# Inflammation and body composition in over- and undernutrition:

## Overweight and cancer cachexia

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# Summary

This thesis focuses on the association between inflammation and body composition and consists of one cross sectional study in healthy overweight and in lean subjects (paper I), and one prospective study in patients with pancreatic cancer (papers II and III). In **paper I**, the relationship between inflammatory markers and body composition measured with anthropometry and bioelectrical impedance analysis (BIA) was examined in healthy individuals. Increasing hip circumference (HC) was associated with increasing levels of leptin and C-reactive protein (CRP) in both groups. CRP increased with increasing body mass index (BMI), also in the lean group. HC may be a proxy for fat percent measured with BIA in lean and overweight individuals, and may be useful for identification of people at risk of developing overweight-related disorders. In **paper II** the level of inflammatory markers, body composition, energy intake and development of cachexia was examined. Pancreatic cancer patients had higher levels of inflammatory markers compared with a healthy reference population at inclusion, and the levels increased as death approached. Weight, fat- and muscle mass were reduced during follow-up. At inclusion, there were no differences in the levels of inflammatory markers between patients who were classified as cachectic and those who were not. These results indicate that the increased levels of inflammation may be caused by the tumor and the tumor-host reactions. The **third paper** compared two classifications of cancer cachexia; the 3-factor classification that includes CRP as a criterion, and the consensus classification that includes sarcopenia as a criterion. Patients were categorized as cachectic and non-cachectic according to both classifications. Consistency across definitions was examined, as well as their ability to predict survival. The two classifications of cancer cachexia showed good overall agreement in defining cachectic patients, and cachexia was associated with poorer survival according to both. Inflammation in the body may be caused by fat mass and tumors. Overweight increases the risk for chronic diseases and pancreatic cancer. In cancer patients, the tumor may cause systemic inflammation, and cytokines may trigger weight loss and might be a driving force behind cachexia. Among pancreatic cancer patients, those with neither weight loss, reduced energy intake, systemic inflammation nor sarcopenia have the longest survival. We suggest that clinical interventions should be directed towards optimizing these risk factors in these patients.

# Norsk sammendrag

Denne avhandlingen består av en tverrsnittsstudie av friske overvektige og slanke personer (artikkel I) og en prospektiv studie av pasienter med kreft i bukspyttkjertelen (artikkel II og III). I **artikkel I** ble forholdet mellom inflammasjonsmarkører og kroppssammensetning målt med antropometri og bioimpedans (BIA), undersøkt i friske individer. Større hofteomkrets var assosiert med høyere plasmanivåer av leptin og C-reaktivt protein (CRP) i begge gruppene. CRP økte med økende kroppsmasseindeks, også blant de slanke. Hofteomkrets kan brukes som et surrogatmål på fettprosent, målt med BIA, og kan være nyttig for å identifisere de med risiko for overvektsrelaterte sykdommer. I **artikkel II** ble nivået av inflammasjonsmarkører, kroppssammensetning, energiinntak og utvikling av kakeksi undersøkt. Kreftpasientene hadde høyere nivåer av inflammasjonsmarkører sammenliknet med friske. Nivået av inflammasjonsmarkørene hos kreftpasientene fortsatte å øke fram mot død, samtidig som pasientene fikk redusert vekt, fett- og muskelmasse. Ved inklusjon i studien var det ingen forskjell i inflammasjonsmarkører mellom pasienter som ble klassifisert som kakektiske versus de ikke-kakektiske. Funnene kan tyde på at det er tumoren og kroppens reaksjon på tumoren som er årsak til økte nivåer av inflammasjonsmarkører. I den **tredje artikkelen** ble to klassifikasjoner av cancer kakeksi sammenliknet; 3-faktor klassifikasjonen som inkluderer CRP som et kriterium, og konsensusklassifikasjonen som inkluderer sarkopeni som et kriterium. Vi undersøkte om det var samsvar mellom klassifikasjonene i kategoriseringen av kakektiske pasienter, og hvilken av klassifikasjonene som best predikerte overlevelse. De to klassifikasjonene samsvarte godt i kategorisering av kakektiske og ikke-kakektiske pasienter og kakeksi var assosiert med dårligere overlevelse ifølge begge klassifikasjonene. Både fettmasse og kreft er assosiert med inflammasjon. Overvekt øker risikoen for kroniske sykdommer, inkludert kreft i bukspyttkjertelen. Hos kreftpasientene kan svulsten føre til en økning av inflammasjonsmarkører, deriblant cytokiner som kan påvirke vekttap og som kan være årsak til kakeksi. Pasienter med kreft i bukspyttkjertelen som verken hadde vekttap, redusert matinntak, systemisk inflammasjon eller sarkopeni, hadde lenger overlevelse enn pasienter der disse risikofaktorene var til stede. Kliniske intervensjoner bør derfor rettes mot disse risikofaktorene i denne pasientgruppen.

# List of papers

The papers will be referred to by their Roman numerals.

## Paper I

**Measurements of body fat are associated with markers of inflammation, insulin resistance and lipid levels in both overweight and in lean, healthy subjects**

Nima Wesseltoft-Rao, Kirsten B. Holven, Vibeke H. Telle-Hansen, Ingunn Narverud, Per Ole Iversen, Marianne J. Hjermstad, Ingrid Dahlman, Stine M. Ulven, Asta Bye. *e-SPEN Journal*, 7: e234-e240, 2012.

## Paper II

**Alterations in Inflammatory Biomarkers, Body Composition and Energy Intake in Cancer Cachexia: A Prospective Study in Patients with Inoperable Pancreatic Cancer**

Asta Bye, Nima Wesseltoft-Rao, Per Ole Iversen, Grete Skjegstad, Kirsten Holven, Stine M. Ulven, Marianne J. Hjermstad. (submitted).

## Paper III

**Comparing two definitions of cancer cachexia and their association with survival in patients with unresectable pancreatic cancer**

Nima Wesseltoft-Rao, Marianne J. Hjermstad, Tone Ikdahl, Olav Dajani, Stine M. Ulven, Per Ole Iversen, Asta Bye. *Nutr Cancer*, 673: 472-480, 2015.

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*Oslo, June 2015*

*Nima Wesseltoft-Rao*

# Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| BAT | Brown adipose tissue |
| BCM | Body cell mass |
| BIA | Bioelectrical impedance analysis |
| BMI | Body mass index |
| CRP | C-reactive protein |
| CT | Computer tomography |
| DEXA | Dual X-ray absorptiometry |
| FFM | Fat free mass |
| IFN | Interferon |
| IGF-1 | Insulin-like growth factor-1 |
| IL | Interleukin |
| Kcal | Kilocalories |
| LMF | Lipid mobilizing factor |
| MUAC | Mid-upper-arm circumference |
| MUAMA | Mid-upper-arm muscle area |
| MUMAC | Mid-upper-arm muscle circumference |
| NF-κB | Nuclear factor kappa B |
| OUH | Oslo University Hospital |
| PIF | Proteolysis-inducing factor |
| QoL | Quality of Life |
| SAT | Subcutaneous adipose tissue |
| SGA | Subjective Global Assessment questionnaire |
| T2DM | Type 2 diabetes mellitus |
| TNF | Tumor necrosis factor |
| TSF | Tissue skin fold |
| UCP | Uncoupling protein |

| | |
|---|---|
| VAT | Visceral adipose tissue |
| WAT | White adipose tissue |
| WHO | World Health Organization |
| WHR | Waist-to-hip ratio |

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# 1 Introduction

Overweight individuals and pancreatic cancer patients with cancer cachexia differ in weight and body composition and represent states of over- and undernutrition respectively. However, low-grade chronic inflammation probably contributes to metabolic alterations in both groups. The present study was undertaken to increase the knowledge about body composition and inflammation in over- and undernutrition and thereby contribute to the understanding of the role of inflammation in cancer cachexia.

## 1.1 Obesity and pancreatic cancer

A person is defined as overweight if the body mass index (BMI) is 25-30 kg/m² or obese if the BMI is above 30 kg/m² [1].The prevalence of overweight and obesity is increasing worldwide [2]. Currently 66% of the US population is overweight or obese and similar figures are reported globally, corresponding to 45% in Norway [3]. The main burden of obesity lies in the connection with diseases like insulin resistance and type 2 diabetes mellitus (T2DM) [4]. Adipose tissue plays an important role in the development of these diseases. Increased amounts of adipose tissue may lead to macrophage infiltration into the adipose tissue and a subsequent secretion of pro-inflammatory cytokines and acute phase proteins, such as C-reactive protein (CRP), into the circulation [4, 5]. Furthermore; low-grade inflammation is considered an underlying cause of insulin resistance, T2DM [6, 7] and of many types of cancers [8], including pancreatic cancer [9].

In Norway, the estimated incidence of pancreatic cancer was 7.7% and 6.6% for men and women respectively in 2013 [10]. In general, pancreatic cancer is rare in patients younger than 40 years, and the median age at diagnosis is 71 years [11]. Pancreatic cancer, in which 85% are pancreatic ductal adenocarcinomas, is usually diagnosed at an end stage when the tumor has metastasized into other organs such as the lungs and liver [11, 12]. The mortality exceeds 90%, and is higher in men than in women [11]. Surgery offers the only chance of a potential cure and adjuvant chemotherapy has shown a small increase in survival rates [13]. However only 15-20% of patients are considered candidates for surgery, because localized tumors are often infiltrated into local vessels [11, 14]. Consequently, palliative care, including palliative chemotherapy is the only treatment option for the majority of these patients [15,

16]. At the time of diagnosis, more than 80% of pancreatic cancer patients suffer from nutritional problems [17].

Although obesity is a risk factor for pancreatic cancer [9], these patients are prone to weight loss due to inadequate energy and protein intake which may lead to malnutrition [18]. Malnutrition can be defined as a condition where energy and protein deficiency leads to a reduction in fat mass and fat-free mass (FFM) [19-21]. Recently, it has been proposed that diagnosis of malnutrition should be based on either a low BMI (<18.5 $kg/m^2$), or on the combination of weight loss together with either reduced BMI (age-specific) or a low muscle mass using sex-specific cut-offs [19]. In malnourished patients, adequate symptomatic treatment and specific nutritional support will contribute to weight gain and a better nutritional state [18].

In patients with pancreatic cancer, malnutrition may be generated by different pathophysiological conditions caused by the tumor, such as duodenal stenosis, malabsorption, pancreatic insufficiency, constipation, malaise, taste alterations, early satiety and loss of appetite [15, 22]. The condition may be worsened by side effects from anti-cancer treatment, i.e. chemotherapy, which may cause nausea, vomiting and changes in smell and taste, with frequent aversion to specific foods or food in general [15]. Chemotherapy can also lead to problems in the digestive system, affecting both the ability and desire to eat and also the absorption of nutrients, thus contributing to increasing anorexia [15]. However, chemotherapy may also prolong survival and improve quality of life (QoL), thus the administration of chemotherapy must be balanced against side effects [23, 24].

## 1.2 Cancer cachexia

The word cachexia stems from the Greek words *kako`s* and *he`xis*, meaning “bad condition” [25]. Cancer cachexia emerges from a complex interaction between cancer growth and host response resulting in progressive weight loss that is a consequence of a negative protein and energy balance, often associated with signs of inflammation [15].

The syndrome is multifactorial and characterized by anorexia, fatigue and involuntary weight loss, i.e. ongoing loss of skeletal muscle mass, with or without the loss of fat mass [15, 22, 26]. It is also associated with poor tolerability of cancer treatment, decline in performance status and reduced QoL and survival [27-29]. As opposed to malnutrition, weight loss in

cachexia cannot be compensated by optimal energy intake [27, 28]. In addition, metabolic alterations in cachexia cause greater loss of muscle mass than in malnutrition, where lean body mass is preserved [15, 18].

Loss of skeletal muscle mass and adipose tissue may lead to low albumin levels and fatigue, which limits physical activity and accordingly inhibits protein synthesis [15]. Muscle wasting may be accelerated by a combination of metabolic changes (i.e. increased resting energy expenditure and increased turnover of protein, lipids and glucose), hormonal changes (i.e. insulin resistance), systemic inflammation (i.e. cytokine release) and tumor-inducing factors (i.e. proteolysis-inducing factor (PIF)) [15, 22]. Moreover, the loss of adipose tissue is caused by a combination of the tumor-inducing factor, lipid mobilizing factor (LMF) [22], and cytokines [30] that contribute to a disturbance of lipid metabolism causing increased lipolysis, which is considered a key factor in triggering the loss of adipose tissue [31, 32].

Although several factors involved in the development of cachexia are described, the underlying mechanisms remain unclear [26, 33]. Because of this and because of the fact that cachexia is associated with a wide range of clinical manifestations it is difficult to precisely define this condition. This makes it difficult to estimate the prevalence of cancer cachexia [34]. The prevalence rates in cancer patients vary between 12% and 82% depending on definitions, study designs and patient samples [35-37]. However, even with the same tumor type and stage of disease, one patient may develop cachexia whereas another may not. Such variation may relate, at least in part, to the host genotype [38, 39]. It is estimated that cancer cachexia accounts for about 20% to 40% of all cancer deaths [40, 41]. In order to identify, treat and/or prevent this condition and to develop potential therapeutic interventions, there is an urgent need of a consensus definition that is accepted by clinicians and researchers [26].

### 1.2.1 Definitions of cancer cachexia

Due to the lack of an established definition of cancer cachexia, involuntary weight loss of 10% or more has been used as a diagnostic criterion for cancer cachexia [42]. However, weight loss alone is often not enough to capture the complexity of cachexia, as a substantial loss of muscle mass may be concealed by edema and/or overweight [43]. Furthermore, weight loss is not sufficient to explain deterioration in physical function, which is a characteristic feature of cancer cachexia [37, 44].

A universally accepted definition of cancer cachexia has therefore been requested. In order to follow a consensus process to define cachexia, three expert groups were established in 2006 (Table 1); (i) An expert meeting organized by the Society of Cachexia and Wasting disorders (SCWD) [26], (ii) The Special Interest Group (SIG) on Cachexia-Anorexia in chronic wasting disease created within The European Society for Clinical Nutrition and Metabolism (ESPEN) [45], and (iii) The European Palliative Care Research Collaborative (EPCRC) [25].

**Table 1: Consensus definitions of cancer cachexia**

| **Year of publication** | **Special interest groups and definitions** |
|---|---|
| **2008**<br><br>Evans et al.<br>[26] | *Society of Cachexia and Wasting disorders*<br><br>Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by **loss of muscle with or without the loss of fat mass.** The prominent clinical features of cachexia is weight loss in adults and growth failure in children. Anorexia, inflammation, insulin resistance and increased muscle protein break down 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. |
| **2010**<br><br>Muscaritoli et al.<br><br>[45] | *The Special Interest Group on Cachexia-Anorexia in chronic wasting disease created within The European Society for Clinical Nutrition and Metabolism*<br><br>Cancer 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 patient's **morbidity** and mortality. Contributory factors to the onset of cachexia are anorexia and metabolic alterations, i.e. increased inflammatory status, increased muscle proteolysis, and impaired carbohydrate, protein and lipid metabolism. Considering the wide range of clinical manifestations of cachexia, the staging of this syndrome is warranted. |
| **2010**<br><br>Argiles et al.<br>[25] | *The European Palliative Care Research Collaborative*<br><br>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.** |
| **2011**<br><br>Fearon et al.<br>[44] | *Representatives of the above mentioned expert groups*<br><br>Cancer cachexia is a multifactorial syndrome characterized by an **ongoing loss of skeletal muscle mass (with or without the loss of fat mass)** that cannot be fully reversed by conventional nutritional support and leads to progressive **functional impairment**. The pathophysiology is characterized by negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism. |

The common focus of the definitions from SCWD, ESPEN and EPCRC was loss of skeletal muscle mass (with or without loss of fat mass) and the subsequent loss of functional capacity.

