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Alterations in adipose tissue in colorectal cancer patients

by

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Abstract

Mechanisms underlying fat loss in cancer are not well understood. Knowing the types of fat being lost from adipose tissue may help define interventions to circumvent wasting. The aim of this study was to explore the differences in adipokines and fatty acid composition between visceral adipose tissue (VAT) and subcutaneous (SAT) depots and relate this to fat mass changes (expressed as %change /100d) assessed using computed tomography images. Adipose tissue samples were obtained intraoperatively from advanced colorectal cancer patients (n=16). The findings indicate that fat was more commonly lost than gained. VAT was not preserved in cancer patients throughout the disease progression. Greater amounts of n-6 fatty acids in VAT were associated with greater fat loss in cancer patients. There were higher levels of leptin in SAT than VAT and higher monocyte chemotactic protein-1 (MCP-1) amounts in VAT. Future work will investigate the mechanisms of fat loss in cancer patients.

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List of Abbreviations

ACC	acetyl-CoA carboxylase
AT	adipose tissue
BMI	body mass index
C/EBPα	CCAAT/enhancer binding protein alpha
CRC	colorectal cancer
CRP	C-reactive protein
CT	computed tomography
d	day(s)
DXA	dual energy X-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
FAS	fatty acid synthase
GPAT	glycerol-3 phosphate acyltransferase
HSL	hormone-sensitive lipase
IMAT	intramuscular adipose tissue
IL-1	interleukin-1
IL-6	interleukin-6
kg	kilogram
LMF	lipid mobilising factor
LPL	lipoprotein lipase
MCP-1	Monocyte chemotactic protein-1
ml	milliliter
MRI	magnetic resonance imaging

MUFA	mono-unsaturated fatty acid
mRNA	messenger RNA
ng	nanogram
PUFA	poly-unsaturated fatty acid
PGC-1α	peroxisome proliferators-activated receptor gamma coactivator-1 alpha
PL	phospholipid
RT-PCR	Reverse transcription polymerase chain reaction
SAT	subcutaneous adipose tissue
SCD-1	stearoyl-CoA desaturase-1
SFA	saturated fatty acid
SREBP-1c	sterol regulatory element binding protein-1c
TAT	total adipose tissue
TG	triglyceride
TNF-α	tumour necrosis factor α
UCP-2	uncoupling protein-2
VAT	visceral adipose tissue
VEGF	endothelial growth factor
ZAG	zinc-α2-glycoprotein

CHAPTER 1: Introduction and literature review

1.1 Cancer

The National Cancer Institute of Canada estimated that in 2011, approximately 84,800 women and 93,000 men were diagnosed with cancer. Four types of cancers accounting for more than 50% of all new cancer cases are lung, prostate, breast and colorectal cancer (1). Cancer is defined as a disease in which abnormal cells grow and divide without control and form a mass of tissue. Cancer cells, comprising the tumor can spread to other tissues through the blood and lymph systems, a process termed metastasis. The most common treatment methods are surgery, chemotherapy and radiation therapy or a combination of two or three treatments (2).

1.1.1 Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer type in both males and females and second leading cause of cancer deaths in Canada (1). Overall, men have 1 in 14 chance and women a 1 in 15 chance of being diagnosed with colorectal cancer in their lifetime (1). A number of factors that may increase the risk of colorectal cancer are age, family history of colon cancer, inflammatory bowel disease, previous colonic polyps, and environmental factors (e.g., diet, obesity, sedentary life style, smoking, and alcohol consumption) (3). A diet high in processed and red meat, high in fat, and low in fiber may contribute to CRC risk (3). Previous studies have demonstrated an association between body mass index (BMI) and increased risk for colorectal cancer, particularly in men (4-5). It

was estimated that in Canada obesity accounts for 16.2% and 12.5% of colorectal cancer cases in men and women, respectively (6).

The stage of a cancer describes the severity and progression of cancer and is considered as an important predictor of survival and treatment. Site, size and number of tumors, lymph node involvement, cell type, tumor grade and the presence or absence of metastasis are common elements of staging systems (1). Staging in CRC is the process used to identify if cancer has spread within the colon or to other parts of the body (7). For CRC, five stages have been considered (summarized in Table 1-1) (7).

1.1.2 Cancer Cachexia

Cachexia comes from a Greek term, *kachexia*, meaning 'bad condition'. Cachexia has been recently defined as a "complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass" (8). Presence of cachexia could be considered if a patient has a weight loss of at least 5% in 12 months or less, and three of following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index, abnormal biochemistry including increased inflammatory markers CRP (>5.0 mg/l), IL-6 (> 4.0 pg/ml), anemia, and low serum albumin (8). Recently, diagnostic criteria and classification of cancer cachexia was expanded to include three stages of cachexia: precachexia, cachexia and refractory cachexia (9). Precachexia is characterized by anorexia, metabolic alterations and weight loss less than 5%, depending on cancer type and stage, systemic inflammation, and reduced food intake, may develop to cachexia stage. Cachexia is a stage with weight loss more

than 5% or BMI<20 and weight loss >2% or sarcopenia and weight loss >2%. The last stage, refractory cachexia, may happen because of very advanced, progressive cancer or the lack of response to anticancer therapy. It is associated with low performance status and an expected survival less than 3 months (9). Advanced cancer stage, older age and the tumour type, with the highest frequency at diagnosis being in pancreatic and gastric cancer, are related most commonly to wasting (10). Cachexia predicts a poor prognosis, shorter survival, treatment failure and toxicity, diminished performance status, and decreased quality of life (11-13).

Cachexia is considered as a multifactorial disorder. It seems that various factors such as tumour products, pro-inflammatory cytokines, host-tumour interactions, increased energy expenditure, anorexia may have role in the development of cachexia (13). Cachexia results from interactions between the host and the tumour (13). The tumor secretes pro-inflammatory cytokines which in turn stimulate the host inflammatory response and the production of pro-cachectic factors by tumours. Lipid mobilising factor [LMF] is considered as tumour-specific cachectic factor, which exerts catabolic effects on host adipose tissue (14). In response to tumour, host cells produce cytokines especially interleukin-6 (IL-6), interleukin-1 (IL-1), and tumour necrosis factor α (TNF-α). The liver response is increased production of positive acute-phase proteins, such as C-reactive protein (CRP) and fibrinogen. However, the plasma levels of negative acute phase proteins such as albumin are decreased in cachectic patients.

The effect of these mediators on adipose tissue will be discussed later in this chapter.

1.1.3 Obesity paradox

The Obesity paradox is a phenomenon in which obese patients with chronic diseases have higher survival rates compared to healthy weight and lower weight patients (16-18). Although some studies support the relationship between obesity and higher risk of cancer development, recurrence and treatment toxicity, others suggest an association between obesity and better survival in cancer (19). It seems that excess adipose tissue in obese cancer patients may provide fuel to bridge the gap between decreased intake and elevated requirements so a slight gain or stabilization of weight in cancer may be beneficial for longer survival (19). However, controversy remains regarding obesity as a prognostic factor in cancer and there is a lack of human studies due to inherent differences in controlling patient's weight loss during cancer.

Myers et al. followed 3834 men for 7 years and reported 314 deaths with 93 of them caused by cancer (19). Among these cancer deaths, 78 patients lost weight and 15 had maintained or gained weight. Weight loss was associated with higher mortality and weight gain was related to lower mortality when compared to weight stable groups. However, this study did not adjust for cancer types and BMI was used as an obesity index, rather than direct measurement of body composition (19).

On the other hand, Serrano et al. studied 213 patients who underwent surgery for CRC and monitored them up to 24 months. BMI was not a significant

prognostic factor of in CRC patients in their study (20). It seems that BMI may not be relate to long-term survival in CRC patients as it does not differentiate between different body fat distribution, nor proportions of fat mass and fat free mass. Other measurements of obesity which focus on abdominal obesity and the composition of fat gain or loss in cancer are needed to be investigated to increase our understanding of the effect of obesity on survival and the role of adipose tissue in cancer survival.

1.2 Adipose Tissue

Adipose tissue (AT) plays a dynamic role in body energy regulation, homeostasis, controlling appetite, insulin sensitivity, angiogenesis, inflammation and fat metabolism. Adipose tissue contains 82-88% fat in the form of triglyceride (TG), 2-2.6% protein, and 10-14% water (21). AT contains adipocytes, as major components, and non-adipocytes including inflammatory cells, immune cells, pre-adipocytes and fibroblasts (22).

Lipolysis and lipogenesis are two major pathways that regulate fat mass. These two pathways are controlled in adipocytes by short and long term signals. Hormones in blood provide short term signals; long term regulation is facilitated by alterations in size and number of fat cells. As mature and differentiated adipocytes can not divide, alterations in fat cell numbers depend on the differentiation of preadipocytes and apoptosis (23). Alterations in lipolysis and lipogenesis have substantial effects on fat mass and free fatty acids flux. Obesity, for example, is associated with alterations in adipose tissue function, structure and secretory properties. Hyperplasia (increased cell numbers) and hypertrophy

(increased cell size) are two mechanisms involved in adipose tissue growth in obesity (24). Hypertrophy precedes hyperplasia in obesity progression to enable AT to store more fat in adipocytes (25). Adipocyte hyperplasia appears to occur during AT development in preadipocytes (25).

Lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) are two major enzymes involved in fatty acid turnover in human adipose tissue (26). LPL hydrolyzes TG-rich lipoproteins and the resulting fatty acids are taken up by AT for storage or oxidation (27). HSL hydrolyzes TG in AT, releasing fatty acids bound to albumin, which are taken up by various tissues, mainly skeletal muscle, liver and heart (28).

