktminskning närmare 30 % är inte förenligt med liv.

Syfte: Vårt mål var att undersöka effekten av insulin på glukosmetabolismen och innehåll av energimetaboliter i muskeln hos patient med pankreascancer (PC) och om förändringar i nedbrytning av fett, antal fettceller, produktion av fett eller cytokiner koncentration och/eller infiltration av vita blodkroppar in i fett, kunde redogöra för några av de funktionella förändringar som observerats i fettväv i cancer kakexi (CK). Målet var också att se om volym som fastställs genom datortomografi (DT) kan ge information om distribution och mängd av muskler, subkutan och abdominal fettväv hos patienter som nyligen fått diagnosen cancer kakexi.

Material och metoder: Venösa blodprov togs på patienten, glukosbelastning utfördes, fettvävnadsbiopsier erhölls under lokalbedövning, energiförbrukning och mätning av kroppssammansättning gjordes vid patientens kliniska laboratorium. För PC group och tre kontroll grupper, var muskelbiopsier tagna i operationssalen och frystes omedelbart eller transporterades färsk direkt till laboratorium och analyserades för glykogen, adenosintrifosfat och phosphocreatine innehåll. Glukostransport kontrollerades i mänskliga muskeltrådar och muskelceller från råtta. För patienter med CK och två cancer kontroll grupper, vikt stabila (VS) och med stopp i mag- tarmkanalen (SM), alla med nydiagnostiserad sjukdom, var fettvävnadsbiopsier transporterades färska till forskningslaboratorium för mätning av 1) nedbrytning av fett, samt mätning av ämnet som styr nedbrytning av fett, hormonkänslig lipas (HSL) och HSL genuttryck, 2) genuttryck för inflammation markörer och mätning av inflammation i blodet och

cytokiners sekretion i fettväv, 3) volym och nummer av fett celler. Slutligen var muskel- och fettvolym mätt med DT.

Resultat: Patienter med CK har förlorad10% av sin vanliga vikt, VS gruppen 3% och SM gruppen 17%. Insulinstimulerad glukostransport, muskelglykogen och adenosintrifosfat innehåll var sänkt hos patienter med PC jämfört med kontrollgruppen, och insulinstimulans gav inte signifikant ökning i produktion av glykogen in vitro hos patienter med PC. Media konditioneras med PC celler påverkade inte glukos transport i rått celler. Cirkulerande nivåer av citokin och naturlig nedbrytning av fett var ökade i CK-gruppen jämfört med kontrollerna. I CK gruppen visade resultatet mer stimulation av fettnedbrytning jämfört med i kontrollgruppen, mängd av HSL protein och gen ökade jämfört med kontrollgruppen. Det fanns inga skillnader i mRNA uttryck av cytokiner eller sekret i fettvävnad och produktion av fett. Fettcellerna minskade i storlek, men deras antal var normala i CK gruppen jämfört med VS gruppen. Fettvävnad minskade i CK och SM grupperna. Både enligt DT och i mätning av kroppssammansättning visade CK gruppen en selektiv minskning av abdominell fettvävnad.

Slutsats: Fettvävnadsförlust är en framträdande del av cancer kakexi syndromet och startar före muskelförtvining. Insulinmotstånd för aktiv transport glukos i skelettmuskulaturen hos patienter med pankreascancer är inte direkt kopplade till faktor/faktorer från bukspottkörtelcancer eller viktminskning. Det är en ökad nedbrytning av fett, inte inflammation samt ökad apoptos eller minskad lipogenes, som är involverad i förlust av fettvävnad i cancer kakexi. Det finns ett ökat uttryck och aktivitet av hormonkänslig lipas (HSL), som ger ökad hastighet av nedbrytning av fett hos patienter med cancer kakexi. Även om cancerpatienter med gastrointestinal obstruktion, vid tidpunkten för diagnos, nästan har förlorat dubbelt så mycket kroppsvikt jämfört med patienter med cancer kakexi, så har den senare gruppen förlorat mer av abdominell fettvävnad.

8 ÚTDRÁTTUR

Inngangur: Orðið kakexía kemur frá grísku orðunum kako og hexis, og þýðir slæmt ástand eða að vera þróttlítill.

Sú hugmynd að æxli gefi frá sér efnið sem getur valdið krabbameins kakexíu, kom fyrst fram í grein sem birtist árið 1962. Áður var talið að líkaminn brenndi fitu og vöðvavef, í því skyni að "fæða" æxlið.