With the intention to reach a more specific definition of cancer cachexia and a classification based on a formal consensus process, representatives from the three groups participated in a Delphi process in 2009. An international consensus definition was reached in 2011 [44] (Table 1): "*Cancer cachexia is a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without the loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. The pathophysiology is characterized by negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism"*. A set of criteria for a classification was defined of which one of the following had to be present: weight loss more than 5% over the past 6 months (in absence of simple starvation), BMI <20 kg/m$^2$ and any degree of weight loss >2%, or appendicular skeletal muscle index consistent with sarcopenia and any degree of weight loss >2% (Table 2).

Previously, several diagnostic criteria were proposed (Table 2). Between 2002 and 2004, the North Central Cancer Treatment Group (NCCTG) [46-48] classified cachexia as involuntary weight loss, anorexia or impaired oral nutritional intake, in phase III studies. In 2006, Fearon et al [37] incorporated systemic inflammation in a three-factor classification of cancer cachexia based on a study of weight-losing pancreatic cancer patients. The presence of two out of the three factors i.e. weight loss, reduced food intake and systemic inflammation, was shown to have impact on objective (i.e. hand-grip strength and Karnofsky performance score) and subjective (i.e. physical function, dyspnea and fatigue) function [37]. Furthermore, in 2009, the Screening Nutritional Risk in Oncology (SCRINIO) working group suggested a classification that could be feasible for clinical practice, until more specific diagnostic measures were available. They proposed four stages of severity of cancer cachexia based on weight loss of less than 10% (pre-cachexia) or more than 10% (cachexia) and on the absence or presence of three symptoms i.e. anorexia, early satiety or fatigue. The classifications are either asymptomatic pre-cachectic (class 1), symptomatic pre-cachectic (class 2), asymptomatic cachexia (class 3) or symptomatic cachexia (class 4) [33]. The criteria for classification in the consensus definition include elements from all of the previous criteria, but food intake, identification of inflammation and symptom mapping are not included.

**Table 2: Overview of proposed diagnostic criteria of cancer cachexia**

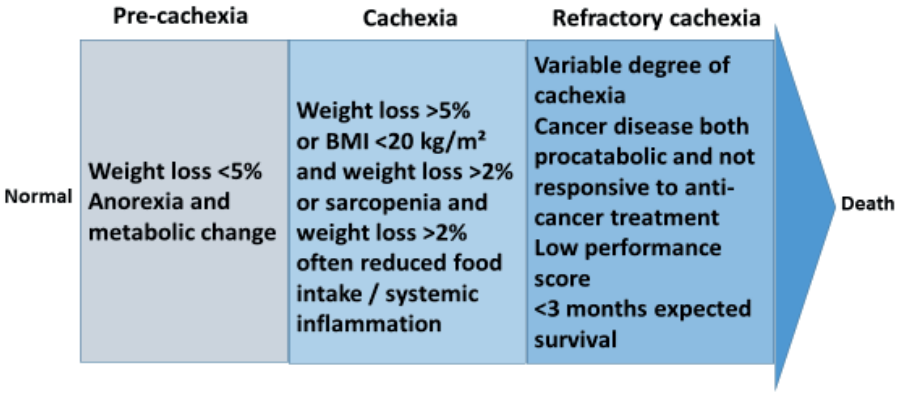
| Year of publication and author | Studies and Classifications |
|---|---|
| **2002-2004**<br><br>Jatoi et al. [46-48] | The North Central Cancer Treatment Group studies.<br><br>• weight loss: 2% in 2 months or 5% in 6 months<br>• anorexia: visual analogue scale >3/10, (0 no problem and 10 maximal problem)<br>• reduced food intake: <75% than normal or <20 kcal/kg body weight/day |
| **2006**<br><br>Fearon et al. [37] | Definition of cancer cachexia: effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis.<br><br>Minimum 2 of the 3 factors should be met:<br><br>• Weight loss >10%<br>• Low food intake (<1500 kcal)<br>• CRP >10 mg/l |
| **2008**<br><br>Evans et al. [26] | Cachexia: a new definition.<br><br>Classified by: weight loss of at least 5% in 12 months or less (or BMI <20 kg/m²), plus three of the following criteria:<br><br>• decreased muscle strength (lowest tertile)<br>• fatigue (physical and/or mental weariness resulting from exertion)<br>• anorexia (limited food intake: <75% than normal or <20 kcal/kg body weight/day)<br>• low fat free mass index (lean tissue depletion measured by MUAMC[1] or DEXA[2])<br>• abnormal biochemistry[3] (increased inflammatory markers, anemia, low serum albumin) |
| **2009**<br><br>Bozzetti et al. [33] | Defining and classifying cancer cachexia: a proposal by the SCRINIO working group.<br><br>weight loss (<10%, pre-cachexia; >10%, cachexia) and at least one cachexia-related symptom[a]:<br><br>• anorexia<br>• fatigue<br>• early satiety |
| **2011**<br><br>Fearon et al. [44] | Definition and classification of cancer cachexia: an international consensus.<br><br>One out of 3 criteria should be met:<br><br>• weight loss <5% over past 6 months (in absence of simple starvation); or<br>• BMI <20 kg/m², and any degree of weight loss >2% or<br>• appendicular skeletal muscle index consistent with sarcopenia[b] and any degree of weight loss >2% |

[1]MUAMC = mid-upper-arm muscle circumference <10$^{th}$ percentile for age and gender, [2]DEXA = Dual X-ray absorptiometry: men <7.25 kg/m²; women <5.45 kg/m², [3] increased inflammatory markers: CRP: >5.0 mg/l, IL-6 >4.0 pg/ml, anemia: <12g/dl, low serum albumin <3.2 g/dl, [a]Symptoms were classified according to a 4-point score; 1=no symptoms, 2= mild symptoms, 3= moderate symptoms, 4= severe symptoms, [b]CT imaging: men <55 cm²/m², women <39 cm²/m².

It was emphasized that cachexia can develop progressively through various stages (Figure 1). The spectrum starts with pre-cachexia, then develops to cachexia and finally to refractory cachexia. The progression can be modulated by factors like cancer type and stage, systemic

inflammation, reduced food intake and response to anticancer therapy. Not all patients experience all stages [49]. Cancer cachexia has traditionally been associated with advanced disease [34]; however, it is now recognized as an early occurrence in the development of cancer [50].

**Figure 1: Stages of cancer cachexia**




***Modified from Fearon et al. [44]***

The international consensus definition is considered a framework that can be modified over time [44]. Thus, efforts to validate this definition in clinical studies are important [25, 44]. Wallengren et al. [35] compared the consensus definition [44], and the classifications by Fearon et al. [37] and Evans et al. [26] in 405 patients with different cancer diagnoses in advanced stages. The conclusion was that weight loss, fatigue and markers of systemic inflammation were most strongly associated with adverse outcomes like reduced QoL, functional decline, more symptoms and shorter survival. Based on the results, an alternative three-factor classification was developed; weight loss >2% since before onset of disease, fatigue >3 points (on a 1-10 scale), and CRP >10 mg/l. Blum et al [51] also validated the

consensus definition in a heterogeneous sample of patients with advanced cancer. They concluded that the combination of weight loss and low BMI showed the strongest association with short survival.

Considering the limited treatment options for cancer cachexia, emphasis is put on pre-cachexia, in which multi-modal interventions may slow down weight loss [49]. However, discrepancy in the prevalence of pre-cachexia was found in two recent studies in patients with advanced cancer and no significant difference in survival was found between pre-cachexia and cachexia [51, 52]. Moreover the clinical relevance of pre-cachexia has been questioned, as an unpublished study by Blauwhoff-Buskermolen et al. showed that the use of the present framework depicted in Figure 1 identified very few patients i.e. 0.5-2% [53].

## 1.3 Inflammation

Inflammation is the body's defense against infections or damaged tissue following i.e. surgical trauma or tumor surgery [54, 55], and is the body's attempt to remove the harmful stimuli and to initiate the healing process (6).

The localized inflammatory responses co-occur with a large number of systemic and metabolic changes referred to as the acute phase response [54]. Primarily the response involves the innate immune system through secretion of cytokines and interleukins by activated macrophages. Clinically, the acute phase response is characterized by inflammation, anorexia, increased vascular permeability, vasodilatation and increased cardiac output, as well as neuro-endocrine and metabolic changes [54]. CRP is a well described acute phase protein and is known for its clinical application as a marker for systemic inflammation [56, 57]. CRP levels reported in healthy individuals is usually less than 3 mg/l, [58, 59], but can rise 1000-fold in severely infected individuals [54]. Therefore, the CRP levels can indicate the severity of an inflammatory response [60] and CRP >10 mg/l is used as an indication of the acute phase response [61].

Acute inflammation has a relatively short duration, lasting from minutes to a few days [62]. Important proteins involved in the inflammatory response are the cytokines, which is a large group of small proteins [63, 64] and include the pro-inflammatory molecules interleukin (IL) -1 , IL-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and the anti-inflammatory cytokine IL-10 [65]. These proteins may activate and influence each other [66]. TNF-α as

well as IL-1β and IL-6, activate the NF-κB transduction pathway and thus stimulate further cytokine production [67, 68]. IL-10, on the other hand, inhibits the actions of e.g. IL-6, as the special physiological role of IL-10 is to prevent and limit tissue damage by limiting overwhelming immune reactions [69].

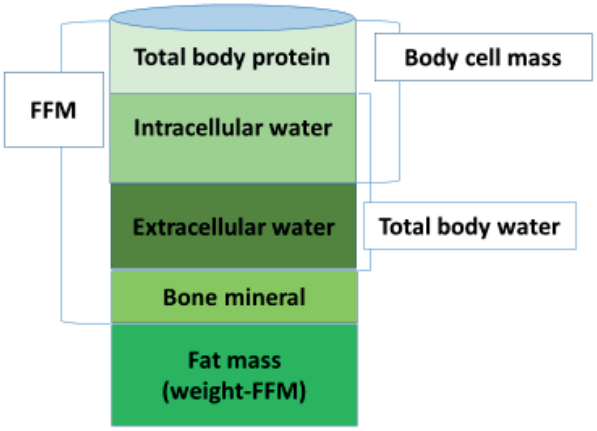
The inflammatory response in obesity differs in duration and intensity from an inflammatory response caused by pathogens/infections. When infectious inflammation involves short-lived, high amplitude responses [62], adipose tissue inflammation occurs at low levels for years to decades, and therefore the term chronic low-grade inflammation is used to describe the inflammation caused by obesity [4, 6, 7]. Obesity causes a recruitment and infiltration of macrophages to the adipose tissue, and is the initial event in obesity-induced inflammation [70, 71]. They secrete a variety of cytokines, such as TNFα, IL-1β and IL-6, which can act locally in a paracrine manner, or they leak out of the adipose tissue [65], causing a systemic effect (endocrine action) on insulin sensitivity in insulin target cells, which again may lead to insulin resistance [6, 7]. Furthermore, obesity is also linked to adipocyte hypertrophy and hypoxia, aggravating the inflammatory state [70, 72]. Moreover, sustained inflammatory signaling, insulin resistance and hypoxia cause a microenvironment favorable for tumor development [72, 73].

Obesity-induced activation of intracellular pathways, such as NF-κB are major mediators in pancreatic cancer, causing elevated concentrations of TNFα, IL-1β, IL-6, IL-10 and growth factors [74]. In the presence of a tumor, cytokines are also secreted by skeletal muscle (e.g. the myokine TGFβ) lymphocytes and the tumor itself [30, 34, 74] thus cytokine levels increase with tumor progression [75]. Increased levels of cytokines act locally in hypothalamus and are thought to be responsible for anorexia, elevated resting energy expenditure and weight loss [76]. They may also cause other symptoms like fatigue, anemia and protein degradation [39]. Whether these symptoms occur as independent processes or are direct results of the inflammatory process of cancer cachexia is not fully understood [15]. TNF-α is also called cachectin [39] and is known to play a critical role in cancer cachexia [65, 77]. IL-6 is recognized as the main mediator of the hepatic acute phase response in humans and a driving force of systemic inflammation in cancer cachexia [39]. Moreover, it is known to inhibit synthesis and enhance catabolism of lipids and proteins in adipocytes and myocytes respectively [65].

## 1.4 Body composition and disease

Body composition refers to the distribution and amount of FFM and fat mass (i.e. adipose tissue) in the human body [43]. The FFM is everything that is not fat mass (Figure 2) [78] and consists of extracellular and intracellular water, bone mineral and total body protein, including visceral protein and skeletal muscle mass. Intracellular water and total body protein are components of the body cell mass (BCM). The BCM is the protein-rich compartment that is affected during catabolism. Loss of BCM (i.e. skeletal muscle mass) [78, 79], which occurs with ageing and diseases like cancer, chronic heart disease and pulmonary disease, has important implications for QoL, physical function and survival [76, 80].

***Figure 2: Body composition compartments***




***Schematic diagram of FFM, total body water, intracellular water, extracellular water and BCM. Modified from Kyle et al.*** **[78]**.

### 1.4.1 Skeletal muscle mass and adipose tissue

Skeletal muscle activity influences metabolism by producing and consuming energy [81]. Skeletal muscle mass is controlled by the balance between protein synthesis and proteolysis. Protein synthesis is mainly regulated by insulin, insulin-like growth factor-1 (IGF-1) and activity, while proteolysis is regulated by inflammation and inactivity [82]. Skeletal muscle also act as an endocrine organ by producing and releasing inflammatory markers, termed myokines, which exerts local effects on signaling pathways involved in muscle metabolism and endocrine effects on other organs [65, 83]. Myokine IL-6 is normally released by contracting skeletal muscle cells, and is important for muscle metabolism during exercise [83]. It mediates the anti-inflammatory properties of exercise by modulating TNF-α levels and stimulating IL-10 production. Myokine IL-6 does not activate the NF-κB signaling pathway but other pathways promoting insulin sensitivity [83]. In contrast to this, elevated levels of serum IL-6 synthesized by adipocytes and immune cells are associated with insulin resistance [84].

The adipose tissue is an important metabolic organ, consisting of white adipose tissue (WAT), dominated by white adipocytes and brown adipose tissue (BAT), dominated by brown adipocytes [85]. There are two representative types of WAT: Visceral adipose tissue, which is mostly localized in the abdominal cavity [86], and subcutaneous adipose tissue, which lies immediately under the skin and in the intramuscular fat. Subcutaneous adipose tissue is largely found in the lower trunk (e.g. hips) [85]. The abdominal cavity is drained by the portal vein [7], and thus visceral obesity may cause a release of non-esterified fatty acids directly into the portal vein and to the liver, causing metabolic alterations and insulin resistance [84].