1.2.1 Adipose tissue fatty acid composition

Among various factors including age, sex, genetics, the type of fat depots, the major determinant of AT fatty acid composition is dietary intake (29). AT fatty acid composition is considered as a good marker of dietary polyunsaturated fatty acids (PUFAs) intake (30, 31). However, there is discrepancy in the association between dietary saturated fatty acids (SFAs) or monounsaturated fatty acids (MUFAs) and AT content. Studies reporting fatty acids values as a percentage of the AT total fatty acid or percentage of dietary fatty acids found SFAs (except odd-numbered fatty acids) and MUFAs do not reflect dietary intakes as these fatty acids are synthesized endogenously (30, 31). On the other hand, Field et al. reported a positive correlation between subcutaneous AT composition and diet SFAs in 20 healthy subjects (32). Higher levels of SFAs in SAT TGs correlated with higher intakes of SFAs. It should be noted that intakes

were expressed as grams of fatty acids per kg of body weight (g/kg body weight) and AT fatty acids as a % w/w (32).

Changes in dietary fatty acid intake is reflected in the composition of FAs in stored TGs and structural phospholipids (PLs). Alterations in membrane fatty acid composition influences membrane associated functions. Field et al. found a stronger relationship between dietary intake and AT TGs content compared to PLs (32). The major fatty acids including 16:0, 16:1, 18:0, 18:1, and 18:2 n-6 constitute more than 90% of AT TGs whereas the main FAs in AT PLs are 16:0, 18:0, 18:1, 18:2 n-6, 20:4 n-6, and 20:5 n-3 (32).

1.2.2 Abdominal Visceral and Subcutaneous Adipose Tissue composition

The pattern of fat distribution may influence risk of diseases as excessive abdominal visceral fat accumulation compared to subcutaneous, is associated with metabolic disorders including hyperglycaemia, hyperinsulinaemia, hypertriglyceridema, impaired glucose tolerance, hypertension and some types of cancers (33). VAT refers to fat within the abdominal muscular wall (around abdominal viscera in mesentery and omentum) and SAT is fat found under the skin outside the abdominal muscle wall (34). Subcutaneous adipose tissue accounts for about 80% of total body fat, however, VAT comprises up to 10-20% of total fat in men and 5-8% in women (35). In obesity, a higher proportion of visceral fat and relatively low abdominal subcutaneous fat may lead to lipodystrophy (36).

VAT and SAT differ in the type of fat cells, anatomic location, endocrine function, lipolytic activity, response to insulin and cytokine production (37-39).

Large adipocytes, characteristically found in VAT, are insulin resistant and hyperlipolytic (37). Catecholamines have greater effects on lipolysis in VAT compared to SAT in humans. A study in 24 subjects undergoing elective cholecystectomy reported a higher sensitivity of lipolysis to catecholamines in adipocytes isolated from VAT compared to SAT. This suggests that in the presence of stress hormones, the location of VAT enables direct delivery of free fatty acids to liver, so increased lipolytic activity in VAT in response to catecholamines, may lead to more free fatty acids reaching the liver to cause toxic effects (38).

1.2.3 Adipokines

Adipokines are proteins synthesized and secreted from adipocytes. Adipokines act both locally and distally, contributing to body homeostasis by regulation of appetite, energy balance, insulin sensitivity, lipid and carbohydrate metabolism, angiogenesis, inflammation and immunity. Adipokines act on other tissues and organs such as skeletal muscle, adrenal cortex, brain and sympathetic nervous system. Both adipocytes and non-adipocyte cells secrete adipokines (40, 41). Adipokines play an important role in the pathophysiology of cancer in obesity. In obesity, particularly visceral adiposity, infiltration of immune cells into the adipose tissue may lead to changes in local adipokine secretion. Obesity and cancer cachexia are characterized as inflammatory conditions and higher expression of pro-inflammatory cytokines have been observed. Altered production of some cytokines such as tumour necrosis factor alpha (TNF-α), Interleukin-6 (IL-6), and leptin by adipose tissue and other tissues contribute to the systemic

inflammation in obesity and some types of cancer (40). A study in subjects with and without cancer demonstrated the potential role of adipokines in the regulation of growth and proliferation of tumours. Plasma leptin levels were lower in cancer cachectic patients compared to non cachectic cancer patients. Plasma adiponectin levels were similar in both groups (42). Moreover, the inhibitory effects of adipokines on appetite and food intake, stimulatory effects on lipolysis and proteolysis and gluconeogenesis may contribute to wasting in cachectic patients (42).

Leptin is produced predominantly by subcutaneous adipose tissue adipocytes. Leptin is considered to be a pro-inflammatory adipokine (43). Many factors influence leptin synthesis and secretion in adipocytes such as insulin, TNF-α, glucocorticoids, reproductive hormones, and prostaglandins (43, 44). In humans, the main factor which influences plasma leptin concentration is adipose tissue mass. Higher concentration of serum leptin in obese individuals is associated with increased fat mass and cell size (41).

Elevated leptin and decreased adiponectin levels in obesity may play important roles in development of obesity related cancers. Leptin may influence cell growth, proliferation, apoptosis, and angiogenesis or act as a mitogen (45). A study using colonic epithelial cell lines derived from the mouse demonstrated that leptin can induce epithelial cells to produce factors such as vascular endothelial growth factor (VEGF) that may drive angiogenesis, vascular development and therefore contribute to cancer progression (46).

Tessitore et al. measured leptin plasma levels and adipose tissue mRNA expression in 23 breast cancer patients and 103 controls matched for BMI. They reported that plasma levels and AT expression of leptin were significantly higher than controls (47). In a later study, they measured plasma leptin levels and AT mRNA levels in 87 gynaecological and breast cancer patients. They found that in these types of cancers, increased leptin levels is associated with hormonal status but not to cachexia as the levels of plasma sex hormones and their receptors in adipose tissue were elevated (48).

Circulating leptin concentrations in gastrointestinal (GI) cancer patients have been reported to be lower than healthy subjects and is associated with body fat mass, not the presence of an inflammatory response (49, 50). A study in 26 advanced gastrointestinal cancer patients and 11 age and gender matched healthy controls established that BMI and percent fat mass were lower and IL-6 and CRP levels were higher in cancer patients than in controls. Total leptin was measured by radioimmunoassay methods and total body water, determined by bioelectrical impedance, were used to calculate body fat percentage. Leptin levels were low in cancer groups and significantly correlated with fat mass (49).

Adiponectin is a protein expressed exclusively in adipocytes. The important roles of adiponectin include anti-atherogenic, anti-inflammatory and insulin sensitizing effects (51). In contrast to most adipose tissue derived proteins, plasma adiponectin levels are lower in obesity, metabolic syndrome, and some types of cancers compared to healthy subjects (51). Plasma levels of adiponectin have been shown to increase with weight loss (51). Moreover, adiponectin and

leptin work together to increase peripheral tissues sensitivity to insulin (52). Human AT cultures demonstrated that adiponectin inhibits production and release of a number of pro-inflammatory cytokines including TNF-α, IL-6, IL-8. Conversely, TNF-α and IL-6 are inhibitors of adiponectin production and release by adipocytes (53).

Low adiponectin levels in obesity or cancer, may increase risk of carcinogenesis or tumor progression (54). By inhibiting the production of TNF-α in macrophages and its action in endothelial cells or by activation of AMP-activated protein kinase, adiponectin has been demonstrated to play a role in regulation of tumour cell proliferation, and growth (54). A study in 60 patients with non-metastatic CRC and 30 subjects without diabetes mellitus or BMI<28 kg/m^2 as controls demonstrated that cancer patients had lower adiponectin levels compared to control subjects (55). Patients were followed from the time of surgery for at least 3 years and serial measurement of serum adiponectin levels was obtained. Kaplan-Meier Analysis showed that low adiponectin level was a predictor of tumour recurrence regardless of tumour stage (55).

TNF-α is a pro-inflammatory cytokine produced mainly by macrophages and lymphocytes, but also adipocytes (45). TNF-α increases adipocyte lipolysis, decreases lipogenesis and impairs TG storage in adipose tissue by inhibiting free fatty acid uptake, and downregulates lipoprotein lipase expression (56). In mature white adipocytes, TNF-α is reported to regulate cell size (57). As the adipocytes mass increase in obesity, it produces more TNF-α, which in turn reduces cell size (57). Culture of human adipocytes shown that TNF-α induces insulin resistance in

adipocytes by increasing lipolysis and FFA release from adipose tissue (58). Moreover, in 3T3-L1 adipocytes TNF-α can bind to specific TNF receptors and inhibit insulin receptor signaling and activity which results in insulin resistance (59).

IL-6, a circulating cytokine, is secreted by many cell types, including immune cells, skeletal muscle, adipose tissue, and fibroblasts (60). Diet induced weight loss significantly decreases IL-6 levels in adipose tissue and serum. IL-6 acts as a proinflammatory cytokine by increasing secretion of IL-1 and TNF-α level. IL-6 also stimulates hepatic production of C-reactive protein, which is considered a marker of inflammation (56).

A critical review on 36 studies in CRC patients shown that plasma IL-6 levels were higher in CRC patients than control and was considered as a prognostic indicator in CRC patients. There was a positive association between elevated IL-6 circulating levels, elevated tumour stage, size, metastasis and decreased survival (61). The pattern of adipokine production differs between visceral and subcutaneous depots (28). VAT produces more adiponectin, CRP and pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 (28). On the other hand, subcutaneous fat is a main source of leptin (44). Visceral fat produces three fold more IL-6 than subcutaneous fat (62). Although both IL-6 and TNF-α are expressed by adipose tissue, there are important differences in their systemic release (63). SAT may preferentially release leptin and IL-6 (63). Mohammad-ali et al. (1997) found that TNF-α was not secreted by SAT so SAT may not influence LPL action, lipolysis, or insulin signaling through endocrine

mechanisms (63). In contrast, IL-6 is released into the systemic circulation and is able to provide a systemic signal (63).