Kakexía er heilkenni sem er erfitt að skilgreina. Flestir þekkja það sem megrun krabbameinssjúklinga. Langt gengin kakexía einkennist af alvarlegu þyngdartapi, lystarleysi, slappleika, blóðleysi og bjúg. Krabbameinskakexía er algeng hjá sjúklingum með krabbamein í meltingafærum og er samheiti yfir öll þau einkenni sem ekki eru tengd stærð æxlisins eða krabbameinsmeðferðinni þ.e. lyfjameðferð, geislameðferð eða skurðaðgerð. Þó að þessir sjúklingar fái mikla næringu, þá tekst ekki að snúa ferlinu við. Rýrnun öndunarvöðva hjá sjúklingum með kakexíu, stuðlar að allt að 50% dauðsfalla þessara sjúklinga. Þegar þyngdartap er yfir 30%, af þyngd fyrir veikindi, þá á sjúklingurinn sjaldan langt eftir ólifað.

Markmið: Markmið okkar var í fyrsta lagi að rannsaka áhrif insúlíns á efnaskipti sykurs og myndun orku í vöðvum sjúklinga með briskrabbamein (BK). Í öðru lagi að kanna hvort að breytingar á niðurbroti eða myndun fitu, fjöldi fitufrumna eða myndun bólguefna og/eða flutningur hvítra blóðkorna inn í fituvef, gæti skýrt út þær breytingar sem verða í fituvef hjá sjúklingum með kakexíu. Að lokum var athugað hvort magn fitu og vöðvavefs, mælt með tölvusneiðmynd, getur gefið upplýsingar um dreifingu og/eða rýrnun vöðva og fituvefs hjá sjúklingum sem nýlega hafa greinst með kakexíu.

Efni og aðferðir:
Vöðvasýni voru tekin úr sjúklingum með briskrabbamein og þremur samanburðarhópum og magn glykogens, adenósíns þrífosfats og fosfokreatíns var mælt. Sykurflutningar voru rannsakaðir í vöðvum manna og í vöðvafrumum rotta. Bláæðablóðsýni og fituvefjapróf úr sjúklingum með krabbameinskakexíu (KK) og tveimur samanburðarhópum, öðrum með stöðuga þyngd (ÞS) og hinum með stíflu í meltingarvegi (SM), voru rannsökuð með það að markmiði að 1) einangra fitufrumur og reikna út fjölda og stærð; 2) mæla niðurbrot fitu og efnið sem stýrir því, hormón-næmur lípasa (HSL) 3) mæla framleiðslu á fitu, prótein og genatjáningu og seytun bólguefna i fituvef.
Einnig var orkunotkun og samsetning líkamans mæld og fitu- og vöðvamagn var mælt með tölvusneiðmynd.

Niðurstöður: Sjúklingar í KK hópnum höfðu lést um 10% af þyngd sinni fyrir veikindi, SM hópurinn 17% og ÞS hópurinn 3%. Það var minna af insúlínörvuðum sykurflutningi, vöðva glycogen og adenósíns þrífosfat innihaldi, hjá sjúklingum með BK en hjá samanburðarhópunum. Insúlín örvaði ekki framleiðslu á glycogen hjá sjúklingum með BK. BK hefur ekki áhrif á flutning sykurs i vöðvafrumum. Magn bólguefna í blóði og náttúrulegt niðurbrot fitu var meira i KK hópnum miðað við ÞS og

SM hópana. Í KK hópnum var meiri örvun á niðurbroti fitu miðað við ÞS og SM hópana, meiri tjáning af HSL mRNA og próteinum. Það var enginn munur milli hópanna á framleiðslu á fitu, á tjáningu mRNA bólguefna eða seytingu þeirra í fituvef. Fitufrumur minnkuðu í stærð, en fjöldi fitufrumna var sá sami í KK miðað við ÞS og SM hópana. Magn fituvefs var minna í KK og SM hópum, bæði þegar fita var rannsökuð með tölvusneiðmynd och mælingum á samsetningu líkamans. Tölvusneiðmynd sýndi sértæka minnkun á fituvef inni í kviðarholi hjá KK hópnum miðað við ÞS hópinn. Enginn munur var á magni vöðvavefs á milli hópanna.

Ályktun: Eyðing fituvefs er áberandi í krabbameinskakexíu heilkenni og byrjar fyrr en eyðing vöðvavefs. Það er aukið viðnám fyrir insúlínörvun á virkum sykurflutningi í vöðvum sjúklinga með briskrabbamein og það er ekki tengt efni/efnum sem koma þaðan eða þyngdartapi. Það er aukið niðurbroti fitu, en ekki bólga í fituvef, aukið sjálfsmorð fitufrumna eða minnkuð myndun fitu, sem er orsök fitueyðingar hjá sjúklingum með krabbameinskakexíu. Það er aukin tjáning og virkni hormóna-næma lípasa (HSL), sem veldur þessu. Þótt krabbameinssjúklingar með stíflu í meltingarvegi, hafi við sjúkdómsgreiningu, tapað nær tvöfalt meiri líkamsþyngd borið saman við sjúklinga með krabbameins kakexíu, þá hefur seinni hópurinn minna af fitu inni í kviðarholinu.

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