In addition to adipocytes, the WAT also consists of inflammatory cells. In lean subjects, these inflammatory cells are involved in maintaining a normal response to insulin [71, 87]. As obesity develops, these adipocytes start to secrete chemokines that attract monocytes into the adipose tissue to develop into macrophages that cause low grade chronic inflammation as described above [88]. The main function of white adipocytes is to store energy (as triacylglycerol) in large cytoplasmic lipid droplets. Most adipose tissue in humans is WAT, and this is also the main site of energy storage in the body [85, 88]. Brown adipocytes also store triacylglycerol, but in contrast to white adipocytes they have a high number of mitochondria and many small cytoplasmic droplets [85]. The main function is to regulate thermogenesis by transferring energy from food into heat with its characteristic protein,

uncoupling protein (UCP)-1 [85]. In animal models of cancer cachexia, chronic inflammation (i.e. cytokines and myokines) has shown to cause an increase of UPC-1 expression and a following switch from WAT to BAT, called WAT browning [65, 83, 89]. WAT browning takes place in the initial stages of cachexia, before skeletal muscle atrophy and may be responsible for an increase in energy expenditure and loss of adipose tissue [89, 90].

### 1.4.2 Adipose tissue as an endocrine organ

The white adipocytes secrete hormones, termed adipokines that regulate food intake (i.e. leptin) and modulate immunity and inflammation [91]. They also effect various metabolic pathways such as growth hormone signaling, insulin sensitivity and lipogenesis [76].

The hormones may act locally and/or at a systemic level and provide an important link between obesity, insulin resistance, and related disorders [86], as they modulate energy metabolism, influence insulin sensitivity [92], and affect the innate immune system by either suppressing or activating cytokine production [86].

**Leptin** is a product of the obese gene secreted by adipocytes in proportion to fat mass. It has been recognized to play a major role in long-term regulation of body mass by affecting the balance between two pathways that control energy expenditure and food intake within the hypothalamus; the orexigenic pathway that stimulates energy intake, and the anorexigenic pathway that inhibits energy intake [18, 22]. Leptin crosses the blood-brain barrier in a process that is highly regulated; low leptin levels are compensated by an increase in appetite and a decrease in energy expenditure, and high leptin levels are followed by reduced appetite and stimulation of fat oxidation and increased energy expenditure [76].

Leptin has been shown to upregulate the production of inflammatory cytokines [86]. Leptin binding proteins e.g. CRP can bind leptin in blood, and limit leptin receptor binding and transport across the blood-brain-barrier [76]. In cases of increased body fat, this may cause a desensitizing of leptin, a condition known as leptin resistance [76].

**Resistin** is another adipokine known to be positively correlated with BMI, but although BMI is a significant predictor of insulin resistance, resistin is not [93]. Many aspects of the role of resistin on insulin resistance in humans are unclear, however it is recognized to inhibit insulin action and increase insulin resistance [86]. Moreover, resistin may play a role in cell

proliferation and differentiation, and chronic inflammatory reactions associated with overweight/obesity [86], by up regulating cytokine production via the NF-κB pathway [65].

**Adiponectin** on the other hand is inversely associated with overweight and abdominal obesity. This hormone is inversely associated with fat mass and hence high levels are found in lean individuals [93]. Adiponectin may protect against insulin resistance and atherosclerosis by decreasing blood glucose concentrations, and by limiting circulating fatty acids and triglycerides in muscles and liver [93].

## 1.5 Motivation for this thesis

It is generally agreed that cytokines induce and are the driving forces behind cancer cachexia, and that muscle mass is reduced during the cancer cachexia trajectory. The syndrome is thought to be present in the majority of patients with pancreatic cancer, but little is known about how cytokines and the specific secretion of adipokines from adipose tissue are associated with body composition and how this changes during the disease trajectory. Tumors, as well as dysfunctional adipose tissue and muscle tissue, probably cause an environment that enhances the production of cytokines, adipokines and myokines. However, for most of the inflammatory markers, there are no established reference values, but obesity evidently leads to a chronic low-grade inflammation and causes an increase in pro-inflammatory cytokines followed by alterations in energy metabolism.

The consensus definitions of cachexia recognize muscle loss as a hallmark and include this as one of the classification criteria. However, the suggested set of criteria is not well validated in clinical studies and their consistency in defining cachectic and non-cachectic cancer patients is rarely investigated. The fact that we lack a universally accepted and validated cachexia definition may have therapeutic consequences, as the condition is not recognized at an early stage, and because optimal interventions to treat or postpone cachexia are not implemented.

The main motivation for this study was to study the relationship between inflammatory markers and body composition in healthy individuals within different BMI categories. We also wanted to study cancer cachexia and the role of inflammation in a cohort of patients with pancreatic cancer. Finally, we wanted to study the consistency of two definitions of cancer cachexia in the same patient group and their ability to predict survival.

# 2 Aim

The overall aim of this thesis was to gain more knowledge about cancer cachexia and to investigate the relationship between body composition and inflammation in two different samples; healthy individuals and patients with pancreatic cancer. To address this aim we specifically asked:

I What is the relationship between fat- and lean body mass and inflammatory markers and adipokines in healthy individuals, both overweight and lean? (paper I)

II How do inflammation and fat- and muscle mass develop from the time of diagnosis towards death in pancreatic cancer patients? How is the inflammatory profile in non-cachectic and cachectic patients, defined according to the 2011 consensus definition? (paper II)

III Is there a consistency between two established definitions of cancer cachexia in the classification of cachectic patients and in their ability to predict survival? (paper III)

# 3 Materials and methods

This thesis consists of two separate studies, one in healthy individuals and one in patients with pancreatic cancer (Table 3).

**Table 3: Designs of the studies**

| | **Study I** | **Study II** | |
|---|---|---|---|
| **Design** | Cross-sectional study | Prospective study | |
| **Paper** | **I** | **II** | **III** |
| **Participants** | 47 healthy overweight (BMI 26-49 kg/m² ) and 40 healthy lean individuals (BMI<25 kg/m²) | 20 pancreatic cancer patients | 45 pancreatic cancer patients |
| **Methods** | Anthropometry<br><br>Bioelectrical impedance<br><br>Venous blood samples | Anthropometry<br><br>Bioelectrical impedance<br><br>Venous blood samples<br>Hand grip strength<br><br>24 hour recall | Computed tomography imaging<br><br>CRP from routine blood testing<br><br>Height from medical records<br><br>Self- reported weight from questionnaires from a hospital database |
| **Location** | Oslo and Akershus University College of Applied Sciences | Oslo University Hospital, Ullevål | Oslo University Hospital, Ullevål |

## 3.1 Participants

The reference data collected in study I was collected at a single time point in a cross-sectional study conducted at Oslo and Akershus University College of Applied Sciences. The overweight individuals were subjects available for baseline analysis in a contemporary intervention trial performed in 2009 [94]. They were approached through mass media and selected in accordance with certain inclusion criteria. In 2010, a reference group of lean subjects was recruited in the same way as the overweight individuals. The study aimed to

investigate associations between inflammation markers, adipokines and fat mass in a healthy overweight or lean population (study I).

In October 2008, Oslo University Hospital (OUH) established a multidisciplinary research program on pancreatic tumors including all patients who were admitted to the Department of surgery, gastric medicine or oncology with suspected or documented solid or cystic pancreatic or periampullary neoplasms (study II). Standard evaluation included medical history, physical examination, routine laboratory tests, tumor markers, and contrast-enhanced helical CT of the abdomen and thorax in three vascular phases [95, 96]. The aim was to increase the knowledge about pancreatic tumors and the consequences on patient reported outcomes and to improve the treatment and follow-up of the patients. After written consent, clinical data, diagnostic and treatment related-issues, results from blood tests, tumor tissue and cyst fluid and patients' self-reported symptoms were collected prospectively once a month until death.

Data from study II formed the basis for papers II and III. Paper II stems from a prospective study of inoperable pancreatic cancer patients who were referred to the Palliative Care Unit at OUH-Ullevål between March 2010 and January 2012. Patients were first included in the pancreatic project, then asked to participate in study II. Body composition, inflammatory markers and food intake were registered once a month from the time of diagnosis until death, and associations with inflammatory markers were examined. Paper III is based on a prospective study of pancreatic cancer patients, referred between January 2008 and December 2011. Some of the patients from study II were included in paper III. In addition, data were retrieved from patient records and the database of the pancreatic project. The aim was to explore consistencies between two definitions of cancer cachexia and their association with survival.

## 3.2 Methods

### 3.2.1 Measurements of body composition

#### Anthropometry

Changes in body composition may show the integrated effect of a period of metabolic imbalance [79]. Anthropometric measurements are measurements of the size, weight and proportions of the human body, and are useful tools for localization and determination of the amount of fat and muscle mass. Anthropometry can be used as a quick assessment of nutritional status and to identify undernutrition, overweight and obesity [97].

Classical anthropometric measurements include weight, height, waist circumference, hip circumference, triceps skin fold (TSF) thickness, mid-upper-arm circumference (MUAC) and hand grip strength. Weight is measured by a weight scale and height is measured by a wall-mounted stadiometer. BMI is calculated as weight (kg)/height squared ($m^2$). Waist and hip circumferences are measured with a standard non-stretch tape. Waist circumference and the waist-hip circumference ratio (waist circumference divided by hip circumference) (WHR) are useful indicators of abdominal obesity (i.e. visceral and subcutaneous fat), and hip circumference may reflect the amount of subcutaneous fat in the lower trunk (i.e. hip and buttocks) [98, 99]. Waist circumference has been more strongly associated with total body fat measured with DEXA than WHR, and it has been more closely related to metabolic disturbances associated with abdominal obesity [100].

Skin fold thickness at the triceps muscle i.e. TSF, is measured by a Harpenden caliper and indicates the amount of subcutaneous fat. The mid-upper-arm circumference (MUAC) can be measured with a standard non-stretch tape. The MUAMC can be calculated from these measurements with the equation: MUAC – ($\pi$ x (TSF/10)) = MUAMC ($cm^2$). The combination of the measurements provides a simple estimate of subcutaneous fat mass and muscle mass, respectively [100]. Hand grip strength assesses upper extremity muscle strength and is measured using a dynamometer, which can test hand grip strength up to 90 kg. In community studies of older adults poor hand grip strength has been related to poor nutritional status defined as low BMI [100].

### Body composition analysis: bioelectrical impedance analysis and Computer Tomography

Impedance and imaging can be used to provide accurate estimates of body composition, as the methods permits separation of skeletal muscle and adipose tissue.

The use of bioelectrical impedance analysis (BIA) is widespread both in healthy subjects and patients [78] and is widely used in clinical research for analyses of nutritional status (16). BIA allows the determination of the FFM and TBW in subjects without significant fluid and electrolyte abnormalities [78]. BIA is practical, safe, cost-effective, portable, quick and convenient for the patient, and easy to use [43, 101]. The method relies on population-specific regression equations, is shown as a good tool in large-scale epidemiologic studies [102]. In papers I and II, weight and body composition were measured with a Tanita scale (BC-418 MA, Tanita Corp., Tokyo, Japan), operating at 50 kHz.

BIA involves attaching electrodes to the hands and feet of a person and sending a small, and clinically negligible, electrical signal through the body [98, 100]. The method measures resistance from the electrical signal based on the fact that FFM has a higher water- and electrolyte content than fatty tissue and therefore has a higher electrical conductivity and lower impedance [43, 100]. BIA measures TBW, which is then used to estimate FFM and fat mass. Standardized procedures must be used to obtain BIA measurements. Hydration status, recent physical activity and consumption of food or beverages, are among the factors that can affect the validity and the precision of the measurements [78, 100]. However, BIA has shown limited applicability in those with BMI >34 kg/m² as it may overestimate FFM and underestimate fat mass [43].

Computer Tomography (CT) is based on the relationship between the degree of attenuation of an X-ray beam and the density of the tissue through which the beam has passed. From this relationship, a two dimensional radiographic image of the underlying anatomy of the scan area can be constructed [103]. CT scans used for analysis in paper III were performed for initial cancer staging and routine diagnostic purposes. Patients who had an abdominal CT scan taken within 30 days before or 10 days after diagnosis were selected for the study. One CT image, at the level of the third lumbar vertebra (L3) was assessed, and was the image in which both transverse processes were first clearly visible [104]. The muscles in the area of the L3 encompass psoas, erector spinae and quadratus lumborum, as well as transversus abdominus, external and internal oblique abdominals, and rectus abdominus [104]. The scans

were analyzed with the software program Slice-O-Matic V4.3 (Tomovision, Montreal, Canada) which enables specific tissue demarcation using Hounsfield unit (HU) thresholds that are based on a linear scale using water as reference (0 HU). Skeletal muscle was quantified by HU thresholds of -29 to +150 [105]. The L3 region is chosen as a landmark for CT images because the skeletal muscle area 5 cm above the L4-L5 has been shown to correlate strongly with total body skeletal muscle volume in a healthy population [32]. Muscle area was normalized for height in meters squared ($m^2$), and reported as muscle index ($cm^2/m^2$). The same was done for fat mass, which was reported as fat mass index. Indices of FFM and fat mass ($kg/m^2$) were calculated by using regression equations developed by Mourtzakis et al [106]. All CT images were identified and analyzed by the same single trained observer. Cut-offs in the consensus definition of cancer cachexia are gender specific and set at 55 $cm^2/m^2$ for men and 39 $cm^2/m^2$ for women [44].

### 3.2.2 Preparation of blood samples

Blood samples from healthy lean and overweight participants were prepared using identical procedures (paper I). Venous blood samples were collected after an overnight fast (> 12 hours) between 8 am and 10 am by authorized health personnel. Serum from the overweight and lean was obtained from silica gel tubes, kept on ice and centrifuged (1500 *g* for 12 min), aliquoted and stored at -80 ºC until further analyses, or kept in room temperature for at least 30 min, until centrifugation and immediately prepared for subsequent analyses. Plasma was obtained in EDTA tubes, kept on ice, centrifuged (2000 *g*, 4ºC for 10 min), within 15 min. The same procedures were applied for blood samples from the pancreatic cancer patients (paper II), except that patients were not asked to fast, serum was stored at room temperature for at least 45 min until centrifugation, and plasma samples were centrifuged (2500 *g*, for 15 min), within 10 min. All blood samples from these patients were stored at -80 until further analysis.

Serum levels of the adipokines resistin (paper I), leptin and adiponectin (papers I and II) and the cytokines IL-10, INFγ and TNF-α (paper II), and plasma levels of IL-6 and IGF-1 (paper I and II) were measured by enzyme immunoassays from R&D Systems (Minneapolis, USA) according the manufacturer's instructions. All analyses were performed in duplicates. The coefficients of variation for intra-assay and inter-assay variability were <5% and <10%, respectively, for all analyses. Standard blood chemistry (including CRP) and lipid parameters

of healthy individuals were measured in serum or in EDTA plasma at Fürst Medical Laboratory (Oslo, Norway) using routine methods (paper I). Results from standard blood chemistry and CRP of pancreatic patients were retrieved from the medical records (papers II and III).

### 3.2.3 Evaluation of food intake

#### Subjective global assessment

The Subjective Global Assessment (SGA) [107, 108] is a validated questionnaire that measures nutritional status based on the features of medical history; e.g. weight changes and dietary intake, and physical examination; e.g. loss of subcutaneous fat and muscle wasting (Appendix 1). The SGA has been found to be highly predictive of nutrition-associated complications in different patient groups including cancer patients [109]. In paper III one item from the SGA was used. (i.e. the question; "as compared to normal, I would rate my food intake during the past month as either unchanged, more than usual or less than usual). Food intake reported as less than usual in the SGA was considered as an energy intake less than 1500 kcal/day according to previous studies [107, 110].