Monocyte chemotactic protein-1 (MCP-1) is a pro-inflammatory chemokine, involved in the recruitment of monocytes to sites of inflammation and mainly produced by macrophages and endothelial cells (64). Gerhardt et al found that MCP-1 is also produced and released by human preadipocytes and mature adipocytes (65). However, collagenase digestion of human visceral adipose tissue suggested that the majority of MCP-1 released by adipose tissue explants is derived from the non-fat cells such as macrophages (66). Culture of human visceral and subcutaneous adipose tissue explants demonstrated that VAT releases more MCP-1 than SAT. Moreover, obese subjects produce higher MCP-1 compared to normal weight subjects (67, 68). Incubation of human adipose tissue explants by various cytokines for 48 h in fresh medium showed that proinflammatory cytokines such as IL-6, IL-1ß and TNF-α increase MCP-1 release by AT (67). 3T3-L1 adipocytes and adipose tissue from mice demonstrate that MCP-1 is involved in regulation of insulin sensitivity (69). By increasing the expression of several adipogenic genes, MCP-1 can induce adipocyte dedifferentiation. (69).

A direct role of adipose tissue MCP-1 in cancer progression has not yet been clarified. TNF-α increases MCP-1 production by cultured adipose tissue and leading to higher macrophage infiltration into adipose tissue. It has been suggested that adipose tissue from cancer cachectic patients may produce higher amounts of MCP-1 (70).

Overall, infiltration of inflammatory cells, primarily macrophages, into WAT or changes in fat mass in cancer promotes the local production of inflammatory mediators, such as cytokines, from adipocytes, which in turn initiates a negative set of effects on adipocyte function. Higher production of cytokines such as TNF-α, IL-6 and IL-1β by adipose tissue in response to tumour presence may lead to increased lipolysis and systemic inflammation with higher circulating levels of IL-6, TNF-α and lower adiponectin levels in cancer. Both IL-6 and TNF-α inhibit LPL activity preventing fat cells from taking fatty acids from lipoproteins, Whereas TNF- α stimulates hormone-sensitive lipase. Collectively, these alterations lead to increased lipolysis and fat loss in cancer (71).

1.3 Body composition

Body composition, defined as a description of body fat and lean mass proportions such as bone and muscle, can be assessed by different methods (72). Body mass index (BMI) has been used frequently as an easy measurement of human body composition. BMI correlates with total body fat and is considered as an index of fatness associated with higher risk of chronic diseases such as diabetes, hypertension and some types of cancer. However, BMI does not take into account the proportions of AT, lean tissues and their location which are clinically important (73).

Previous studies found that the drug distribution in the body in the elderly (74), obese (75) or cancer patients (76) is related to the proportion of fat and lean tissues. Moreover, specific tissues accumulation or loss of fat is associated with specific health outcomes such as the relationship between visceral AT

accumulation and insulin resistance (77) or loss of muscle and physical disability (78). These all exemplify the need for valid and precise method to assess different components of the body which are associated with different health risks.

Methods for body composition assessment have been validated in different populations. Dual energy X-ray absorptionary (DXA), magnetic resonance imaging (MRI), and computed tomography (CT) have been considered as standard methods in the evaluation of human body composition in aging and chronic diseases research (79). CT image analysis has the potential to discriminate and quantify muscle, adipose tissue, organs and is capable of providing estimation of the amount of fatty infiltration in muscle and organs which has effect on insulin resistance and muscle dysfunction (80, 81). Shen et al. found that single slice tissue areas might be useful in estimating the whole body volumes of muscle and adipose tissue (82). CT analysis is not frequently available in healthy populations as radiation exposure is a major concern. High cost, and required high technical skills are some disadvantages of this method (80). However, in an oncologic population, CT images are a routine part of treatment and are available from patient records as a chart review.

1.3.1 Visceral and subcutaneous depots and cancer

Classical anthropometric measures such as waist circumference, waist to hip ratio cannot distinguish between different body compartments. Direct imaging methods such as computed tomography and MRI, and ultrasound are different methods used to estimate visceral and subcutaneous depots in different populations. However, CT imaging is becoming the gold standard for

measurement of fat mass in cancer. Studies found that visceral obesity measured by CT imaging is a risk factor for colorectal carcinogenesis (83, 84). A Japanese study in 108 patients with early-stage colorectal neoplasia, including 22 with early cancer found that visceral fat area, not subcutaneous, was significantly positively associated with colorectal cancer risk. Abdominal CT was performed in patients and control groups, comprised of patients with negative screening results. Patients with greater visceral fat mass (>129 cm^2) but not those with greater subcutaneous fat mass had higher chance of early colorectal cancer. Visceral adiposity and related insulin resistance promote carcinogenesis as VAT produces several adipokines which are involved in the development and progression of cancer (84). In the study by Yamamoto et al. (84), all patients were at the early stage of the disease, however using CT image analysis in a Turkish study of 54 patients with colorectal adenoma and carcinoma and 50 healthy control subjects showed that the CRC patients tended to have a smaller visceral fat area than control subjects (73 ± 48 cm^3 vs. 94.6 ± 45.2). The major limitation of their study was that they assessed both colorectal cancer and colorectal adenoma patients together as a cancer group so their results have been influenced by cancer induced weight loss (85).

The prognostic significance of visceral adiposity in 161 patients with resectable colorectal cancer was investigated by analyzing preoperative CT images and determining the ratio of visceral fat area to subcutaneous fat area. Patients were followed more than 1 year after surgery. Patients were categorized into high visceral fat area to subcutaneous fat area (>50 percentiles) and low

visceral fat area to subcutaneous fat area (<50 percentiles) ratio. Overall, survival did not differ between overweight and normal weight patients and BMI and visceral adiposity did not relate to survival of patients (86).

Ogiwara et al. (1994) investigated the relationship between VAT area and cancer cachexia in 13 cachectic gastrointestinal cancer patients by CT scanning within 3 months before death. Sixteen nonobese subjects without gastrointestinal cancer were selected as the control group. There was no significant difference in visceral and subcutaneous area between cancer patients without weight loss and cachectic cancer patients (87). Cachectic patients, with weight loss greater than 10% within 6 months, had a significantly smaller VAT area than control group (43.9 ± 42.4 cm^2 vs. 93.4 ± 56.0 cm^2, $P<0.05$) which suggests that VAT was not preserved in cachexia (87).

Overall, then, CT image analysis suggests that visceral adiposity has a major role in colorectal cancer development. On the other hand, in cachectic cancer patients, visceral adipose tissue is not preserved and the severity of fat loss increases approaching death.

1.4 Loss of adipose tissue

1.4.1 Adipose atrophy in cancer

Extensive loss of AT in cancer cachexia has been observed. CT image analysis of cancer patients and morphological examination in cancer cachectic mice has demonstrated adipose atrophy in both human and animal models (87, 88). Intensity of AT loss increases close to death (89) and total body fat and

adipose tissue loss have been demonstrated to be a predictor of shorter survival (89, 90).

Using subcutaneous adipose tissue from the abdominal region of gastrointestinal cancer patients (n=13 cachectic and n= 14 without cachexia), a reduction in fat cell volume (504 ± 146 pico litres vs. 331 ± 137), but not fat cell numbers was observed in cachectic groups compared to weight stable cancer patients. Patients with weight loss more than 5% of the habitual weight during the previous 3 months or more than 10% loss during the previous 6 months were considered as cachectic patients (91). The total number of fat cells in the body was calculated as the amount of body fat divided by the mean fat cell weight. Elevated lipid mobilization from subcutaneous adipose tissue, evidenced by elevated levels of plasma glycerol and FA and up regulation of genes involved in energy turnover pathways including Krebs TCA cycle and oxidative phosphorylation, was the reason for fat loss. Moreover, the expression of inflammatory genes assessed by Real Time-PCR was unchanged in SAT of both cachectic and non cachectic patients, which suggests that subcutaneous adipose tissue is not involved in increased systemic inflammation in cachectic patients. There was no significant difference in lean body mass between the cachectic and weight stable groups which suggests that changes in fat mass is not secondary to changes in lean body mass (91). Similar studies in cancer patients indicate that SAT does not contribute to the systemic inflammatory reaction and increased lipolysis, not inflammation, or adipocytes cell death, is the reason for fat loss in cancer (92).

A retrospective study by Murphy et al. (2010) assessed loss of AT in colorectal and lung cancer patients and reported losses of fat at 7 months from death. Serial CT images of patients collected in the last 500 days were analyzed to calculate AT rate of change during this period. One hundred eight patients with at least 2 CT images in the last 500 days of life between diagnosis and death were selected for this analysis. As the timing of CT images differs between patients, rate of change was expressed as % change/100 days to enable to comparison between patients. Approaching death, total AT, VAT and SAT mass decreased in cancer patients with intense loss of approximately 10kg of fat mass/100 days close to death (89). Moreover, loss of adipose tissue was associated with shorter survival and the rate of loss accelerated approaching death. Intramuscular adipose tissue was the only depot that increased in cancer patients close to death (89). In a study from the same cancer center, longitudinal CT image analysis, demonstrated alterations in body composition in CRC patients throughout the cancer trajectory that would not be captured without the use of CT imaging. Increased liver and tumor mass and decreased muscle and fat mass were observed 3 months approaching death in cancer patients (93).

Bing et al. (2006) investigated bearing the MAC-16 tumor morphological and molecular characteristics of AT of mice with cancer cachexia. They found that the presence of a tumour alters AT mass and function (94). Substantial changes in adipose tissue plasticity in mice with cancer cachexia compared with ad libitum-fed and pair-fed controls were observed in morphologic evaluation by electron microscopy (94). Cells containing a large lipid droplet with a thin

peripheral rim of cytoplasm and the peripherally located nucleus were the characteristics of control mice adipocytes. Circular adipocytes with a moderate reduction in cell size and slightly increased collagen fibrils were the characteristics of adipose tissue from pair-fed animals. Adipose tissues from tumour-bearing mice contain shrunken adipocytes of different sizes with an expanded interstitial space. More detailed examination in this group has shown severe reductions in adipocyte size (94). Overall, this suggests adipose tissue atrophy in human and animal models of cancer cachexia.

1.4.2 Mechanisms and mediators of adipose tissue loss

Many studies have demonstrated that an enhanced catabolic response and disrupted anabolic processes may be the main reasons for fat loss (88). Elevated lipolysis seems to be a major factor of fat loss in cancer (95). Studies have shown that lipolytic activity (fasting plasma glycerol or fatty acids) is increased in patients with cancer cachexia compared to weight stable cancer patients (96). Elevated activity and expression of HSL mRNA and protein, a major enzyme involved in the lipolysis of adipose tissue TGs (95), several factors produced by tumours or host tissue in response to the tumour burden such as proinflammatory cytokines, i.e. TNF-α, IL-1β, IL-6 and lipid mobilizing factor zinc-α2-glycoprotein (ZAG), are suggested to promote fat loss in animal and human models of cachexia (88).

A study in tumour bearing mice shown that LPL activity of AT was decreased to levels found in starved animals (97). The lipoprotein lipase activity was expressed as μmol fatty acid released per hr per g, of tissue. Reduced AT

LPL activity was associated with reduced breakdown of plasma lipoproteins and increased flux of lipid into the circulation (97). However, unlike starvation, in cancer patients mRNA and protein levels of HSL were elevated which may be an indicator of increased utilization of stored fat (98).

A study in tumour bearing mice showed impairment in the capacity of adipose tissue for fat synthesis and storage. There were major reductions in mRNA levels of adipogenic transcription factors including CCAAT/enhanceer binding protein alpha (C/EBPα), CCAAT/enhancer binding protein beta, peroxisome proliferator-activated receptor gamma, and sterol regulatory element binding protein-1c (SREBP-1c) in adipose tissue and lipogenic enzymes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase-1 (SCD-1) and glycerol-3 phosphate acyltransferase (GPAT). Increased mRNA levels of peroxisome proliferators-activated receptor gamma coactivator-1 alpha (PGC-1α) and uncoupling protein-2 (UCP-2) has demonstrated that lipid utilization is increased in white fat of cachectic mice (94).

Increased fat cell lipolysis, not decreased lipogenesis or adipocyte cell death have been suggested as main causes of fat loss in SAT. Subcutaneous samples were obtained from 10 weight-stable patients, 13 cachectic (involuntary weight loss >5% within a 6-month) patients with cancer, and from 5 patients without cancer (benign diagnosis). Plasma glycerol and fatty acid concentrations per kg of body fat, as an in vivo index of lipolysis, increased significantly in the cachectic group compared with the other 2 groups. Serum levels of IL-6 and TNF-α were measured and they found that TNF-α level were similar in the 3 groups,

however there was an increase in circulating levels of IL-6 in cachectic groups (92).

Numerous alterations in adipose tissue fat metabolism including increased lipolysis, elevated fatty acid oxidation, and alterations in expression of genes involved in fat metabolism, and adipocyte development collectively play a role in fat loss in cancer patients (99). Moreover, any alteration in the function of major lipolysis-regulating hormones such as insulin and catecholamines may contribute to the wasting (95). A better understanding of mechanisms involved in adipose atrophy in cancer cachexia will help to find effective interventions for fat loss in cachexia.

1.5 Summary

Alterations in adipose tissue fat metabolism, changes in expression of genes involved in fat metabolism and adipocyte development play roles in fat loss in cancer patients. It has been discovered recently that fat is at least as important as muscle in cancer trajectory and colorectal cancer patients who are obese, live longer. Alterations in fat mass and composition between visceral and subcutaneous depots are not the same and little is known regarding these alterations throughout the cancer trajectory. The prognostic significance of these depots needs to be investigated in large populations throughout the cancer progression. Further elucidation of fatty acid composition, adipokines and fat mass alterations of these fat stores and understanding the association between visceral and subcutaneous fatty acid composition and fat mass changes may lead to design interventions for preventing adipose atrophy in cancer.

Tables

Table 1-1. Colorectal cancer stages

Stage	Characteristics
0	Cancer is found in the inner lining of the colon or rectum
1	Cancer has spread from the inner lining into the middle layers of the colon or rectum wall
2	Cancer has spread outside the colon or rectum to nearby tissues
3	Cancer has spread outside the colon or rectum to nearby lymph nodes
4	Cancer has spread outside the colon or rectum to another part of the body

Available from Canadian Cancer Society 2010 (7).

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CHAPTER 2: Research plan

2.1 Rationale

CRC is the third most common cancer in the developed world (1). Advanced CRC is characterized by malnutrition and cachexia, which includes loss of muscle and adipose tissue (2). CT image analysis has revealed adipose atrophy in cachectic cancer patients (3-5). Fat loss relates to shorter survival in cancer (4, 6). The majority of CRC cancer patients are overweight or obese, so the importance of fat mass and whether cancer patients should lose or maintain their fat stores through cancer progression requires investigation.

Mechanisms underlying fat loss are not well understood although inflammation is thought to contribute. Identifying types of fatty acids within visceral and subcutaneous depots and characterizing the relationship between fat mass, fat loss, adipose tissue fatty acids as well as inflammatory proteins involved in fat metabolism will enhance our understanding of aberrations in adipose tissue mass, composition and functional activity and help define targets for interventions to stabilize fat mass.

2.2 Objectives and Hypotheses

The objectives of this thesis are as follows:

1) To characterize the concentrations of adipokines including MCP-1, leptin, adiponectin, IL-6 and TNF-α in visceral and subcutaneous adipose tissues of humans undergoing surgery for colorectal liver metastases and to assess the differences in adipokines levels between these two depots.

2) To assess the fatty acid types and amounts of TG and PL in VAT and SAT of CRC patients and to explore the differences between these two depots at the time of surgery.

Hypothesis: It is hypothesized that:

1) Compared to subcutaneous adipose tissue, visceral will exhibit higher levels of adiponectin, IL-6, TNF-α and MCP-1 and lower amounts of leptin.
2) Saturated fatty acids will be higher in visceral adipose tissue triglycerides compared to subcutaneous. MUFAs will be higher in triglycerides stored in subcutaneous adipose tissue compared to visceral. Visceral adipose tissue will contain more PUFAs in the phospholipids compared to subcutaneous adipose tissue.

This objective is investigated in chapter 3.

Investigated in Chapter 4:

1) To assess whole body fat mass and changes in visceral, subcutaneous, and intramuscular adipose tissue areas using CT images obtained at 2 different time points in CRC patients.
2) To relate the fatty acid composition to changes in fat mass (expressed as % change/100d)

Hypothesis: It is hypothesized that:

1) More patients will lose fat than gain fat.
2) Subjects losing fat will have higher n-6 PUFAs in their depots compared to those gaining.

This work represents a first step in exploring the relationship between adipose tissue fatty acid composition and rate of fat mass changes in colorectal cancer patients.

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CHAPTER 3: Adipokine levels and fatty acid composition of adipose tissue in colorectal cancer patients

3.1 Introduction

Fat metabolism of advanced cancer patients is not well characterized. Increased lipolysis (1), elevated fasting plasma glycerol and free fatty acids, and oxidation of free fatty acids, are evidence of altered lipid metabolism in advanced cancer patients (2). Abnormalities in lipid metabolism may lead to fat and weight loss which correlates with poor prognosis and shorter survival (3).

Adipose tissue fatty acid composition is considered as a good marker of long- term dietary fatty acid intake as well as a biomarker of fatty acid status in different conditions. Adipose tissue fatty acids (FAs) have been used to explore the possible role of FAs as potential risk or protective factors (4). Previous animal and human studies have identified differences in types of fatty acid in adipose tissue sampled from visceral and subcutaneous fat depots. These studies indicate that visceral adipose tissue (VAT) contains more saturated fatty acids than subcutaneous adipose tissue (SAT) (5, 6).

Depot-specific characteristics of subcutaneous and visceral adipose tissue are described in detail in Chapter 1. Only a few studies with a limited number of patients have been conducted on adipose tissue fatty acid composition in cancer patients. No significant difference in the fatty acid composition of triglycerides (TGs) and phospholipids (PLs) between cancer and control groups have been reported in previous studies (7,8). A study in breast cancer patients indicated that adipose tissue fatty acid composition is related to diet rather than to the stage of

the disease. Fatty acid composition of breast adipose tissue (outside the tumour) from patients with breast cancer and benign breast disease were analysed and cancer staging was performed based on TNM classification (7). Self administrated food frequency questionnaire were used to assess dietary intakes of fatty acids. In this study there were no significant differences between fatty acid compositions of TGs in patients with early (0, I, II) versus advanced stages (III, IV) of cancer (7). The difference between visceral and subcutaneous fatty acid composition in colorectal cancer patients has only been explored in one study. Fatty acid content of VAT and SAT was assessed by gas chromatography in 8 men and 11 women and was compared between sites and between patients. Subcutaneous abdominal adipose tissue was reported to be a good indicator of visceral adipose tissue composition as VAT and SAT fatty acid composition within individuals was similar (9).

Adipose tissue is an active tissue that produces various adipokines. The expression and secretion of many adipokines has been reported to differ between visceral and subcutaneous depots (10, 11). Studies indicate elevated inflammation in VAT compared to SAT (10). It has been reported that VAT is a more active producer of adipokines including IL-6, TNF-α, and adiponectin. On the other hand, leptin is produced mainly by SAT (11). Adipocytes are the main site of leptin and adiponectin production, however IL-6 and TNF-α are produced mainly by non-fat cells that reside in the adipose tissue (11). Studies comparing adipokine secretion and release by these two depots have yielded inconsistent results. The important reasons for these discrepant results may be differences between subjects

age, gender, BMI, sample size of study or body fat distribution across ethnic groups (12). The majority of studies in cancer focus on adipokine levels in plasma, however the correlation between plasma adipokines levels and AT adipokines protein or mRNA levels differ depending on the adipokines measured (13). Measuring mRNA or protein levels, especially for leptin and adiponectin, in AT provides a better understanding of the expression of these genes.