#### 24-hour recall

The 24-hour recall method was used in paper II for registration of dietary intake. The interview was conducted face-to-face and the patients were asked to remember and report all the foods and beverages consumed in the preceding 24 hours. The quantities eaten were estimated by the patient and described in household measures as the number of units consumed (e.g. cups, glasses, spoons, number of slices, pieces, decilitres). A photographic booklet with portion sizes was used. Tables of food portion sizes were used to translate household measures to weights [111]. Food intake estimated as $\leq$20 kcal/kg per day was classified as anorexia [26, 112].

## 3.3 Statistics

The PASW 18 (SPSS Inc., Chicago, II, USA) (Papers I and III) and IBM SPSS statistics v 21 (Armonk, NY, USA) (Paper II) were used for all statistical analyses. In paper III the software program GraphPad Prism (version 6, La Jolla, CA, USA) was used. Sample size calculations were not performed in any of the studies partly because of the descriptive study designs and partly because of lack of adequate background information to perform such calculations. Statistical significance was accepted for *P*-values <0.05.

Standard descriptive statistics (percentage, mean, median SD/range etc.) were used in all studies.

Comparison of groups in paper I was tested for significant difference with the independent samples t-test when comparing overweight and lean participants, and ANOVA was performed when comparing tertiles of body composition and inflammatory markers. In paper II the Mann Whitney U test was used for comparison of cytokines and adipokines at study entry vs ≤3 months before death, and the Wilcoxon signed rank test was applied when comparing cachectic vs non-cachectic participants. In paper III comparisons between the two classifications of cancer cachexia was performed by a 2x2 contingency table and McNemar's test. Fisher's exact test was used to compare mortality within three months from diagnosis for the two cachexia classifications. The significance level was set at P <0.05.

Univariate linear regression analyses were applied to quantify the relationship between BIA and anthropometric measurements of body fat. A stepwise reduction model procedure was conducted, where the F-ratio test was used. The variables age and sex were adjusted for to avoid confounding effects (paper I).

Survival was defined as time from the date of a histologically verified diagnosis to the date of death (papers II and III), and was analyzed using Kaplan-Meier curves and the log rank test (paper III).

## 3.4 Ethical considerations

The studies in this thesis were approved by the Regional Committee of Medical Health Research Ethics South East Norway and by the Norwegian Social Science Data Services, and performed in accordance with the Declaration of Helsinki [113]. All study subjects gave written informed consent prior to participation.

In all studies, and particularly so in studies with patients with advanced stage cancer and short life expectancy, patient burden must be minimized. It is, however, also considered important that research is conducted in these groups. Thus, it is important to optimize symptom assessment and reduce respondent burden in frail patients. To comply with this, extra blood samples for specific biomarkers (interleukins etc.) were drawn at the same time as routine blood samples if possible. The CT scans used for assessment of sarcopenia, were the same as those taken for initial diagnosing and staging, and the pancreatic cancer patients were not asked to fast. Anthropometric measurements and the 24-hour food recall interviews were performed at the same day as other appointments at the OUH to avoid extra traveling.

# 4 Summary of results

### Paper I: Measurements of body fat are associated with markers of inflammation, insulin resistance and lipid levels in both overweight and in lean, healthy subjects

A high percentage of body fat in overweight subjects is associated with enhanced levels of adipokines, cytokines and risk markers for lifestyle-related disorders. The aim of this cross-sectional study was to investigate associations between body composition and inflammatory markers among healthy individuals in different BMI categories. Anthropometry (waist- and hip circumference, WHR and TSF) and fat mass (kg and %), measured by BIA, were correlated with inflammatory markers (i.e. IL-6, CRP, IGF-1) and adipokines (i.e. leptin, adiponectin, resistin) in 47 healthy overweight adults (BMI 26-49 kg/m²) and 40 lean (BMI 17-25 kg/m²) adults, matched for age and sex. The main findings were that hip circumference was significantly associated with BIA-assessed fat mass (%) in both lean and overweight individuals. An increase in hip circumference was associated with higher plasma levels of leptin and CRP in both groups. Interestingly, CRP increased with increasing BMI, also in the lean group.

To conclude, an increase in fat percentage was associated with increased levels of inflammatory markers (i.e. CRP) and adipokines (i.e. leptin) in overweight persons, but also among persons within the normal range of BMI. Hip circumference stood out as a surrogate measure for fat mass (%) in subjects within different BMI categories, and may be useful for identification of fat percentage in both overweigh and lean individuals. The results may indicate that adherence to a healthy lifestyle to prevent a high percentage of body fat is important for both lean and overweight people in order to limit low-grade inflammation in the body, and thus reduce the risk for overweight-related chronic diseases.

### Paper II: Alterations in inflammatory biomarkers, body composition and food intake in pancreatic cancer patients with cachexia: a prospective study

Chronic inflammation is proposed as an underlying biological mechanism for development of cancer cachexia, but the levels of inflammatory markers and associations to cancer cachexia have not been established. The aim of the study was to study changes in the levels of inflammatory markers, FFM, fat mass and energy intake in an unselected cohort of pancreatic cancer patients with or without cachexia, as they approached the terminal stage of their disease.

As there are no reference values for inflammatory markers, biomarkers and measures of FFM and fat mass from healthy lean individuals were used as reference material. Twenty patients with newly diagnosed inoperable pancreatic cancer were included after being included in the pancreatic project. Cachexia was classified according to the consensus definition of cancer cachexia and sarcopenia was assessed as MUAC <18 cm² (female), <32 cm² (men). FFM, fat mass, cytokines, CRP and adipokines, and food intake were measured prospectively from inclusion and up to a maximum one year. It was shown that at inclusion, eleven (55%) patients were classified as cachectic, and there were no significant differences in CRP, cytokines and adipokines, FFM, fat mass and energy intake between the cachectic and non-cachectic patients. During the disease trajectory, FFM and fat mass decreased slightly. Most inflammatory markers increased, however, INF-γ remained stable, while leptin and IGF-1 was reduced. All changes were insignificant except for the elevation of IL-10 levels.

To conclude, patients with advanced pancreatic cancer experience an ongoing inflammation, and at the same time they lose fat mass and FFM. However, patients classified as cachectic immediately after diagnosis did not have higher levels of inflammatory markers than patients classified as non-cachectic. Inflammation in the cancer patients may be caused by the presence of the tumor and may eventually cause symptoms similar to cachexia. This makes it difficult to distinguish between symptoms caused by the cancer and symptoms caused by cachexia. Moreover, increased inflammation may stimulate fat and muscle mass depletion. For this reason, an optimization of cancer treatment is essential in pancreatic cancer patients.

### Paper III: Comparing two classifications of cancer cachexia and their association with survival in patients with unresected pancreatic cancer

Cancer cachexia is characterized by reduced weight and muscle mass, poor treatment tolerance and short survival. The two most common classifications of the condition have been the 3-factor classification requiring presence of two or more of the following three factors; weight loss ≥10%, food intake ≤1500 kcal/d, and CRP ≥10 mg/l, and the consensus classification requiring either weight loss >5% the past 6 months, or BMI <20 kg/m² or sarcopenia, together with ongoing weight loss >2%. Thus, one definition includes inflammation as a criterion, while the other definition includes reduced muscle mass as one of the criteria. The aim of the study was to examine the consistency between the two classifications in defining patients as cachectic or not, and to investigate the association with survival in a palliative cohort of unresected pancreatic cancer patients. Forty-five patients were included. Sarcopenia was assessed by lumbar skeletal muscle index determined by CT-imaging (men <55 cm²/m² and women <39 cm²/m²). Values for height, weight, CRP and survival were extracted from the pancreas data base and the patients' medical records. Food intake was assessed by SGA (anorexia was determined by self-reported food intake less than usual). It was found that the agreement for cachexia and non-cachexia was 78% across the two classifications. Survival was significantly shorter in cachectic patients compared to non-cachectic patients according to both classifications. However, the difference in survival between cachectic and non-cachectic patients according to the consensus classification was significant only after dividing the non-cachectic group into a pre-cachectic and a non-cachectic group (i.e. separating the patients with high CRP and low food intake from the non-cachectic group).

To conclude, the two classifications showed good overall agreement in defining cachectic patients in this cohort with pancreatic cancer, and cachexia was associated with poorer survival according to both. Our findings suggest that the classification including systemic inflammation (i.e. CRP >10 mg/l) as a criterion was a better predictor of survival. Patients with weight loss, reduced energy-intake, sarcopenia and indications of metabolic change (i.e. CRP >8 mg/l) had the poorest survival. Thus, clinical interventions should be directed to optimize these known risk factors.

# 5 Discussion

In this thesis, the association between inflammation and body composition was investigated in a healthy overweight and lean population and in a cohort of pancreatic cancer patients with or without cachexia.

## 5.1 Methodological considerations

### 5.1.1 Study designs

In the field of preventive medicine and palliative care, observational studies are important to e.g. generate hypotheses or build evidence for identifying best hospital practices. The method involves the direct observation of individuals in their natural setting, thus "exposures" or interventions on subjects are determined by individual preferences, hospital practice patterns or policy decisions [114, 115].

#### Cross-sectional studies

A cross-sectional study is an observational study in which exposure and outcome are determined simultaneously for each subject, often described as taking a snapshot of a group of individuals. The primary intention with this study design is screening, hypothesis generating and to estimate prevalence [115]. The study design has been used to understand the prevalence of various conditions or associations of different characteristics: e.g. visceral obesity and life style related disorders [115, 116]. However, one of the problems with cross-sectional studies is that because the exposure and outcome are measured simultaneously, there is no evidence that the exposure (e.g. fat mass) causes the outcome (e.g. CRP: paper I). Thus, causal inferences cannot be drawn. However, if the exposure is a characteristic such as gender and the outcome has developed over time the temporal association between the exposure (e.g. male) and outcome (e.g. FFM: paper I) is more convincing. Otherwise, if the exposure is not an inherited trait, causality is unclear [115, 117]. In addition, cross-sectional studies provide only an instant picture of a situation and the result may be different at another point in time. The marker for systemic inflammation, CRP may for example fluctuate and can be elevated for reasons other than elevated fat mass or cachexia [36]. Another problem with cross-sectional studies is that it evaluates prevalence rather than incidence of an outcome, and thus

excludes people who develop the outcome (e.g. cardiovascular disease) but die before study entry. Thus, there is a bias toward including people with better survivorship in the study, for example metabolically healthy obese individuals, which may cause a healthy adherer effect [118]. Moreover, it is important to rule out alternative explanations for the study results (e.g. exercise or diet) [115]. Nevertheless, cross-sectional studies can provide new information concerning associations between fat mass and lean mass and inflammatory or clinical markers, which we aimed for in study I.

Cross-sectional studies are the most frequently applied study design in palliative care research, supplying much of our knowledge about cancer cachexia. However, cross-sectional studies should preferably be followed by longitudinal studies in order to confirm hypotheses generated in the former [117].

### Cohort studies

In a cohort study, a study population is selected, then it is determined who are exposed or not exposed to a factor and then the subsequent development is evaluated over time. The studies can be either retrospective or prospective [115] and study II (paper II and III) has a prospective design. In paper II, patients with advanced pancreatic cancer were selected (i.e. exposed), and compared to a reference population (i.e. not exposed). Energy intake, body composition and inflammatory markers were observed from study entry and close to death. In this paper, we also investigated levels of inflammatory markers in patients defined as cachectic (i.e. exposed) vs those defined as non- cachectic (i.e. not exposed). In paper III, the same study population was observed. Information was obtained to determine who were cachectic (i.e. exposed) and who were not cachectic (i.e. not exposed), and the prevalence of the condition was associated with survival in the cachectic vs. the non-cachectic patients [115]. The problem that may arise when classifying patients as cachectic or non-cachectic according to the consensus classification, is that some of the patients who are classified as cachectic at study entry respond to chemotherapy or other treatment and thus gain weight, increase FFM or skeletal muscle mass. These patients may be classified as non-cachectic during the disease course. Other patients may be classified as non-cachectic at study entry but lose weight, FFM or skeletal muscle mass during the disease course and may be defined as cachectic later in the disease trajectory. Appetite and CRP may also change during this time interval. It has been shown that cachexia has a significant impact in terms of morbidity and

mortality [119]. However, since the prevalence and incidence of cachexia may vary from diagnosis until the time of death, it is challenging to determine the impact of cachexia on morbidity and mortality in patients who are receiving anti-cancer treatment. The incidence of the condition is normally assessed at diagnosis or study entry; however, patients should be monitored several times during a follow up.

There are three categories of systematic errors related to observational studies: selection bias, information bias and confounding. Selection bias may affect the extent to which one can generalize from people who were included in the study to people in general (i.e. external validity), and information bias and confounding cannot rule out the possibility that external variables may have influenced the results (i.e. internal validity) [120, 121].

### 5.1.2 Study samples, strengths and weaknesses

Participants in paper I were selected through advertisements in newspapers and mass media in the surroundings of Oslo and Akershus in the eastern part of Norway. The strength of this approach is that this was an efficient way to recruit and evaluate a large sample of individuals in a short time period. The use of specific inclusion and exclusion criteria when recruiting the participants, may limit the possibility of generalizing the results to a broader population. The reason for this is that the participants in paper I did not necessarily represent the general population between 30 and 70 years in this area. People who volunteer to participate in health surveys tend to be more health conscious and better educated than the rest of the population, thereby introducing a selection bias in the direction of a healthy adherer effect [118]. This may reduce the external validity of the results. The healthy volunteers in paper I were collected in order to investigate the relationship between inflammation and body composition in individuals with different BMI in a healthy population, but also to serve as a reference population in paper II. However, matching by age and sex was not possible as the healthy volunteers were younger than the pancreatic cancer patients with a median age of 53 and 48 years in the healthy men and women respectively, relative to 67.5 years among the pancreatic cancer patients. There was also an uneven sex distribution in the two groups, i.e. the reference group included 35% women while there were 25% females in the pancreatic cancer group. Notably, some of the variables reported in paper II, such as adipokines can be effected by age; leptin may be reduced and adiponectin elevated in elderly people [122]. Therefore, the observed differences in adipokines may not only be because of the cancer.

In paper II, inoperable pancreatic cancer patients were recruited from an out-patient palliative unit. The strength of this approach is that one can study disease progression and the natural development of the outcomes [115]. However, there are several issues to consider when evaluating these studies. During the recruitment period, only 20 patients were included in the study, thus we have a small sample size in paper II. Patients were not included or not willing to participate for several reasons; i.e. some were too tired to participate or died before inclusion, while others were treated at their local hospitals and they were not followed at OUH. A small number was included in the study but dropped out during the follow-up. In longitudinal studies, missing data is inevitable, and in the palliative care setting missing data is often attributed to worse health status [123, 124]. This means that the data is not missing at random, and may thus introduce a bias: a healthy adherer affect [115, 118]. A third source of missing data is that patients do not answer all the questions in a questionnaire, for example "pre-illness weight" (paper II). Unless this applies to specifically sensitive questions, occasionally missing data is most often at random, and is of lesser importance regarding generalizability. However, as in this case, the missing data can result in biased estimates and loss of statistical power due the small sample size. The best way to deal with this statistically is to impute the available longitudinal data of that person [124]. This procedure was done in paper II, with the first observation carried backward method.