Although the number of studies investigating fatty acids and adipokines in cancer is limited, it suggests that there might be some variation in adipose tissue adipokines and fatty acids composition of cancer patient compared to healthy subjects or patients with benign tumours. It seems important to identify the alterations, and causes of these changes in adipose tissue composition throughout the cancer progression to plan effective nutritional interventions. The objective of this study was to assess adipokines protein levels and fatty acid composition of visceral and subcutaneous adipose tissue triglyceride and phospholipids in colorectal cancer patients and compare the proportions of adipokines and fatty acids in these two depots.

3.2 Methods

3.2.1 Study design and patient population

The study was approved by the Alberta Cancer Board Research Ethics Board (Edmonton, Alberta, Canada). All patients were recruited from the Tom Baker Cancer Centre, University of Calgary Medical Clinic, Foothills Medical Center and Peter Lougheed Hospital (Calgary, Alberta, Canada) between January 2008 and December 2010. Eligible patients were identified and asked to

participate by their physician or surgeon. All patients with a gastrointestinal or hepatopancreatobiliary tumor who understood and signed informed consent were eligible to participate in this study. Adipose tissue (visceral and subcutaneous) samples were collected at the time of surgery. Subcutaneous fat was harvested from the periumbilical area; visceral adipose tissue (VAT) was collected from subomental fat at the surgical site. Samples were snap frozen in liquid nitrogen and stored at -80˚C until analysis. Frozen samples were shipped on dry ice in batches to Dr. Mazurak's lab.

The Alberta Cancer Registry is a computerised database, designed for the collection of data regarding all cancer cases in the province of Alberta. This database was searched for information about cancer site, morphology, clinical and demographic characteristics for each of the subjects. Patients with a primary diagnosis of CRC that were matched to available samples from patients were selected for this study. Analysis was performed on patients who had provided both subcutaneous and visceral and did not undergo chemotherapy treatment to surgery (n=16). As there was some missing information in cancer registry, additional information was obtained from ARIA Manager (V 8.6), which is information and image management system that aggregates patient data into an electronic medical chart. Each patient's chart was reviewed and additional data including age, gender, BMI, liver metastases, stage, date of sample collection, chemotherapy, and computed tomography (CT) scans available were collected (n=16).

3.2.2 Adipose Tissue Fatty Acid Analysis

3.2.2.1 Lipid Extraction

Frozen adipose tissues were ground in liquid nitrogen using mortar and pestle until crushed into a fine powder (14). Lipids were extracted using modification of the Folch procedure (15), by adding 1ml chloroform/methanol (2:1 vol/vol) to each tube, sonicating, then adding 3 ml chloroform/methanol (2:1) and 0.8ml of 0.025% $Cacl_2$ sequentially, with vortexing after each step. Samples were stored overnight at 4C to allow layers to separate. The following day, the bottom layer was drawn up with glass pipette, transferred into a clean test tube. The top layer was washed with 1ml C/M/H_2O (86:14:1), and vortexed. After layers separated, the second bottom layer was added to the first bottom layer, and dried down under nitrogen gas. Chloroform/methanol (2:1; 300 µl) was added to each dried tube and vortexed.

3.2.2.2 Thin Layer Chromatography

Thin layer chromatography plates (SILICA GEL G 20X20 cm, Analtech Inc) were prepared in chloroform wash in G tank and then dried in oven 110˚C for one hour. The developmental chamber was lined with Whatman filter paper and solvent (80:20:1 petroleum ether [PE]/ diethyl/ethyl ether [De]/acetic acid [glacial; HAC]) was added to the tank and allowed to equilibrate for one hour. After spotting, plates were run in a solvent system until solvent reached 1-2 cm from the top of the plate. Plates were dried, sprayed with 0.1% 8-anilino-1-naphthalenesulfonic acid (ANSA). Phospholipids and triglyceride bands identified under UV light, scraped and added to clean methylation tubes.

3.2.2.3 Lipid Isolation

For quantification, 50µL C17:0 [20.45 mg/200ml] and 100 µL C15:0 [10.2 mg/100 ml] were added as standard to PL tubes and TG tubes respectively. KOH in methanol (0.5N) was added to TG samples and tubes were left in heating block for 1 hour at 110˚C. Both PL and TG tubes were methylated using 1 ml BF_3 (14%; in methanol) and 2 ml hexane. Tubes were heated in heating block for 1 hour. After 1 hour, tubes were cooled at room temperature. Distilled deionized H_2O (1ml) was added to each tube and tubes were vortexed. The top layers were transferred to small tubes (microvials) and dried under nitrogen. After adding 2 ml hexane to each dried tubes, tubes were capped well and stored at -80˚C until analysis.

3.2.2.4 Gas liquid chromatography

Samples were analyzed by automated gas liquid chromatography (Varian CP-3400) on a fused silica BP20 capillary column. Fatty acid methyl esters were separated by an automated gas-liquid chromatograph, varian model 3900 equipped with a vista 8400 autosampler. The system used a bonded phase fused silica capillary column, BP20: 25mm X 0.25 OD SGE product. Helium was used as the carrier gas at a flow rate of 2.6 ml/minute using a splitless injector. The GLC oven temperature of 175˚C was increased to 215˚C at 20˚C/minute and held for 23 minutes followed by a second stage temperature increase to 240˚C at 5/minute for a total analysis time of 76 minutes. These conditions separate most of saturated monounsaturated and polyunsaturated fatty acids from 14 to 24 carbon chain lengths. Percent content of saturated (SFA), monounsaturated

(MUFA), polyunsaturated (PUFA), total n-6 and n-3 fatty acids were analyzed at each timepoint.

3.2.3 Adipokines protein analysis

3.2.3.1 Tissue preparation

Visceral and subcutaneous samples were first weighed and mixed with NP40 Cell Lysis Buffer (Invitrogen Corporation, Frederick, MD, USA) in a 1:2 ratio for homogenization. Tissue was homogenized on ice using a glass on glass homogenizer. The samples were then centrifuged at 20 000xg for 10 minutes at 4ºC. Lipid fractions were discarded and the supernatant fraction separated from the pellet. Supernatant fractions were stored at -80ºC prior to analysis.

3.2.3.2 Adipokines quantification

Supernatant fractions were diluted in phosphate buffered saline and protein was quantified using Pierce BCA Protein Assay (Thermo Scientific, Rockford, IL, USA). Absorbance was read by spectrophotometry at 562 nm. Tissue homogenate was standardized for protein content based on the sample with the lowest protein content and analyzed for presence of IL-6, leptin, MCP-1, and TNF-α (Human Adipocyte Milliplex kit, Millipore, Billerica, MA, USA) using Luminex xMAP technology (Bioplex-200, Bio-Rad Laboratories, Mississauga, ON, Canada). Adiponectin was quantified by enzyme-linked immunosorbent assay (Human Adiponectin ELISA, Millipore, Billerica, MA, USA).

3.2.4 Statistical analysis

Data are expressed as mean ± SD. Two sample t-tests were used for comparison of mean values. Statistical significance was determined at $P \leq 0.05$.

However, we used a Bonferroni correction to adjust the P value ($P < 0.01$) for number of t-tests performed (total=4) on the fatty acids and adipokines comparison between obese versus non-obese and women versus men. Statistical analyses were performed using SigmaPlot 12.0 for Adipokines and SPSS (SPSS for Windows, version 17.0, SPSS, Chicago, IL) for fatty acid analysis.

3.3 Results

3.3.1 Patient population

Colorectal cancer patients who had both visceral and subcutaneous adipose tissue samples were selected for this study (n=16). Patients were in stage III or IV and all had liver metastases. Subject characteristics including age at sample collection, gender, presence of liver metastases, and BMI are shown by gender in Table 3-1. There were no significant differences in age or BMI between males and females. Despite generally advanced disease, the majority of patients were overweight or obese (mean= 27.9 ± 5.2). Not all subjects had a weight loss history available.

3.3.2 Adipose tissue triglyceride fatty acid composition

The fatty acid composition of TGs in visceral and subcutaneous adipose tissue regions are shown in Table 3-2. The most abundant fatty acids in TGs in each of these two depots were 18:1 n-9, followed by 16:0 and 18:2 n-6. The types of fatty acid found in TGs in VAT did not differ significantly from those in SAT, with the exception of 16:1 n-9 which was more abundant in VAT. No significant difference was observed in the proportion of total saturated fatty acids,

monounsaturated fatty acids, or n-3 and n-6 polyunsaturated fatty acids between the two depots.

Patients were divided into groups based upon BMI as this factor has been considered as confounding factors in CRC studies. No significant difference was noted in the proportion of saturated and monounsaturated fatty acids of VAT or SAT between overweight/obese and non-obese groups (Table 3-3 and 3-4). In both VAT and SAT, there was a trend for obese patients to have higher n-6 FAs compared to non-obese patients. Except for higher amounts of MUFAs in women, no significant differences were found in fatty acid composition of VAT and SAT TGs between men and women groups. Fatty acid composition of TGs in VAT and SAT by sex are represented in Table AP1 and AP2.

3.3.3 Adipose tissue phospholipid fatty acid composition

The fatty acid composition of PLs in visceral and subcutaneous adipose tissue is shown in Table 3-5. The fatty acid compositions of PLs in VAT differ significantly from that of SAT, with respect to SFAs and PUFAs. There was a significantly higher proportion of SFAs in SAT contributed by higher proportions of 14:0, 16:0, 18:0. The higher proportions of n-6 FA in visceral adipose tissue PLs were attributable to 20:3 n-6, 20:4 n-6, and 22:2 n-6.

There were no significant differences in fatty acid composition of VAT and SAT PLs between overweight/obese and non-obese (Table 3-6 and 3-7). Men compared to women tended to have higher proportion of PUFAs in visceral depots (Table AP3 and AP4).