In paper III only patients with CT scans taken 30 days prior to and 10 days after diagnosis were included, and for that reason a large number of patients were excluded in this study. In spite of that, the mean survival after diagnosis of the excluded and included patients was similar, 39 and 37 weeks respectively. This means that a systematic difference and a selection bias between the included and excluded group is unlikely, and that the sample is representative for this particular population. Although the statistical power is reduced due to the small sample size, the strength of the study is that the data set is complete, with no missing values. This is rarely achieved in palliative care research [123].

### 5.1.3 Validity of methods

#### Food intake, SGA and 24-hour recall

The question from the SGA that evaluates nutritional intake is; "as compared to normal, I would rate my food intake during the past month as either unchanged, more than usual or less than usual". The answer to this question has not been fully validated against prospective records of energy intake. A study of 22 patients however, found that self-reported reduced food intake from the Patient-Generated SGA (i.e. PG-SGA) was associated with reduced calorie intake Furthermore, when a patient claimed to eat less than usual, energy intake was evaluated to be <1500 kcal/d [110]. In paper III we therefore evaluated the answer from SGA of energy intake "less than usual" to be <1500 kcal/day; and used this value to classify anorexia in paper III. This estimation is in accordance with Fearon et al. [44], who suggests that patient's own estimate of their overall food intake in relation to their normal food intake could be used as an assessment of a patients food intake [44]. However, although a patient reports to eat less or more than usual, this may not correspond to actual energy intake, due to an underestimation or an overestimation of own food intake, respectively.

The 24-hour interview is often structured, using specific probes, which is a tecqnick that helps the respondent in remembering all the foods and drinks consumed in the preceding day [125]. In paper II probing was especially useful in collecting necessary details, such as how foods were prepared (e.g. butter on toast), and foods not originally reported (e.g. snacks or beverages). However, the patients may not have reported their food intake accurately due to lack of knowledge, memory or the specific interview situation. The patients may also have underestimated or overestimated their own food intake.

#### Methods for measurement of body composition in healthy individuals and in cancer patients

The BIA is commonly used in clinical practice where quick evaluations of nutritional status and measurements of FFM and fat mass are needed [126]. The reliability of BIA depends on the use of body composition prediction equations that are adapted to the study subjects and based on the inclusion of various parameters (i.e. weight, height, sex, age, race) [127]. However, the equations are validated in heathy populations and may as such be influenced by disease [126]. This also means that body composition measurements may be imprecise in the

presence of abnormal distribution of body compartments (e.g. ascites, dialysis, lipodystrophy) or in persons with extreme weights (e.g. cachexia, severe obesity) [127]. In this thesis, BIA was used on overweight individuals (paper I) and cancer patients (paper II). In order to standardize the measurements, all participants in paper I were asked to refrain from alcohol use and heavy exercise 24 hours before measurements, and all measurements were performed after a minimum of 12 hours fasting and after urination. However, these standardizations were not requested in the cancer patients due to the severity of the disease. This difference may have influenced the results as some patients may had just eaten while others had not, which may have resulted in increased FFM estimates from the BIA. Nevertheless, BIA is often used in clinical settings and for research purposes and is feasible on a population level [90, 102]. The method was therefor considered a suitable method for the purpose of our study.

Magnetic resonance imaging (MRI), DEXA and CT scans are commonly used to measure muscle mass in cancer patients [128]. In cancer patients, CT scans are used for initial diagnosis and to monitor disease progression [23]. These scans are often stored and accessible in a digital format which makes it possible to evaluate the patient's body composition during disease progression. In our cohort (paper III), we experienced that although CT scans were repeatedly conducted, the time span between CT scans were not the same for all patients. Many patients were therefore excluded due to the lack of CT scans in the time span 30 days before or 10 after diagnosis. In several studies, CT scans completed within 30 days of the patients' initial visits are considered to accurately measure muscle mass at presentation [129]. Sarcopenia has been associated with functional impairment, disability and reduced survival [130, 131], and is classified as reduced quantity of skeletal muscle mass or more than two standard deviations below the muscle mass typical of healthy adults [132]. In paper III, sarcopenia was assessed by CT images, but in paper II CT scans were not available from all participants within the requested time span. Hence, we had to assess sarcopenia by mid-upper-arm muscle area (MUAMA), as suggested in the latest consensus definition of cancer cachexia [44]. The consistency of CT images and MUAMA in their ability to assess sarcopenia, was not evaluated in this thesis.

#### Statistics and confounders

A confounder is a characteristic which is associated with the exposure in the population and with the outcome, and not in the causal pathway between the exposure and outcome [120]. A confounder may under- or overestimate the association that is investigated, and may even change the direction of the effect on the outcome measures. Confounders may be handled by matching, by restriction (i.e. exclusion criteria), or by adjustment in the statistical analysis. In paper I, specific inclusion and exclusion criteria were used. We also matched the overweight and lean individuals for age and sex to avoid the confounding effect by these two factors on variables, such as adipokines, as it is known that age and sex influence these markers [122]. Matching does not completely control for confounders, thus the variables were also adjusted for possible effects of confounders by logistic regression. We were not able to do a matching between the cancer group and the healthy reference population in paper II. Since no reference values for inflammation markers exist, the comparison of inflammatory markers in these two groups gives us an indication of the magnitude of elevation or reduction in inflammatory markers in the cancer group. However, we did not adjust for age and sex in these comparisons, thus these variables may act as confounding factors in paper II.

## 5.2 General discussion

### 5.2.1 Inflammation and body composition

#### Adipokines and fat mass in healthy individuals

Adipose tissue secretes adipokines (i.e. leptin and adiponectin) that play a role in overweight-related disorders [86]. In paper I, it was shown that overweight subjects had significantly higher levels of leptin, compared to the lean subjects, and that the levels of adiponectin were significantly lower in the overweight group compared to the lean group. The findings are consistent with previous studies [92]. It was also shown that leptin levels increased significantly across tertiles of fat percent in the overweight and the lean group, respectively, and that women had significantly higher levels of leptin than men. These results are as expected, as leptin is secreted by fat mass [86] and overweight individuals have significantly more body fat mass (kg and %) than lean individuals, and women have significantly more body fat mass (kg and %) than men. Furthermore, although there is an inverse relationship

between adiponectin and adiposity [133], adiponectin levels increased significantly from low to high fat percent in both overweight and lean individuals, and was higher in women than in men (paper I). These findings may be explained by the protective role of adiponectin and its attempt to maintain an anti-inflammatory profile in plasma although fat percent increased [133]. In lean, the fat percent measured by BIA was associated with hip circumference and TSF, suggesting that the fat is located subcutaneously in lean individuals. Furthermore, in the lean we found that an increase in hip circumference was significantly associated with higher levels of leptin. Since leptin is normally secreted by subcutaneous adipose tissue [122] these results were as expected.

### Inflammatory markers and fat mass in healthy individuals

Adipose tissue may be an underlying source of cytokines, causing CRP production in the liver [4, 5]. The overweight women in our study had the highest levels of whole body fat mass (kg and %), and also the highest circulating concentration of CRP. The concentration of CRP increased across all tertiles of BMI, and interestingly, it increased significantly in the lean group. This confirms findings from other studies of an association between fat mass and inflammation in lean individuals [134]. Leptin induces the production of for example reactive oxygen species [135], and it has been shown that lower-body fat mass (i.e. subcutaneous fat) plays an important role in systemic oxidative stress, which again may induce a dysregulation of the production of adipokines and inflammation, also in lean subjects [136]. This may explain the increasing levels of inflammation when going from low to high BMI.

### Inflammatory markers in relation to body composition in pancreatic cancer patients

Adipokines and cytokines regulate each other. The leptin receptor belongs to the class 1 cytokine receptor family and leptin itself has pro-inflammatory properties as it has structural similarities with IL-6 [76]. Moreover, leptin also stimulates the production of IL-6 and TNF-α [86]. The anti-inflammatory effect of adiponectin is by stimulating the production of IL-10 and inhibiting the production of TNF-α. However although IL-10 is considered anti-inflammatory, increased IL-10 is associated with weight loss and worsened prognosis in chronic disease [137].

Plasma leptin level is proportional to fat mass and has been related to loss of body fat loss [138]. In our study, leptin levels correlated significantly with fat mass in the healthy adults as well as fat mass index in pancreatic cancer patients (papers I and II). Furthermore, loss of fat mass was accompanied by a decline in leptin levels (paper II, table II).The concentrations of leptin and IL-6 are closely linked since leptin stimulated the production of IL-6 [86, 139], and thus, both leptin and IL-6 were positively correlated to fat mass (papers I and II) and negatively correlated to FFM index in healthy individuals. In contrary, this correlation was opposite in the cancer patients (paper II). In diseases with chronic inflammatory conditions, inflammation causes a reduction in leptin levels [91], which may be part of the reason for the reduced circulating concentration of leptin in pancreatic cancer patients (paper II). Thus, the decline in leptin levels may be caused by both reduced fat mass and increased inflammation. Furthermore, it has been shown that leptin levels decrease with age, in women [122]. Our patients had a median age of 67.5 years, while median age of the reference population was 50.5 years i.e. about 17 years younger, thus age may, at least in part, explain some of the changes in the levels of leptin and other adipokines in our study.

Whereas leptin levels were low and continued to decline throughout the observational period, the levels of adiponectin were higher among the patients than in the healthy individuals at study entry, and concentration of adiponectin continued to increase towards death. This is in accordance with results obtained from a case control study in patients with pancreatic cancer [140]. High levels of adiponectin in pancreatic cancer may be part of a compensatory response to insulin resistance or weight loss which often occurs during the course of the disease [140]. High levels of adiponectin has also been shown to promote pancreatic cancer cell growth in pancreatic cancer patients, however the association between circulating adiponectin and pancreatic cancer remain controversial and require further exploration [141].

### Tumor microenvironment and inflammation

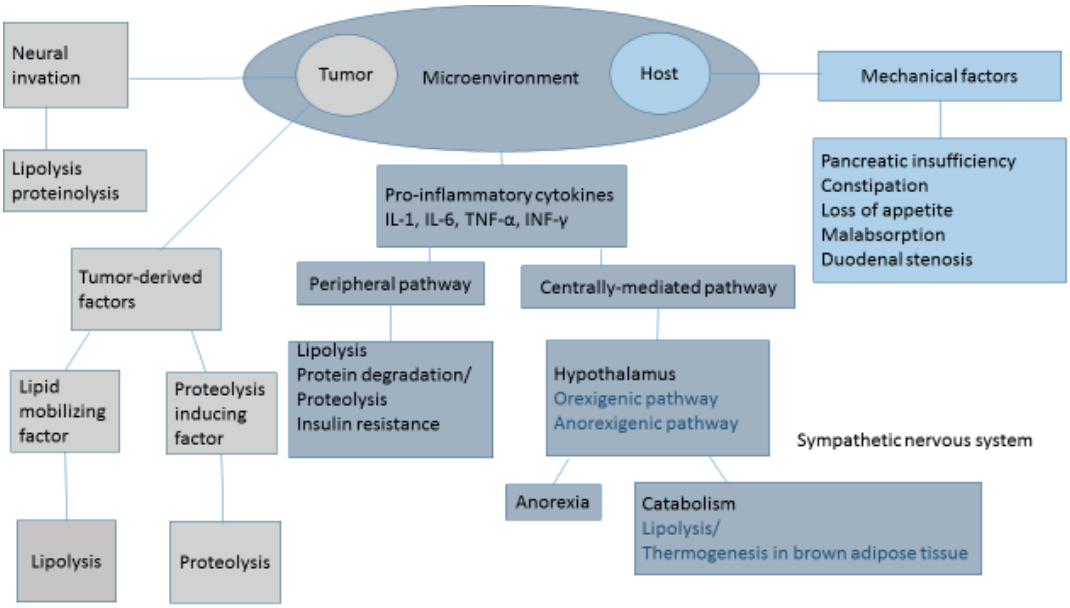
Tumors and the tumor microenvironment may also cause a dysregulation of the production of cytokines, which stimulate an activation of white blood cells and other immune cells, resulting in the release IL-6 and TNF-α [11, 142]. Previous studies have shown increased levels of IL-6 and TNF-α in cancer patients losing weight [34, 77]. This was confirmed in paper II, as the patient group had higher levels of circulating IL-6 and TNF-α than the reference population at study entry, and the levels increased during the disease course. In paper II, the levels of IL-6 and TNF-α correlated positively with FFM index in the cancer

group, but negatively in the healthy adults. Considering an elevated cytokine production in the patient group, this may explain correlations that were the opposite of that in healthy adults. In the presence of a tumor, the body reacts with a systemic inflammatory response in the brain, liver and skeletal muscle, which leads to symptoms like anorexia, reduced food intake, fatigue and pain as well as altered metabolism. These symptoms may again lead to weight loss, poor performance status and reduced survival. Interestingly, these symptoms are similar to symptoms of cachexia [35] and may arise from the tumor itself [142] Thus, it is essential to treat the underlying cause; the cancer. However, in patients with advanced pancreatic cancer, there is no cure, thus palliative treatment including palliative chemotherapy is important to improve QoL, relieve symptoms and prolong life [22].

Systemic inflammation driven by pro-inflammatory cytokines is assumed to play an important role in the development of cancer cachexia [37, 142]. The acute phase response is a sign of systemic inflammation and is clinically often measured by increased CRP and decreased albumin levels [34, 142]. Paper II shows an insignificant increase in CRP and a significant decrease in albumin levels, indicating an inflammatory response in the cancer patients [34, 77]. It has been suggested that there is a local cytokine production in peripheral blood mononuclear cells and in adipose tissue, especially SAT, which may have systemic effects on metabolism and weight loss in cancer patients [31, 143]. Plasma levels of inflammatory markers were not significantly different in the cachectic versus the non-cachectic patients, as defined with the consensus definition. One explanation may be that the magnitude of local inflammation is not detectable in plasma [31]. Moreover, in patients defined as non-cachectic because they have no weight loss or sarcopenia, local inflammation can be an underlying cause of FFM depletion due to protein degradation or proteolysis [22]. Thus, these patients may develop cachexia later on in the disease trajectory.

Taken together, inflammation in cancer patients may arise from the tumor and may cause changes in the body composition of the patients. This notion is in line with previous assumptions (Figure 3) [22].

**Figure 3: Mechanisms of the tumor microenvironment in pancreatic cancer patients**




*Modified from Tan et al. [22]*

### 5.2.2 Body composition in pancreatic cancer patients.

#### Depletion of fat mass

We demonstrated that pancreatic cancer patients lose both muscle and fat during the disease trajectory (paper II), which confirms findings from a previous study in pancreatic cancer patients [144]. At study entry, the cancer group had less fat mass (FMI and fat %) than the reference group. Furthermore, fat mass was lost during the disease trajectory. A prospective study in cancer patients referred for palliative care showed that decreased body weight was explained by loss of fat mass [138]. Others claim that adipose tissue wasting can be detected even before changes in food consumption [31]. It has also been demonstrated that fat mass is lost more rapidly than lean tissue in cancer patients losing weight [39], and that the loss of fat mass, both VAT and SAT precedes the loss of skeletal muscle mass [90]. These findings may

explain why weight losing cancer patients have lower adipose tissue mass than weight stable cancer patients and healthy reference populations [90, 145], as in our study (paper II).