3.3.4 Adipose tissue adipokines composition

The cellular protein levels of 5 adipokines including IL-6, TNF-α, leptin, adiponectin and MCP-1 were compared between visceral and subcutaneous adipose tissue. Adipokine amounts were variable between subjects but overall, there was no significant difference between adiponectin, IL-6, TNF-α amount in these depots. SAT had higher levels of leptin compared to VAT (Table 3-8). For TNF-α, values were below the level of quantification in 11 subjects of 16; therefore these values were deleted from the analysis. MCP-1 levels were significantly higher in VAT compared to SAT. No significant difference was found between the sexes (Table AP5). MCP-1 was more highly expressed in VAT compared to SAT, including in non-obese groups (Table 3-9). In both overweight/obese and non-obese groups leptin levels were higher in SAT vs. VAT. However, when we compared overweight/obese and non-obese groups within each depot, overweight/obese patients had more leptin compared to non-obese patients.

3.4 Discussion

There are few data on lipid metabolism in cancer in general and specially in CRC patients (7, 16), thus the adipose tissue fatty acid profile of cancer patients is not well established. The present pilot study provides, for the first time, data on fatty acids and adipokines that characterize visceral and subcutaneous adipose tissue in CRC patients at advanced stages of cancer. Understanding the fatty acid composition of these depots as a first step may be important in determining different metabolic behaviours of visceral and subcutaneous adipose tissue.

No significant differences were observed in fatty acid composition of TG between visceral and subcutaneous depots in CRC patients except for 16:1 n-9. Schoen et al. (9) reported a significant correlation between visceral and subcutaneous fatty acid content in 19 patients undergoing exploratory laparotomy. Fourteen subjects underwent surgery for colorectal cancer. They did not find any consistent pattern for one site compared the other and the variability in fatty acid content was greater between individuals compared to depots within an individual. However studies reported higher amounts of saturated fatty acids in visceral adipose tissue compared to subcutaneous (5, 17).

Fatty acid composition of adipose tissue has been considered as a marker of long term dietary fatty acid intake. However, there is great variability in the association between dietary saturated (except for odd-numbered fatty acids) and mono-unsaturated fatty acids and adipose tissue content (18). Polyunsaturated fatty acids (PUFAs: n-6 and n-3) are the best markers of dietary intake as these fatty acids can not be synthesized endogenously (19). A study in breast cancer patients by Zhu et al. suggested that changes in adipose tissue fatty acids composition may be more related to diet than cancer (7). The present study was retrospective in nature, so we were unable to collect information on patients' dietary intake which limits our discernment of our results. Another limitation of this study is that adipose tissue samples were collected at a single time patient and patients were all at stage III or IV, so we could not compare the fatty acid composition of these two depots at two different time points during cancer

progression, or in different stages to see whether cancer may impact on adipose tissue composition.

Differences in fatty acid composition were observed in obese compared to non-obese subjects. Obese patients compared to non-obese had higher levels of PUFAs especially n-6 fatty acids in both VAT and SAT depots. Modification in dietary fat sources may be the way to change the adipose tissue PUFA composition as increased levels of n-6 PUFAs may contribute to increased risk of some types of cancers (20).

A markedly higher proportion of SFAs in subcutaneous PLs and higher percentage of PUFAs in visceral PLs were observed. Adipose tissue membrane fatty acid composition is a major determinant of physical and functional properties of membrane (5) which can affect membrane associated functions including phosphate transport across the inner mitochondrial membrane, glucose transport, insulin receptor functions and the activity of membrane-bound enzymes, transporters, receptors, and prostaglandin production (21, 22). Other studies found that membrane fluidity is an important modifier of cell function (23) and PUFAs in membrane PLs have an impact on cell membrane fluidity and permeability and subsequentlyon physiological functions (24). VAT is metabolically more active than SAT, involved in inflammation, insulin resistance (25), contained more mitochondria per milligram of tissue, and has a higher oxidative phosphorylation activity (26) and higher expression levels of specific insulin signalling proteins (25) so higher PUFAs in VAT PLs is conceivable as it

is associated with greater membrane fluidity and more efficient membrane mediated functions.

There were differences in adipokines protein levels between VAT and SAT. Higher level of leptin in SAT was observed in VAT which is consistent with the literature (11). MCP-1 levels were higher in VAT. This finding is similar to what has been reported previously in obese subjects (10, 11). TNF-α levels were below detectable limits, which is similar to a report by Campbell et al (27). In that study, SAT biopsies were incubated in culture for 3 hours and both mRNA and protein levels of leptin, adiponectin, IL-6 and TNF-α were measured respectively by RT-PCR and ELISA technique (27). mRNA levels of leptin, adiponectin, TNF-α and IL-6 were detectable but protein levels for TNF-α and IL-6 were below the level of quantification which suggests that gene expression does not associate with protein synthesis. However, other studies have confirmed correlations between human adipose tissue mRNA and protein levels for several genes including those we measured in this study (28, 29).

This is the first study which assesses both adipokines protein levels and fatty acid composition of visceral and subcutaneous adipose tissue with respect to sex and weight which are two confounding factors in cancer related studies. A major limitation of this part of this study was that we did not collect data about patients' dietary intake and also we obtained adipose tissues at a single time point in the trajectory. Thus it is not known whether cancer progression after surgery may affect adipokines and fatty acid composition of these two depots.

Tables

Table 3-1: Patient demographics and anthropometrics

Characteristic	Women	Men	P-value
Total N (%)	5 (31.2)	11 (68.7)	
Age (years) at sample collection	58.4 ± 6.8 (50-68)	59.2 ±11.3 (38-77)	0.89
Body Mass Index, Kg/m^2	27.8 ± 8.4 (19.5- 38.5)	28 ± 3.8 (21-34.9)	0.95
Liver metastases	All	All	
Post-surgery days (till September 2011)	881 ± 312	856 ± 180	

Mean ± SD, two sample t-test, $P<0.05$.

Table 3-2: Fatty acid composition of Triglyceride in visceral and subcutaneous adipose tissue of colorectal cancer patients

Fatty acid	VAT (% of total)	SAT (% of total)	P-value
C14:0	3.4 ± 1.3	2.9 ± 0.9	0.14
C16:0	22.5 ± 2.5	22.4 ± 2.5	0.92
C16:1	5.3 ± 1.5	4.2 ± 1.0	0.02
C18:0	4.3 ± 1.2	4.3 ± 1.5	0.98
C18:1(9)	48.4 ± 3.6	49.1 ± 6.3	0.68
C18:2(6)	12.7 ± 2.5	13.7 ± 6.7	0.58
C18:3(3)	0.9 ± 0.3	1.0 ± 0.3	0.68
C20:4(6)	0.3 ± 0.1	0.3 ± 0.1	0.53
C20:5(3)	0.1 ± 0.1	0.1 ± 0.0	0.70
C22:6(3)	ND	ND	
ΣSFA	30.8 ± 4.3	30.1 ± 4.2	0.64
ΣMUFA	54.5 ± 3.0	54.2 ± 6.7	0.86
ΣPUFA	14.7 ± 2.6	15.7 ± 6.8	0.57
Σn-6	13.5 ± 2.5	14.4 ± 6.9	0.57
Σn-3	1.2 ± 0.4	1.2 ± 0.4	0.99

Mean ± SD, paired student's t-test, n=16, $P<0.05$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; ND, not detected.

Table 3-3: Fatty acid composition of Triglyceride in visceral adipose tissue of non-obese and overweight/obese groups

Fatty acid	Non-obese (% of total)			Overweight/ Obese (% of total)			P-value
C14:0	4.3	±	1.7	3.1	±	1.0	0.08
C16:0	22.9	±	3.0	22.4	±	2.5	0.69
C16:1	6.3	±	1.9	4.9	±	1.1	0.07
C18:0	4.6	±	1.3	4.2	±	1.3	0.57
C18:1(9)	47.5	±	5.7	48.8	±	2.6	0.51
C18:2(6)	11.4	±	2.0	13.6	±	2.2	0.07
C18:3(3)	0.7	±	0.1	0.1	±	0.3	0.08
C 20:4(6)	0.3	±	0.1	0.3	±	0.1	0.99
C20:5(3)	0.1	±	0.1	0.1	±	0.1	0.79
C22:6(3)	ND			ND			
ΣSFA	32.3	±	5.3	30.2	±	4.0	0.37
ΣMUFA	54.6	±	4.5	54.5	±	2.5	0.94
ΣPUFA	13.1	±	1.4	15.8	±	2.1	0.02
Σ n-6	12.0	±	1.7	14.5	±	2.1	0.04
Σ n-3	1.0	±	0.4	1.3	±	0.4	0.20

Mean ± SD, unpaired student's t-test, n=16, $P<0.01$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND not detected.