### Depletion of fat-free mass

The underlying mechanisms of the loss of skeletal muscle have been suggested to be catabolic hormones such as glucocorticoids (which was not measured in our study) and chronic inflammation, but also inhibition of anabolic pathways, such as the ones controlled by IGF-1 [144, 146, 147]. Furthermore, in a longitudinal study in cancer patients, IGF-1 was predicted by lean body mass [138]. In line with this, we found lower IGF-1 levels in the patients than in the reference group, and the levels continued to decline, indicating loss of anabolic factors in the cancer patients. Loss of this anabolic-stimulating hormone may contribute to the progression of muscle atrophy, which is a characteristic feature in cancer cachexia, but also in aging and sarcopenia [148, 149]. In addition, low IGF-1 is associated with reduced protein intake. In paper II, the patients had lower protein intake than recommended for this group [150]. Furthermore, poor nutritional status has been connected to reduced IGF-1 levels in cancer patients [149], thus negative energy balance and fat loss may lead to reduced production of IGF-1 and anabolic stimuli.

Compared to the healthy reference group, the patients had lower lean body mass (FFMI and MUAMA), and experienced a loss of both FFMI and MUAMA during the disease trajectory. It has been shown that FFM is lost from arm tissue while that of leg and trunk compartments increase [138, 151]. These findings are partly confirmed in our study (paper II), where arm muscle measured by MUAMA was reduced. FFM was also slightly reduced. Four patients gained weight throughout the disease course, whereas tree of these gained FFM (data not shown). However, it has been speculated that this may be due to an increase in the FFM in internal organs such as the liver and spleen [151], rather than to an increase in skeletal muscle mass. Another possibility is attributed to BIAs limited ability to provide a direct estimate of FFM in cancer patients. MUAMA is perhaps a better indicator of muscle mass changes because it is not affected by body water.

Collectively, fat accumulation at the time of diagnosis may contribute to cancer progression [90]. The pro-inflammatory cytokines IL-1β, TNF-α and IL-6 produced by the tumor or host tissue due to tumor presence, lead to both local and systemic inflammation and may contribute to fat mass depletion due to for example increased lipolysis, and FFM depletion

due to proteolysis [22, 31]. Reduced energy intake and leptin levels may inhibit IGF-1 production in pancreatic cancer patients. Consequently, it leads to a reduction of anabolic stimuli and loss of skeletal muscle mass. The anthropometric measure, MUAMA seems to be a good measurement of changes in muscle mass in weight losing pancreatic cancer patients.

Two of the processes taking place during the progression of pancreatic cancer are an increase in inflammatory markers and the loss of body mass, both FFM and fat mass. The underlying causes of these two processes are not fully elucidated in this thesis; however, there are indications that the processes may be initiated by the presence of the tumor itself. Although these changes in inflammatory markers and body composition were observed, only IL-10 increased significantly throughout the disease course. The reason for the lack of a significant increase in inflammatory markers may be the small sample size in this study. However, enhanced IL-10 production is perhaps an indication of the severity of the disease, or it could be an indication of a disruption of anti-inflammatory mechanisms [34, 65].

### 5.2.3 Cachexia definitions and diagnostic criteria

#### Consistencies between two central definitions of cancer cachexia in their ability to predict prevalence and survival time

In paper II we found a good overall agreement between the latest consensus definition, which includes reduced muscle mass [44] as a criterion, and the 3-factor definition, which includes systemic inflammation (i.e. CRP >10 mg/l) [37]. Both classifications of cachexia predicted poorer survival when patients were classified at inclusion. However, the consensus definition only predicted significantly reduced survival in the patients when the non-cachectic patients were divided into a pre-cachectic and a non-cachectic group (paper III). Patients with no weight loss or signs of anorexia and metabolic change (i.e. CRP >8 mg/l) had longer survival than pre-cachectic and cachectic patients. Similarly, a study from 2010 [152] demonstrated that cancer patients with no weight loss and no anorexia had significantly longer survival than patients with either anorexia or weight loss or the combination of the two. These findings may help us in the further characterization of cachexia.

### Skeletal muscle mass

The common focus of the definitions proposed to classify cachexia is on the loss of skeletal muscle mass due to negative protein balance and the resultant loss of physical function [25, 153]. Skeletal muscle mass depletion and systemic inflammation (CRP >10mg/l) are strong predictors of overall survival and prognosis [104, 129]. In a study of cancer patients referred to a palliative care program, it was shown that patients with systemic inflammation had less muscle mass than patients without systemic inflammation and that muscle mass was lost at an accelerated pace during the disease trajectory. In addition they had shorter survival than patients without systemic inflammation [80]. It was also found that patients with pancreatic cancer had less muscle mass compared to patients with biliary tract- or colorectal cancer in the end of life [80]. The 3-factor definition was developed in pancreatic cancer patients and may therefore be better suited for detecting cachexia in this patient group only. However, validation of this definition in clinical studies lacks. We used the consensus definition to classify the patients at diagnosis (paper II); however, it is difficult to use this definition throughout the disease trajectory because one of the criteria is weight loss. Some patients responding to chemotherapy may actually maintain and/or gain muscle mass during such therapy [154, 155]. Moreover, the patients with cachexia are heterogeneous as some patients lose weight rapidly while others remain weight stable or gain weight [151, 156]. Consequently, a patient defined as cachectic at diagnosis, may gain muscle mass and thus may be defined as non-cachectic during the disease trajectory. Thus, the weight loss is not completely irreversible prior to reaching its refractory phase [50] and the focus of initiating anti-cachectic treatment, should be at the time of cancer diagnosis when the patient is in the pre-cachectic or the cachectic phase [157].

### Other diagnostic criteria

Although cytokines are known to be the driving force behind cachexia, and as has been detected in previous studies [158], we did not find any differences in inflammatory markers between cachexia and non-cachexia, classified by the 2011 consensus definition of cancer cachexia (paper II). The reason for the discrepancy between our study and previous studies may be that the latter did not define cachexia by sarcopenia and weight loss as we did in paper II, but by weight loss [42] or low BMI alone [158]. According to the consensus definition, only one out of three classification criteria should be met. Thus patients may be assigned to a cancer cachexia group purely on the basis of weight loss >5% in the past 6 months, or BMI

<20 kg/m² and weight loss >2% (whereas the time frame of weight loss is not specified) [44, 159]. As the majority of patients with advanced cancer have lost weight prior to diagnosis, patients may be classified as cachectic on the basis of BMI <20 kg/m² and weight loss >2% in an unlimited amount of time. The rate of weight loss within a specified timeframe is considered important [144, 159], and should therefore be specified in the diagnosis. Although CRP assessment is favored, the cut-off of inflammation is also not specified in the consensus definition. In paper II we used a cut off of 8 mg/l as in a previous study [52]. The reference of this cut-off is not given other than that it was assessed by experts. The prevalence of cachexia may differ according to which CRP cut-offs and to which criteria of the cachexia classifications that are used: while using the first criterion of the consensus classification (i.e. weight loss ≥5% during the last 6 months), Bozzeti et al. found that 60% of the patients were defined as cachectic. When they used the second criterion (i.e. weight loss 2% or 5% in combination with BMI <20 kg/m²) the prevalence was only 36%. This means that the classification identifies different groups of patients as cachectic, and that there is still a need for an agreed and validated classification of cancer cachexia [160]. A further validation in clinical studies is important. Martin et al [161] showed that patients with involuntary weight loss in addition to loss of muscle mass and low muscle attenuation (i.e. fat infiltration), have reduced survival regardless of overall body weight.

Studies have shown that patients with weight loss experience more symptoms like loss of appetite. Along with early satiety, loss of appetite may be responsible for a reduced dietary intake [25]. Considering pre-cachexia, no concrete directions in the consensus classification are given to how anorexia should be measured. In our study, energy intake was assessed (papers II and III). Patients who reported that they ate less than usual (paper II) or who had an energy intake <1500 kcal/d (paper III) were assumed to have anorexia. Mean dietary intake was measured to 1449 kcal/d based on a 24-hour recall (paper II). Although the general energy intake was low, patient perception of their own food intake was not associated with the estimated energy intakes (data not shown). Both energy and protein intake were higher in cachectic than in non-cachectic patients in our study (paper II). This is possibly because some patients who experience appetite and/or weight loss still manage to eat to avoid low food intake and weight loss [162]. Additionally, the focus of care in these patients is palliative and catabolic agents such as the corticosteroids may be selected to obtain a brief appetite spurt [156], however, weight gain is mainly caused by increases in water and fat [50].

### 5.2.4 Clinical considerations

#### Healthy adults

To keep a stable weight and to engage in physical activity for >30 min/day is important in both lean and overweight individuals, and may be beneficial in order to stay metabolically healthy and to avoid life style related chronic disorders [163]. The anthropometric measurements TSF and hip circumference may be appropriate in order to measure fat accumulation in lean individuals, and waist and hip circumference are perhaps more appropriate in overweight individuals.

#### Pancreatic cancer patients

In our study weight loss and sarcopenia (consensus classification), and reduced food intake and increased CRP (3-factor classification) were most frequently measured (paper III). Since these factors (i.e. weight loss, reduced food intake, systemic inflammation and sarcopenia) are frequently observed [42, 152, 161, 162], clinicians should be watchful to these complaints.

In cases of nutritional risk (i.e. weight loss or risk of malnutrition), early involvement of dieticians and nutrition assessment programs is essential. Both appetite loss and food intake should be assessed in this process [22, 162]. Nutritional support involves dietary advice and oral nutritional supplementation, which can significantly increase oral caloric and protein intake, and have had positive effects on weight and appetite, respectively [22]. It is estimated that energy intake should increase by 300-400 kcal per day and the protein intake by up to 50%. However, these nutritional goals are difficult to achieve in cancer patients receiving anti-cancer therapy. In these situations, parenteral nutrition can be one way of reaching the nutritional goals and may have led to prolonged survival [50]. Although specialized nutritional supplements are associated with weight stabilization by partly reversing fat loss, the metabolic changes preclude reversal of muscle wasting [50]. Thus, the nutritional response is limited. Therefore, it is now widely recognized that cachexia is best managed with a multimodal approach, including nutrition, physical activity and anti-inflammatory medication [22, 164].

Physical activity may induce muscle anabolism and thus resistant exercise may antagonize muscle atrophy. It has been shown that exercise is feasible in cancer patients and that it may improve physical performance [50]. In terms of targeting the inflammatory response, anti-

inflammatory agents such as NSAIDs, Thalidomide and omega-3 fatty acids have been investigated. NSAIDS evidently decrease the production and release of acute phase proteins and pro-inflammatory cytokines. However, Thalidomide is teratogenic and associated with potentially adverse effects and omega-3 fatty acids have shown conflicting results [22, 50], thus further investigation is warranted.

Taken together our findings in paper II and III support previous suggestions of a basic standardized approach to cachexia treatment. First, to target the tumor by anti-neoplastic therapy; second, to target systemic inflammation by anti-inflammatory drugs or nutrients; third, to target all secondary causes of cachexia, such as pain or nausea; fourth, to optimize energy intake by nutritional support, and finally physical activity must be recommended and adequate support given [50]. Anti-cachexia therapy should be initiated in a pre-cachectic phase, during palliative cancer therapy (i.e. supportive oncology), when the patients is responsive to treatment rather than in the refractory phase [50, 157], emphasizing that early recognition of cancer cachexia is essential.

Moreover, since fat mass depletion reflects negative energy balance and may occur prior to the detection of appetite loss, TSF may be an adequate method to estimate fat mass depletion in pancreatic cancer patients and may be one of the methods included in the early detection of cancer cachexia. Furthermore, MUAC and MUAMA may be methods for estimation of early lean tissue depletion [35].

# 6 Further perspectives

Targets relevant to future treatment of cachexia may be either by antagonizing key mediators of systemic inflammation, such as IL-6 and TNF-α, or by blocking catabolic pathways such as myostatin and activin [22, 164]. Alternative targets include pathways in the central nervous system that control appetite [164].

Nutritional management of cancer cachexia is limited since none of the available therapies has shown long-lasting effects on weight stabilization and improvement in survival [22, 165]. Moreover, there is a lack of knowledge about clinical nutrition within oncology, partly due to little evidence-based therapy [50]. However, since it is not possible to increase or stabilize weight if the patients' nutritional needs are not met; nutritional intervention is an important part of the treatment of cancer cachexia [165]. This in turn makes it necessary to require a greater awareness about nutrition and the increased nutritional needs in patients with advanced cancer among health care professionals. The future management of cachexia in pancreatic cancer patients will likely involve a multimodal approach with nutritional support combined with treatment aiming to modify inflammation and catabolism, in addition to palliative chemotherapy [165]. The balance between improved survival and maintenance of QoL is a key feature in the management of cachexia, thus outcomes such as weight stabilization, physical function and improved QoL are important aims in the foreseeable future [164].

# 7 Conclusions

The overall aim of this thesis was to gain more knowledge about cancer cachexia and to investigate the relationship between body composition and inflammation in two different samples; healthy individuals and patients with pancreatic cancer. By the use of observational studies, the conclusions are:

I An increase in fat percentage is associated with an elevation of adipokines (i.e. leptin) and inflammation (i.e. CRP) in overweight persons, but also among persons within the normal range of BMI. Hip circumference stood out as a surrogate measure for fat mass (%) in subjects within different BMI categories, and may be useful for identification of fat percentage in both overweigh and lean individuals. The results may indicate that adherence to a healthy lifestyle to prevent a high percentage of body fat is important for both lean and overweight people in order to reduce the risk for overweight-related chronic diseases

II Patients with advanced pancreatic cancer experience an ongoing inflammation, and at the same time, they lose fat mass and FFM. However, patients classified as cachectic immediately after diagnosis did not have higher levels of inflammatory markers than patients classified as non-cachectic. Inflammation in the cancer patients may be caused by the presence of the tumor and may cause symptoms similar to cachexia. Moreover, increased inflammation may stimulate fat and muscle mass depletion. For this reason, an optimization of cancer treatment is essential in pancreatic cancer patients.

III The two classifications showed good overall agreement in defining cachectic patients in this cohort with pancreatic cancer, and cachexia was associated with poorer survival according to both. Our findings suggest that the classification including CRP as a criterion was a better predictor of survival. Patients with no weight loss, reduced energy-intake or no sarcopenia and CRP<8 mg/L have the longest survival. Thus, clinical interventions should be directed to optimize these known risk factors.

# References


1. World Health Organization. Obesity and overweight, Fact sheet N°311. 2015. http://www.who.int/mediacentre/factsheets/fs311/en/. Accessed 18 June 2015.

2. Dixit VD: Adipose-immune interactions during obesity and caloric restriction: reciprocal mechanisms regulating immunity and health span. J Leukoc Biol. 2008; 84:882-92.

3. World Health Organization. Global data base on body mass index. 2015. http/apps.who.int/bmi/index.jsp. Accessed 1 June 2015

4. Mraz M, Haluzik M: The role of adipose tissue immune cells in obesity and low-grade inflammation. J Endocrinol. 2014; 222:R113-27.

5. Choi KM, Lee KW, Kim SG, Kim NH, Park CG, Seo HS, Oh DJ, Choi DS, Baik SH: Inflammation, insulin resistance, and glucose intolerance in acute myocardial infarction patients without a previous diagnosis of diabetes mellitus. J Clin Endocrinol Metab. 2005; 90:175-80.