Table 3-4: Fatty acid composition of Triglyceride in subcutaneous adipose tissue of non-obese and overweight/obese groups

Fatty acid	Non-obese (% of total)	Overweight/Obese (% of total)	P-value
C 14:0	3.4 ± 1.3	2.7 ± 0.7	0.17
C 16:0	23.2 ± 3.6	22.5 ± 1.9	0.62
C16:1	4.9 ± 1.4	4.0 ± 0.8	0.14
C 18:0	4.5 ± 1.7	4.2 ± 1.6	0.81
C 18:1(9)	48.6 ± 5.0	49.4 ± 2.0	0.65
18:2(6)	12.3 ± 2.0	14.1 ± 1.8	0.14
C 18:3(3)	0.8 ± 0.1	1.0 ± 0.3	0.39
C 20:4(6)	0.3 ± 0.1	0.3 ± 0.1	0.59
C 20:5(3)	0.0 ± 0.0	0.1 ± 0.04	0.29
C22:6(3)	ND	ND	
ΣSFA	31.8 ± 5.6	29.9 ± 3.6	0.45
ΣMUFA	54.0 ± 4.1	54.1 ± 1.7	0.97
ΣPUFA	14.1 ± 1.7	16.3 ± 1.8	0.08
ΣSUM n-6	13.1 ± 1.8	14.9 ± 1.8	0.11
ΣSUM n-3	1.1 ± 0.2	1.2 ± 0.4	0.36

Mean ± SD, unpaired student's t-test, n=16, P<0.01. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table 3-5: Fatty acid composition of phospholipids in visceral and subcutaneous adipose tissue of colorectal patients

Fatty acid	VAT (% of total)			SAT (% of total)			P-value
C14:0	2.6	±	1.0	3.7	±	1.2	0.01
C16:0	23.4	±	2.9	25.6	±	2.9	0.04
C16:1	1.1	±	1.4	0.5	±	1.1	0.14
C18:0	15.4	±	1.5	17.4	±	2.8	0.02
C18:1(9)	11.7	±	3.8	12.3	±	3.1	0.64
C18:2(6)	7.2	±	2.0	6.2	±	3.0	0.27
C20:0	5.2	±	1.8	3.1	±	2.4	0.01
C20:3(6)	5.0	±	1.9	3.1	±	2.1	0.01
C20:4(6)	4.8	±	1.2	3.3	±	1.8	0.01
C20:5(3)	4.5	±	1.5	2.4	±	1.9	0.001
C22:2(6)	3.7	±	1.8	1.3	±	1.6	0.0
C22:6(3)	ND			ND			
ΣSFA	57.6	±	4.5	62.5	±	4.7	0.01
ΣMUFA	15.2	±	6	17.4	±	3.5	0.41
ΣPUFA	26.3	±	4.6	20.1	±	2.8	0.0006
Σn-6	20.9	±	3.8	14.6	±	4.7	0.0007
Σn-3	5.4	±	1.9	4.7	±	4.1	0.28

Mean ± SD, paired student's t-test, n=16, $P<0.05$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; ND not detected.

Table 3-6: Fatty acid composition of phospholipids in visceral adipose tissue of non-obese and overweight/obese groups

Fatty acid	Non-obese (% of total)			Overweight/Obese (% of total)			P-value
C 14:0	3.1	±	0.6	2.4	±	1.1	0.21
C 16:0	24.5	±	3.3	22.9	±	2.7	0.33
C16:1	1.8	±	1.7	0.8	±	1.1	0.15
C 18:0	14.4	±	1.0	15.9	±	1.5	0.08
C 18:1(9)	10.6	±	6.7	12.2	±	1.7	0.46
C18:2(6)	6.9	±	2.5	7.4	±	1.8	0.64
C20:0	4.5	±	1.6	5.5	±	1.8	0.28
C20:3(6)	3.6	±	1.9	5.6	±	1.6	0.05
C20:4(6)	5.0	±	1.2	4.7	±	1.3	0.67
C20:5(3)	4.1	±	1.4	4.7	±	1.5	0.44
C22:2(6)	3.5	±	1.4	3.8	±	1.9	0.79
C22:6(3)	ND			ND			
ΣSFA	56.2	±	5.2	58.5	±	4.4	0.38
ΣMUFA	12.9	±	6.3	13.3	±	2.3	0.84
ΣPUFA	24.5	±	5.6	27.0	±	4.2	0.33
Σn-6	19.1	±	4.1	21.5	±	3.7	0.26
Σn-3	5.4	±	2.8	5.5	±	1.6	0.95

Mean ± SD, unpaired student's t-test, n=16, $P<0.01$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table 3-7: Fatty acid composition of phospholipids in subcutaneous adipose tissue of non-obese and overweight/obese groups

Fatty acid	Non-obese (% of total)			overweight/obese (% of total)			P-value
C14:0	3.2	±	0.8	3.8	±	1.3	0.34
C16:0	22.5	±	2.7	25.6	±	3.1	0.95
C16:1	0.4	±	0.9	0.5	±	1.2	0.86
C18:0	18.2	±	3.1	17.0	±	2.7	0.45
C18:1(9)	12.9	±	3.6	12.0	±	3.0	0.60
C18:2(6)	6.7	±	2.6	6.0	±	3.2	0.67
C20:0	2.7	±	1.9	3.3	±	2.6	0.63
C20:3(6)	2.6	±	1.9	3.3	±	2.2	0.51
C20:4(6)	2.3	±	2.1	3.7	±	1.6	0.15
C20:5(3)	1.8	±	2.1	2.6	±	1.9	0.47
C22:2(6)	1.0	±	1.0	1.4	±	1.8	0.65
C22:6(3)	ND			ND			
ΣSFA	63.9	±	6.4	68.5	±	8.1	0.38
ΣMUFA	13.5	±	4.8	12.8	±	3.8	0.84
ΣPUFA	18.3	±	4.5	19.7	±	5.7	0.33
Σn-6	12.8	±	3.6	15.4	±	5.8	0.26
Σn-3	5.5	±	2.3	4.3	±	1.8	0.95

Mean ± SD, unpaired student's t-test, n=16, P<0.01. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; ND not detected.

Table 3-8: Adipokines protein levels in visceral and subcutaneous adipose tissue of colorectal cancer patients

Adipokines (ng/ml)	VAT	SAT	P-value
MCP-1	119.7 ± 42	79.5 ± 36.4	0.003
TNF-α	ND	ND	
Leptin	1284.6 ± 964	2329 ± 1491	0.04
IL-6	3.3 ± 2	3.4 ± 1.4	0.39
Adiponectin (pg/ml)	520.8 ± 182	507.8 ± 170.4	0.68

Mean ± SD, paired student's t-test, n=16, $P<0.05$. Abbreviations: MCP- 1, Monocyte chemotactic protein-1; TNF-α Tumor necrosis factor-alpha; IL-6, Interleukin-6; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; ND not detected.

Table 3-9: Adipokines protein levels in visceral and subcutaneous adipose tissue of Overweight/obese and non-obese groups

Adipokines (ng/ml)	Overweight/obese	Non-obese	P-value
	Visceral adipose tissue		
MCP-1	116.4 ± 44	195.8 ± 125	0.07
TNF-α	ND	ND	ND
Leptin	1641.4 ± 923	416.2 ± 363	0.01
IL-6	3.2 ± 2.0	3.9 ± 2.5	0.64
Adiponectin (pg/ml)	538.6 ± 201	522.0 ± 125.2	0.86
	Subcutaneous adipose tissue		
MCP-1	83.8 ± 36.3	67.5 ± 39	0.46
TNF-α	ND	ND	ND
Leptin	2921.0 ± 1433.5	1432.7 ± 1008	0.09
IL-6	3.4 ± 1.4	3.2 ± 2	0.86
Adiponectin (pg/ml)	496.6 ± 167	538.4 ± 203	0.69

Mean ± SD, unpaired student's t-test, n=16, P<0.01. Abbreviations: MCP- 1, Monocyte chemotactic protein-1; TNF-α, Tumor necrosis factor-alpha; IL-6, Interleukin-6; ND, not detected.

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CHAPTER 4: Relationship of adipose tissue fatty acid composition and changes in fat mass in Colorectal Cancer patients

4.1 Introduction

Adipose tissue is composed of different fatty acids with various potential health effects. Experimental data have shown that dietary SFAs and n-6 PUFAs have tumour-enhancing properties in the colon (1). n-6 PUFAs may contribute to carcinogenesis through a variety of mechanisms such as effecting membrane fluidity, enhancing tumour proliferation, receptor availability, and prostaglandin or peroxides formation (2, 3). However, n-3 PUFAs appear to exhibit anti-cancer properties such as promoting anti-tumour immunity and inhibiting cancer initiation, tumour angiogenesis, and metastasis (4). Dietary n-3 fatty acids inhibit the production of cytokines such as TNF-α and interleukin-1 beta (IL-1ß) and suppress the production of the n-6 fatty acids eicosanoid inflammatory mediators (5). As adipose tissue is a biomarker of dietary fatty acid intake, especially for n-3 and n-6 polyunsaturated fatty acids (6), alterations in dietary fatty acid intake and in consequence, changes in adipose tissue fatty acid composition may alter the production of carcinogenic mediators (7, 8) and may affect cancer progression.

Body composition assessment using CT images in patients of advanced cancer reveals loss of adipose tissue as cancer progress. It is known that fat loss occurs in both visceral and subcutaneous regions; however intramuscular adipose tissue is the only fat depot that increases approaching death in cancer patients (9). Rate of adipose tissue loss increases as patients approach death (10, 11). It has been reported that 3 months from death adipose tissue from the visceral region is

lost to a greater degree than subcutaneous fat in patients with gastrointestinal carcinoma (11). Little is known about the prognostic significance of fat mass in cancer; however, studies have reported fat mass to be an important predictor of the length of survival (9, 10).

Investigation of the associations between adipose tissue fatty acid composition and fat mass alterations throughout the cancer progression is complex and has not been explored. It is not known if adipose depots are lost at similar rates or if they differ in rates of loss. Achieving a better understanding of this association may yield effective interventions to preserve fat mass in cancer. The objective of this work was to determine whether the fatty acid composition of subcutaneous and visceral relate to losses of adipose tissue mass in CRC patients.

4.2 Methods

This study was approved by the Alberta Cancer Research Ethics board.

4.2.1 Patient population, demographics and anthropometrics

Among colorectal cancer patients with both visceral and subcutaneous samples available (described in detail in Chapter 3), 7 patients who had at least 2 CT images in the interval between diagnosis (before surgery) and after surgery, were selected for body composition assessment. The present analysis is focused on the relationship between adipose tissue fatty acids and fat mass alterations in CRC patients. To relate body composition to adipose tissue fatty acids, it was important to ensure a defined time period between the collection of adipose tissue and CT image. However, due to small number of subjects, patients with a CT image within 2 to 7 months of a surgery were selected from the population.