6. Petelin A, Bizjak M, Cernelic-Bizjak M, Jurdana M, Jakus T, Jenko-Praznikar Z: Low-grade inflammation in overweight and obese adults is affected by weight loss program. J Endocrinol Invest. 2014; 37:745-55.

7. Park HS, Park JY, Yu R: Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Res Clin Pract. 2005; 69:29-35.

8. Davoodi SH, Malek-Shahabi T, Malekshahi-Moghadam A, Shahbazi R, Esmaeili S: Obesity as an important risk factor for certain types of cancer. Iran J Cancer Prev. 2013; 6:186-94.

9. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS: Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. 2001; 286:921-29.

10. Cancer Registry of Norway. cancer statistics. 2013. http://www.kreftregisteret.no/en/The-Registries/Cancer-Statistics/. Accessed 18 June 2015.

11. Ryan DP, Hong TS, Bardeesy N: Pancreatic adenocarcinoma. N Engl J Med. 2014; 371:1039-49.

12. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, et al: Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010; 467:1114-17.

13. Thomas A, Dajani K, Neoptolemos JP, Ghaneh P: Adjuvant therapy in pancreatic cancer. Dig Dis. 2010; 28:684-92.

14. Binkley C, Simone, D: Pancreatic cancer. In encyclopedia of Gastroenterology. In Johnson L, Editor. Encyclopedia of Gastroenterology. USA: Elsevier; 2004: p. 8.

15. Ronga I, Gallucci F, Riccardi F, Uomo G: Anorexia-cachexia syndrome in pancreatic cancer: recent advances and new pharmacological approach. Adv Med Sci. 2014; 59:1-6.

16. Fazal S, Saif MW: Supportive and palliative care of pancreatic cancer. JOP. 2007; 8:240-53.

17. Wigmore SJ, Plester CE, Richardson RA, Fearon KC: Changes in nutritional status associated with unresectable pancreatic cancer. Br J Cancer. 1997; 75:106-09.

18. Nicolini A, Ferrari P, Masoni MC, Fini M, Pagani S, Giampietro O, Carpi A: Malnutrition, anorexia and cachexia in cancer patients: A mini-review on pathogenesis and treatment. Biomed Pharmacother. 2013; 67:807-17.

19. Cederholm T, Bosaeus I, Barazzoni R, Bauer J, Van Gossum A, Klek S, Muscaritoli M, Nyulasi I, Ockenga J, Schneider SM, et al: Diagnostic criteria for malnutrition - An ESPEN Consensus Statement. Clin Nutr. 2015; 34:335-40

20. Jeejeebhoy KN: Malnutrition, fatigue, frailty, vulnerability, sarcopenia and cachexia: overlap of clinical features. Curr Opin Clin Nutr Metab Care. 2012; 15:213-19.

21. Meijers JM, van Bokhorst-de van der Schueren MA, Schols JM, Soeters PB, Halfens RJ: Defining malnutrition: mission or mission impossible? Nutrition. 2010; 26:432-40.

22. Tan CR, Yaffee PM, Jamil LH, Lo SK, Nissen N, Pandol SJ, Tuli R, Hendifar AE: Pancreatic cancer cachexia: a review of mechanisms and therapeutics. Front Physiol. 2014; 5:88.

23. Huggett MT, Pereira SP: Diagnosing and managing pancreatic cancer. Practitioner. 2011; 255:21-5.

24. Kayl AE, Meyers CA: Side-effects of chemotherapy and quality of life in ovarian and breast cancer patients. Curr Opin Obstet Gynecol. 2006; 18:24-8.

25. Argiles JM, Anker SD, Evans WJ, Morley JE, Fearon KC, Strasser F, Muscaritoli M, Baracos VE: Consensus on cachexia definitions. J Am Med Dir Assoc. 2010; 11:229-30.

26. Evans WJ, Morley JE, Argiles J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K, Lochs H, Mantovani G, et al: Cachexia: a new definition. Clin Nutr. 2008; 27:793-99.

27. de Luca C, Olefsky JM: Inflammation and insulin resistance. FEBS Lett. 2008; 582:97-105.

28. Tisdale MJ: Mechanisms of cancer cachexia. Physiol Rev. 2009; 89:381-410.

29. Tuca A, Jimenez-Fonseca P, Gascon P: Clinical evaluation and optimal management of cancer cachexia. Crit Rev Oncol Hematol. 2013; 88:625-36.

30. Batista ML, Jr., Peres SB, McDonald ME, Alcantara PS, Olivan M, Otoch JP, Farmer SR, Seelaender M: Adipose tissue inflammation and cancer cachexia: possible role of nuclear transcription factors. Cytokine. 2012; 57:9-16.

31. Batista ML, Jr., Olivan M, Alcantara PS, Sandoval R, Peres SB, Neves RX, Silverio R, Maximiano LF, Otoch JP, Seelaender M: Adipose tissue-derived factors as potential biomarkers in cachectic cancer patients. Cytokine. 2013; 61:532-39.

32. Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J, Heymsfield SB, Heshka S: Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. J Appl Physiol. (1985) 2004; 97:2333-38.

33. Bozzetti F, Mariani L: Defining and classifying cancer cachexia: a proposal by the SCRINIO Working Group. JPEN J Parenter Enteral Nutr. 2009; 33:361-67.

34. Seelaender M, Batista M, Jr., Lira F, Silverio R, Rossi-Fanelli F: Inflammation in cancer cachexia: to resolve or not to resolve (is that the question?). Clin Nutr. 2012; 31:562-66.

35. Wallengren O, Lundholm K, Bosaeus I: Diagnostic criteria of cancer cachexia: relation to quality of life, exercise capacity and survival in unselected palliative care patients. Support Care Cancer. 2013; 21:1569-77.

36. Thoresen L, Frykholm G, Lydersen S, Ulveland H, Baracos V, Prado CM, Birdsell L, Falkmer U: Nutritional status, cachexia and survival in patients with advanced colorectal carcinoma. Different assessment criteria for nutritional status provide unequal results. Clin Nutr. 2013; 32:65-72.

37. Fearon KC, Voss AC, Hustead DS, Cancer Cachexia Study G: Definition of cancer cachexia: effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis. Am J Clin Nutr. 2006; 83:1345-50.

38. Deans DA, Tan BH, Ross JA, Rose-Zerilli M, Wigmore SJ, Howell WM, Grimble RF, Fearon KC: Cancer cachexia is associated with the IL10 -1082 gene promoter polymorphism in patients with gastroesophageal malignancy. Am J Clin Nutr. 2009; 89:1164-72.

39. Fearon KC, Glass DJ, Guttridge DC: Cancer cachexia: mediators, signaling, and metabolic pathways. Cell Metab. 2012; 16:153-66.

40. Tisdale MJ: Cachexia in cancer patients. Nat Rev Cancer. 2002; 2:862-71.

41. Fox KM, Brooks JM, Gandra SR, Markus R, Chiou C-F: Estimation of Cachexia among Cancer Patients Based on Four Definitions. Journal of Oncology. 2009; doi:10.1155/2009/693458.

42. Bachmann J, Heiligensetzer M, Krakowski-Roosen H, Buchler MW, Friess H, Martignoni ME: Cachexia worsens prognosis in patients with resectable pancreatic cancer. J Gastrointest Surg. 2008; 12:1193-1201.

43. Prado CM, Heymsfield SB: Lean tissue imaging: a new era for nutritional assessment and intervention. JPEN J Parenter Enteral Nutr. 2014; 38:940-53.

44. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, Jatoi A, Loprinzi C, MacDonald N, Mantovani G, et al: Definition and classification of cancer cachexia: an international consensus. Lancet Oncol. 2011; 12:489-95.

45. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, et al: Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr. 2010; 29:154-59.

46. Jatoi A, Yamashita J, Sloan JA, Novotny PJ, Windschitl HE, Loprinzi CL: Does megestrol acetate down-regulate interleukin-6 in patients with cancer-associated anorexia and weight loss? A North Central Cancer Treatment Group investigation. Support Care Cancer. 2002; 10:71-5.

47. Jatoi A, Windschitl HE, Loprinzi CL, Sloan JA, Dakhil SR, Mailliard JA, Pundaleeka S, Kardinal CG, Fitch TR, Krook JE, et al: Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. J Clin Oncol. 2002; 20:567-73.

48. Jatoi A, Rowland K, Loprinzi CL, Sloan JA, Dakhil SR, MacDonald N, Gagnon B, Novotny PJ, Mailliard JA, Bushey TI, et al: An eicosapentaenoic acid supplement

versus megestrol acetate versus both for patients with cancer-associated wasting: a North Central Cancer Treatment Group and National Cancer Institute of Canada collaborative effort. J Clin Oncol. 2004; 22:2469-76.

49. Fearon KC: Cancer cachexia: developing multimodal therapy for a multidimensional problem. Eur J Cancer. 2008; 44:1124-32.

50. Fearon K, Arends J, Baracos V: Understanding the mechanisms and treatment options in cancer cachexia. Nat Rev Clin Oncol. 2013; 10:90-9.

51. Blum D, Stene GB, Solheim TS, Fayers P, Hjermstad MJ, Baracos VE, Fearon K, Strasser F, Kaasa S, Euro I: Validation of the Consensus-Definition for Cancer Cachexia and evaluation of a classification model--a study based on data from an international multicentre project (EPCRC-CSA). Ann Oncol. 2014; 25:1635-42.

52. van der Meij BS, Schoonbeek CP, Smit EF, Muscaritoli M, van Leeuwen PA, Langius JA: Pre-cachexia and cachexia at diagnosis of stage III non-small-cell lung carcinoma: an exploratory study comparing two consensus-based frameworks. Br J Nutr. 2013; 109:2231-39.

53. Blauwhoff-Buskermolen S, de van der Schueren MA, Verheul HM, Langius JA: 'Pre-cachexia': a non-existing phenomenon in cancer? Ann Oncol. 2014; 25:1668-69.

54. Trey JE, Kushner I: The acute phase response and the hematopoietic system: the role of cytokines. Crit Rev Oncol Hematol. 1995; 21:1-18.

55. Desborough JP: The stress response to trauma and surgery. Br J Anaesth. 2000, 85:109-17.

56. Liang YJ, Shyu KG, Wang BW, Lai LP: C-reactive protein activates the nuclear factor-kappaB pathway and induces vascular cell adhesion molecule-1 expression through CD32 in human umbilical vein endothelial cells and aortic endothelial cells. J Mol Cell Cardiol. 2006; 40:412-20.

57. Wilson AM, Ryan MC, Boyle AJ: The novel role of C-reactive protein in cardiovascular disease: risk marker or pathogen. Int J Cardiol. 2006; 106:291-97.

58. Reeves G: C-reactive protein. Australian prescriber. 2007; 30:74-6.

59. Lachmann HJ, Sengul B, Yavuzsen TU, Booth DR, Booth SE, Bybee A, Gallimore JR, Soyturk M, Akar S, Tunca M, Hawkins PN: Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford). 2006; 45:746-50.

60. Pathak S, Nunes QM, Daniels IR, Smart NJ: Is CRP useful in prognostication for colorectal cancer? A systematic review. Colorectal Dis. 2014; 16:769-76

61. Falconer JS, Fearon KC, Ross JA, Elton R, Wigmore SJ, Garden OJ, Carter DC: Acute-phase protein response and survival duration of patients with pancreatic cancer. Cancer. 1995; 75:2077-82.

62. Sarkar D, Fisher PB: Molecular mechanisms of aging-associated inflammation. Cancer Lett. 2006; 236:13-23.

63. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A: Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014; 13:3-10.

64. Woo P: Cytokines and juvenile idiopathic arthritis. Curr Rheumatol Rep. 2002; 4:452-57.

65. Dalamaga M: Interplay of adipokines and myokines in cancer pathophysiology: Emerging therapeutic implications. World J Exp Med. 2013; 3:26-33.

66. Schroder K, Hertzog PJ, Ravasi T, Hume DA: Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004; 75:163-89.

67. Tamandl D, Bahrami M, Wessner B, Weigel G, Ploder M, Furst W, Roth E, Boltz-Nitulescu G, Spittler A: Modulation of toll-like receptor 4 expression on human monocytes by tumor necrosis factor and interleukin-6: tumor necrosis factor evokes

lipopolysaccharide hyporesponsiveness, whereas interleukin-6 enhances lipopolysaccharide activity. Shock. 2003; 20:224-29.

68. Rodriguez-Yoldi MJ, Gascon S, Barranquero C, Garcia-Barrios A, Osada J: Involvement of intracellular signalling in the IL-1beta inhibitory effect on fructose intestinal absorption. J Cell Physiol. 2014; 230:896-902

69. Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J: Biology of interleukin-10. Cytokine Growth Factor Rev. 2010; 21:331-44.

70. Surmi BK, Hasty AH: Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. Future Lipidol. 2008; 3:545-56.

71. Johnson AR, Makowski L: Nutrition and metabolic correlates of obesity and inflammation: clinical considerations. J Nutr. 2015; 145:1131S-36S.

72. Perez-Hernandez AI, Catalan V, Gomez-Ambrosi J, Rodriguez A, Fruhbeck G: Mechanisms linking excess adiposity and carcinogenesis promotion. Front Endocrinol (Lausanne). 2014; 5:65.

73. Farrow B, Albo D, Berger DH: The role of the tumor microenvironment in the progression of pancreatic cancer. J Surg Res. 2008; 149:319-28.

74. McCall K, Schwartz AL, Schwartz FL: Linking obesity and pancreatic cancer. In: Pancreatic Cancer - Insights into Molecular Mechanisms and Novel Approaches to Early Detection and Treatment. CC BY 3.0 license. © The Author(s); 2014; DOI: 10.5772/58546.

75. Zhang GJ, Adachi I: Serum interleukin-6 levels correlate to tumor progression and prognosis in metastatic breast carcinoma. Anticancer Res. 1999; 19:1427-32.

76. Engineer DR, Garcia JM: Leptin in anorexia and cachexia syndrome. Int J Pept. 2012; doi: 10.1155/2012/287457.

77. Garcia JM, Garcia-Touza M, Hijazi RA, Taffet G, Epner D, Mann D, Smith RG, Cunningham GR, Marcelli M: Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. J Clin Endocrinol Metab. 2005; 90:2920-26.

78. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, et al: Bioelectrical impedance analysis--part I: review of principles and methods. Clin Nutr. 2004; 23:1226-43.

79. Thibault R, Genton L, Pichard C: Body composition: why, when and for who? Clin Nutr. 2012; 31:435-47.

80. Wallengren O, Iresjo BM, Lundholm K, Bosaeus I: Loss of muscle mass in the end of life in patients with advanced cancer. Support Care Cancer. 2015; 23:79-86.

81. Iizuka K, Machida T, Hirafuji M: Skeletal muscle is an endocrine organ. J Pharmacol Sci. 2014; 125:125-31.

82. Gumucio JP, Mendias CL: Atrogin-1, MuRF-1, and sarcopenia. Endocrine. 2013; 43:12-21.

83. Pedersen BK: Muscles and their myokines. J Exp Biol. 2011; 214:337-46.

84. Jung UJ, Choi MS: Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci. 2014; 15:6184-23.

85. Park A, Kim WK, Bae KH: Distinction of white, beige and brown adipocytes derived from mesenchymal stem cells. World J Stem Cells. 2014; 6:33-42.

86. Tilg H, Moschen AR: Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006; 6:772-83.

87. Huh JY, Park YJ, Ham M, Kim JB: Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cells. 2014; 37:365-71.

88. Guilherme A, Virbasius JV, Puri V, Czech MP: Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008; 9:367-77.

89. Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, Swarbrick M, Rose-John S, Rincon M, Robertson G, et al: A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab. 2014; 20:433-47.

90. Ebadi M, Mazurak VC: Evidence and mechanisms of fat depletion in cancer. Nutrients. 2014; 6:5280-97.

91. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW: Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005; 64:1195-98.

92. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ: Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol. 2003; 149:331-35.

93. Meier U, Gressner AM: Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem. 2004; 50:1511-25.

94. Telle-Hansen VH, Narverud I, Retterstol K, Wesseltoft-Rao N, Mosdol A, Granlund L, Christiansen KF, Lamglait A, Halvorsen B, Holven KB, Ulven SM: Substitution of TAG oil with diacylglycerol oil in food items improves the predicted 10 years cardiovascular risk score in healthy, overweight subjects. J Nutr Sci. 2012; 1:e17.

95. Nordby T, Ikdahl T, Bowitz Lothe IM, Fagerland MW, Heiberg T, Hauge T, Labori KJ, Buanes T: Improved survival and quality of life in patients undergoing R1 pancreatic resection compared to patients with locally advanced unresectable pancreatic adenocarcinoma. Pancreatology. 2013; 13:180-85.

96. Nordby T, Ikdahl T, Lothe IM, Anonsen K, Hauge T, Edwin B, Line PD, Labori KJ, Buanes T: Opportunities of improvement in the management of pancreatic and periampullary tumors. Scand J Gastroenterol. 2013; 48:617-25.

97. Simko M CC, Gilbride J: Nutrition assessment A comprehensive guide for planning Intervention. 2nd ed. An Aspen Publication; 1995.

98. Going S HM, Farr J.: Body Composition. In: Ross C, Caballero B, editors. Modern Nutrition in Health and Disease. Philadelphia: Lippincott Williams and Wilkins; 2012. p. 635-48.

99. Seidell JC: Waist circumference and waist/hip ratio in relation to all-cause mortality, cancer and sleep apnea. Eur J Clin Nutr. 2010; 64:35-41.

100. Gibson. R: Principles of Nutritional Assessment. 2nd ed. New York: Oxford University Press; 2005.

101. Wells JC, Williams JE, Fewtrell M, Singhal A, Lucas A, Cole TJ: A simplified approach to analysing bio-electrical impedance data in epidemiological surveys. Int J Obes (Lond). 2007; 31:507-14.

102. Piers LS, Soares MJ, Frandsen SL, O'Dea K: Indirect estimates of body composition are useful for groups but unreliable in individuals. Int J Obes Relat Metab Disord. 2000; 24:1145-52.

103. Ali AN, Rossi PJ, Godette KD, Martin D, Liauw S, Vijayakumar S, Cooper S, Jani AB: Impact of magnetic resonance imaging on computed tomography-based treatment planning and acute toxicity for prostate cancer patients treated with intensity modulated radiation therapy. Pract Radiat Oncol. 2013; 3:1-9.

104. Tan BH, Birdsell LA, Martin L, Baracos VE, Fearon KC: Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. Clin Cancer Res. 2009; 15:6973-79.

105. Wagle VG, Rossi AJ, Roberts MP, Goldman R, Ziter F, Clark WE: Thoracic spinal stenosis associated with renal osteodystrophy. Diagnosis based on magnetic resonance imaging and computed tomography. Spine (Phila Pa 1976). 1993; 18:1373-75.

106. Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE: A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab. 2008; 33:997-1006.

107. Detsky AS, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN: What is subjective global assessment of nutritional status? 1987. Classical article. Nutr Hosp. 2008; 23:400-7.

108. Malecka-Massalska T, Mlak R, Smolen A, Morshed K: Bioelectrical impedance phase angle and subjective global assessment in detecting malnutrition among newly diagnosed head and neck cancer patients. Eur Arch Otorhinolaryngol. 2015; April 10. Epub ahead of print

109. Takao Ohnuma. Manifestations of cachexia. In: Kufe DW, Pollock R, Weichselbaum RR, editors. Cancer Medicine. Hamilton (ON): BC Decker; 2003.

110. Thoresen L, Fjeldstad I, Krogstad K, Kaasa S, Falkmer UG: Nutritional status of patients with advanced cancer: the value of using the subjective global assessment of nutritional status as a screening tool. Palliat Med. 2002; 16:33-42.

111. Lillegaard IT, Andersen LF: Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters. Br J Nutr. 2005; 94:998-1003.

112. Marzola E, Nasser JA, Hashim SA, Shih PA, Kaye WH: Nutritional rehabilitation in anorexia nervosa: review of the literature and implications for treatment. BMC Psychiatry. 2013; 13:290.

113. World Medical A: World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310:2191 94.

114. Togo P, Osler M, Sorensen TI, Heitmann BL: Food intake patterns and body mass index in observational studies. Int J Obes Relat Metab Disord. 2001; 25:1741-51.

115. Carlson MD, Morrison RS: Study design, precision, and validity in observational studies. J Palliat Med. 2009; 12:77-82.

116. Mosca L, Edelman D, Mochari H, Christian AH, Paultre F, Pollin I: Waist circumference predicts cardiometabolic and global Framingham risk among women screened during National Woman's Heart Day. J Womens Health (Larchmt). 2006; 15:24-34.

117. Klepstad P, Kaasa S: The importance and pitfalls of correlational science in palliative care research. Curr Opin Support Palliat Care. 2012; 6:508-13.

118. Shrank WH, Patrick AR, Brookhart MA: Healthy user and related biases in observational studies of preventive interventions: a primer for physicians. J Gen Intern Med. 2011; 26:546-50.

119. Tan BH, Fearon KC: Cachexia: prevalence and impact in medicine. Curr Opin Clin Nutr Metab Care. 2008; 11:400-7.

120. Jepsen P, Johnsen SP, Gillman MW, Sorensen HT: Interpretation of observational studies. Heart. 2004; 90:956-60.

121. Grimes DA, Schulz KF: Bias and causal associations in observational research. Lancet. 2002; 359:248-52.

122. Schautz B, Later W, Heller M, Peters A, Muller MJ, Bosy-Westphal A: Impact of age on leptin and adiponectin independent of adiposity. Br J Nutr. 2012; 108:363-70.

123. Palmer JL: Analysis of missing data in palliative care studies. J Pain Symptom Manage. 2004; 28:612-18.

124. Engels JM, Diehr P: Imputation of missing longitudinal data: a comparison of methods. J Clin Epidemiol. 2003; 56:968-76.

125. Thompson FE, Subar AF: Dietary Assessment Methodology. In: Coulston, A., Boushey, C., Ferruzzi, M, editors. Nutrition in the prevention and treatment of disease. Oxford. Elsevier.p.9-46

126. Haverkort EB, Reijven PL, Binnekade JM, de van der Schueren MA, Earthman CP, Gouma DJ, de Haan RJ: Bioelectrical impedance analysis to estimate body composition in surgical and oncological patients: a systematic review. Eur J Clin Nutr. 2015; 69:3-13.

127. Maisonneuve N, Genton L, Karsegard VL, Kyle UG, Dupertuis YM, Pichard C: [Role of impedance measurement in nutritional screening]. Rev Med Suisse Romande. 2004; 124:611-15.

128. Tsai S: Importance of lean body mass in the oncologic patient. Nutr Clin Pract. 2012; 27:593-98.

129. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, Murphy R, Ghosh S, Sawyer MB, Baracos VE: Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol. 2013; 31:1539-47.

130. Janssen I, Heymsfield SB, Ross R: Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc. 2002; 50:889-96.

131. Metter EJ, Talbot LA, Schrager M, Conwit R: Skeletal muscle strength as a predictor of all-cause mortality in healthy men. J Gerontol A Biol Sci Med Sci. 2002; 57:359-65.

132. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD: Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998; 147:755-63.

133. Heilbronn LK, Campbell LV, Xu A, Samocha-Bonet D: Metabolically protective cytokines adiponectin and fibroblast growth factor-21 are increased by acute overfeeding in healthy humans. PLoS One. 2013; doi: 0.1371/journal.pone.0078864.

134. Arner E, Ryden M, Arner P: Tumor necrosis factor alpha and regulation of adipose tissue. N Engl J Med. 2010; 362:1151-53.

135. Bradley JR: TNF-mediated inflammatory disease. J Pathol. 2008; 214:149-60.

136. Wu B, Fukuo K, Suzuki K, Yoshino G, Kazumi T: Relationships of systemic oxidative stress to body fat distribution, adipokines and inflammatory markers in healthy middle-aged women. Endocr J. 2009; 56:773-82.

137. Ebrahimi B, Tucker SL, Li D, Abbruzzese JL, Kurzrock R: Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. Cancer. 2004; 101:2727-36.

138. Fouladiun M, Korner U, Bosaeus I, Daneryd P, Hyltander A, Lundholm KG: Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care--correlations with food intake, metabolism, exercise capacity, and hormones. Cancer. 2005; 103:2189-98.

139. Tamura S, Ouchi KF, Mori K, Endo M, Matsumoto T, Eda H, Tanaka Y, Ishitsuka H, Tokita H, Yamaguchi K: Involvement of human interleukin 6 in experimental cachexia induced by a human uterine cervical carcinoma xenograft. Clin Cancer Res. 1995; 1:1353-58.

140. Dalamaga M, Migdalis I, Fargnoli JL, Papadavid E, Bloom E, Mitsiades N, Karmaniolas K, Pelecanos N, Tseleni-Balafouta S, Dionyssiou-Asteriou A, Mantzoros CS: Pancreatic cancer expresses adiponectin receptors and is associated with hypoleptinemia and hyperadiponectinemia: a case-control study. Cancer Causes Control. 2009; 20:625-33.

141. Huang B, Cheng X, Wang D, Peng M, Xue Z, Da Y, Zhang N, Yao Z, Li M, Xu A, Zhang R: Adiponectin promotes pancreatic cancer progression by inhibiting apoptosis

via the activation of AMPK/Sirt1/PGC-1alpha signaling. Oncotarget. 2014; 5:4732-45.

142. Roxburgh CS, McMillan DC: Cancer and systemic inflammation: treat the tumour and treat the host. Br J Cancer. 2014; 110:1409-12.

143. Falconer JS, Fearon KC, Plester CE, Ross JA, Carter DC: Cytokines, the acute-phase response, and resting energy expenditure in cachectic patients with pancreatic cancer. Ann Surg. 1994; 219:325-31.

144. Di Sebastiano KM, Yang L, Zbuk K, Wong RK, Chow T, Koff D, Moran GR, Mourtzakis M: Accelerated muscle and adipose tissue loss may predict survival in pancreatic cancer patients: the relationship with diabetes and anaemia. Br J Nutr. 2013; 109:302-12.

145. Dahlman I, Mejhert N, Linder K, Agustsson T, Mutch DM, Kulyte A, Isaksson B, Permert J, Petrovic N, Nedergaard J, et al: Adipose tissue pathways involved in weight loss of cancer cachexia. Br J Cancer. 2010; 102:1541-48.

146. Thomas DR: Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia. Clin Nutr. 2007; 26:389-99.

147. Aapro M, Arends J, Bozzetti F, Fearon K, Grunberg SM, Herrstedt J, Hopkinson J, Jacquelin-Ravel N, Jatoi A, Kaasa S, Strasser F: Early recognition of malnutrition and cachexia in the cancer patient: a position paper of a European School of Oncology Task Force. Ann Oncol. 2014; 25:1492-99.

148. Hall DT, Ma JF, Marco SD, Gallouzi IE: Inducible nitric oxide synthase (iNOS) in muscle wasting syndrome, sarcopenia, and cachexia. Aging (Albany NY). 2011; 3:702-15.

149. Vlachostergios PJ, Gioulbasanis I, Kamposioras K, Georgoulias P, Baracos VE, Ghosh S, Maragouli E, Georgoulias V, Papandreou CN: Baseline insulin-like growth factor-I plasma levels, systemic inflammation, weight loss and clinical outcome in metastatic non-small cell lung cancer patients. Oncology. 2011; 81:113-18.

150. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. 5th ed. Copenhagen; Nordic Council of Ministers; 2014.

151. Lieffers JR, Mourtzakis M, Hall KD, McCargar LJ, Prado CM, Baracos VE: A viscerally driven cachexia syndrome in patients with advanced colorectal cancer: contributions of organ and tumor mass to whole-body energy demands. Am J Clin Nutr. 2009; 89:1173-79.

152. Lasheen W, Walsh D: The cancer anorexia-cachexia syndrome: myth or reality? Support Care Cancer. 2010; 18:265-72.

153. Onesti JK, Guttridge DC: Inflammation based regulation of cancer cachexia. Biomed Res Int. 2014; doi: 10.1155/2014/168407.

154. Stene GB, Helbostad JL, Amundsen T, Sorhaug S, Hjelde H, Kaasa S, Gronberg BH: Changes in skeletal muscle mass during palliative chemotherapy in patients with advanced lung cancer. Acta Oncol. 2015; 54:340-48.

155. Prado CM, Sawyer MB, Ghosh S, Lieffers JR, Esfandiari N, Antoun S, Baracos VE: Central tenet of cancer cachexia therapy: do patients with advanced cancer have exploitable anabolic potential? Am J Clin Nutr. 2013; 98:1012-19.

156. MacDonald N: Terminology in cancer cachexia: importance and status. Curr Opin Clin Nutr Metab Care. 2012; 15:220-25.

157. Laviano A, Fearon KC: The oncology wall: Could Ali Baba have got to the nutrition treasure without using the correct words? Clin Nutr. 2013; 32:6-7.

158. Bilir C, Engin H, Can M, Temi YB, Demirtas D: The prognostic role of inflammation and hormones in patients with metastatic cancer with cachexia. Med Oncol. 2015; 32:497.

159. Baracos VE: Pitfalls in defining and quantifying cachexia. J Cachexia Sarcopenia Muscle. 2011; 2:71-3.

160. Bozzetti F: Nutritional status, cachexia and survival in patients with advanced colorectal carcinoma. Different assessment criteria for nutritional status provide unequal results. Clin Nutr. 2013; 32:876.

161. Martin L, Senesse P, Gioulbasanis I, Antoun S, Bozzetti F, Deans C, Strasser F, Thoresen L, Jagoe RT, Chasen M, et al: Diagnostic criteria for the classification of cancer-associated weight loss. J Clin Oncol. 2015; 33:90-9.

162. Solheim TS, Blum D, Fayers PM, Hjermstad MJ, Stene GB, Strasser F, Kaasa S: Weight loss, appetite loss and food intake in cancer patients with cancer cachexia: three peas in a pod? - analysis from a multicenter cross sectional study. Acta Oncol. 2014; 53:539-46.

163. Jakicic JM, Otto AD: Physical activity considerations for the treatment and prevention of obesity. Am J Clin Nutr. 2005; 82:226S-9S.

164. Fearon KC, Baracos VE: Cachexia in pancreatic cancer: new treatment options and measures of success. HPB (Oxford). 2010; 12:323-24.

165. Balstad TR, Solheim TS, Strasser F, Kaasa S, Bye A: Dietary treatment of weight loss in patients with advanced cancer and cachexia: a systematic literature review. Crit Rev Oncol Hematol. 2014; 91:210-21.