4.2.2 CT Image Analysis

Body composition was measured using secondary analysis of images that were retrieved from the patient clinical record. Total adipose tissue area measurement was conducted by analyzing CT scans at 3rd lumbar vertebra (L3). The third lumbar was selected as a standardized landmark, as adipose tissue areas in single CT image at L3 correlate with whole body fat mass (12, 13). Two consecutive transverse CT images extending from L3 to iliac crest were assessed using Slice-O- Matic (V4.3; Tomovision, Montreal, QC, Canada). Adipose tissue cross-sectional areas were calculated by using standard Hounsfield unit thresholds of -150 to -50 for visceral adipose tissue (14) and -190 to -30 for subcutaneous and intramuscular adipose tissue (15).

Tissue cross-sectional areas (cm^2) were calculated by summing the given tissue pixels and multiplying by the pixel surface area. Mean tissue areas for 2 consecutive images were calculated. Visceral, subcutaneous, intramuscular cross-sectional areas were summed to estimate total AT areas. Regression Equations derived from an advanced cancer patient cohort were used to estimate whole body fat mass.

Whole body FM (kg) = 0.068 * (total adipose at L3 (cm^2)) + 4.142; $r^2 = 0.927$ (12)

The timing and the interval between two scans differ between patients, so the rate of AT change within a specific interval were calculated, expressed as a percentage, and divided by the number of days in each interval to calculate the daily rate of change for each patient. A standard unit was needed to compare fat

loss or gain between individuals, so the daily rate of change was multiplied by 100, and expressed as % change /100d.

4.2.3 Statistical Analysis

Data were presented as mean±SD. $P<0.05$ was considered statistically significant. Statistical analysis was completed using the Student's two-tailed t-test to identify the differences between groups when data were normally distributed. When data is not normally distributed, the statistical analysis was based on nonparametric methods (Mann-Whitney and Wilcoxon). AT mass alterations were tested for the presence of outliers and one patient was eliminated from analysis as the time interval between 2 CT images was less than one month. Spearman's rank correlation tests were used to determine potential relationships between fatty acids and rate of adipose tissue change. Statistical analyses were performed with SPSS (SPSS for Windows, version 17.0, SPSS, Chicago, IL).

4.3 Results

4.3.1 Patient demographics

Among all 16 patients with both VAT and SAT samples available, 7 had two CT images at the time period between before and after surgery. Each patient's characteristics are shown in Table 4-1. There were no significant difference between men and women with regard to BMI (28.9 ± 7 versus 27.1 ± 2.7) or age (58 ± 4.7 versus 55 ± 13.7). All but 2 subjects would be classified as overweight (n=2) or obese (n=3).

4.3.2 Visceral and subcutaneous mass alterations over time and adipose tissue fatty acid analysis

There was variability in lumbar adipose tissue within both genders. In men, adipose tissue area ranged from 279 to 713cm^2 (23.1 to 52.7kg estimated whole body fat mass) in initial CT image and ranged from 152 to 547cm^2 (14.5 to 41.3kg estimated whole body fat mass) in CT images following surgery. In women, adipose tissue area ranged from 135 to 470 cm^2 (13.3 to 36.1kg estimated whole body fat mass) in initial CT image and ranged from 181 to 513 cm^2 (16.4 to 39kg estimated whole body fat mass) in the second CT image. The time interval between two scans was 150 ± 107 days.

From the first to the second CT image, the majority of patients (n=5) were losing fat and just 2 patients were gaining fat. The rates of adipose tissue change ranged widely from intense loss (-39.8%/100d; -6kg fat loss/100d) to gain (6%/100d; +0.75kg fat). In the group losing fat, mean total AT rate of change/100days was -18.8 ± 13.1%/100d whereas for those gaining fat, the mean increase was 5.3 ± 1.5%/100d. Reduction in VAT area as a percentage of total was greater than SAT area changes after surgery (-53.7 ± 44.7%/100d versus -8.6 ± 7.5%/100d) (Table 4-3) and (Figure4-1). Changes in total adipose tissue and each depot at the time interval between two CT images for each patient and collectively are shown in Table 4-2 and Table 4-3.

There was a statistically significant association between the changes in fat mass and proportions of PUFAs within the VAT depot. Patients who were losing fat, had higher proportions of PUFAs in their VAT compared to patients who

were gaining fat (%16.8 vs. %14.5) (P= 0.04). There was a significant correlation between VAT TGs PUFA levels and VAT rate of change/100days (Table 4-4). No correlation was found between fat mass changes and FAs proportions of SAT. Visceral adipose tissue SFA and MUFA concentrations did not differ between fat losing and gaining group (P= 0.42, P= 0.64, respectively). Among all PUFAs, the proportions of n-6 fatty acids in VAT were significantly correlated with fat mass alterations. The mean concentrations of VAT n-6 PUFAs were significantly higher in fat losing group compared to gaining group (mean amounts of 15.4 % in losing group vs. 13.4 % in gaining group).

The relationships between VAT TG n-6 FA levels and rate of change in VAT cross sectional area is depicted in a scatter plots in Figure 4-2. N-6 PUFAs decreased linearly from a maximum of 16.3 (% total fatty acid content) in patients with loss of fat to 13.1 in patients gaining fat. There were no significant correlations between adipose tissue PLs or n-6/n-3 ratio and the fat mass changes in 2 adipose tissue regions.

4.4 Discussion

Previous studies indicated that loss of adipose tissue is an indicator of shorter survival (9, 10). This is the first study to assess the relationship between adipose tissue fatty acid composition and fat mass alterations in cancer patients. It seems that the PUFAs are most related to changes in adipose tissue mass. Adipose tissue n-6 PUFAs proportions were higher in subjects with loss of adipose tissue after surgery in colorectal cancer patients. On the other hand, patients who gained fat had the least amount of n-6 fatty acids in their visceral adipose tissue. This

may support an association between fat mass alterations and proportions of n-6 fatty acids in adipose tissue.

The pattern of fat loss in cancer has not been consistently demonstrated. We found that in general, patients were losing visceral adipose tissue more than subcutaneous. This is similar to what was reported by Ogiwara et al. who reported diminished visceral adipose tissue in gastrointestinal cancer patients in the 3 months before death (11). Murphy et al. indicated losses of total AT, SAT and VAT in colorectal and lung cancer patients approaching death, with IMAT being the only depot which increased as patients approached death (9). Longitudinal CT image analysis in 34 advanced CRC patients demonstrated that the greatest changes in body composition occurred at 4.2 months from death. One month from death, liver and spleen mass increase and skeletal muscle (4.2 kg) and fat (3.5 kg) mass decrease (16).

Studies found n-6 PUFAs stimulate inflammatory reactions in adipocytes. This relationship may be partially explained by the inflammatory eicosanoid compounds that are derived from arachidonic acid (5, 17, 18). n-6 derived eicosanoids can lead to the higher production of proinflammatory cytokines at sites of inflammation (17). Prostaglandin E2 (PGE_2) and leukotriene B4 (LTB_4) as two major proinflammatory eicosanoids, derived from the n-6 fatty acid arachidonic acid (AA), are higher in inflammatory cells (18). PGE_2 can increase IL-6 production and release by macrophages and therefore influence fat mass (19). In vitro cell culture models demonstrated that PGE_2 may contribute to weight loss by inhibiting adipocyte differentiation which was supported by lower

expression of adipocyte differentiation markers such as peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding protein (20). Although PGE_2 and LTB_4 have pro-inflammatory potential, these eicosanoids are derived from membrane PUFAs (19). However in this study we could not find any relation between fat mass changes and PLs PUFAs content. Our study did not addresses the relationship between composition and inflammation so we may not conclude that higher n-6 in AT of losing group compared to fat gaining group may lead to greater fat loss due to higher inflammation. This relationship may alternatively be explained by observations of enhanced expression and function of HSL in adipocytes of weight losing cancer cachectic patients (21). HSL, as a major enzyme involved in AT lipolysis, shows selective hydrolysis of TGs which preferentially releases fatty acids from sn-1 and sn-3 positions of TGs (22). Adipose tissue TGs typically have SFA at sn-1 position and unsaturated fatty acids at sn-2 (23, 24). This may suggest that higher expression of HSL in AT of fat losing group compared to fat gaining group and subsequent hydrolysis of the TGs, can be associated with higher proportions of n-6 FAs in AT in patients losing fat. However, this hypothesis needs to be investigated in future studies.

Our study was designed to investigate the relationship between adipose tissue fatty acids composition and mass changes over the cancer progression in advanced CRC patients. The CT images in this study were from various time points before and after surgery. In many cases, the CT images that were available depict the changes mostly due to surgery not CRC per se, which is a limitation of our study. What was consistent, however, is that those losing fat, lost

predominantly VAT. This may reflect the fat mass changes would be regardless of where a patient is in the treatment trajectory after surgery. This study was designed as a pilot study with a limited sample size. We acknowledge that our results need to be confirmed in larger population of similar patients with a confined time interval between surgery and the CT images, where the impact of surgery does not confound the results. However, the association between n-6 PUFAs amount and fat loss was remarkable even with a small number of patients.

Tables

Table 4-1: Adipose tissue in colorectal cancer patients: Subjects Characteristics

Patient ID (n=7)	BMI (Kg/m^2)	Gender/ Age	[1]Estimated whole body fat mass, kg (CT#1)	[1]Estimated whole body fat mass, kg (CT#2)	% TAT Change/100d	Whole body fat mass Change/100d (Kg/100d)
1	33.5	F/62	36.1	39.0	4.2	1.46
2	27.4	F/53	23.7	21.3	-12.5	-2.14
3	30.0	M/67	52.7	41.3	-11.6	-4.30
4	26.7	M/40	23.1	17.7	-39.8	-6.1
5	19.6	F/54	13.3	16.4	6.3	0.78
6	24.6	M/58	23.5	14.5	-22.9	-2.36
7	35.0	F/61	32.8	29.9	-7.2	-1.85

Abbreviations: AT, adipose tissue; BMI, body mass index; TAT. Total adipose tissue

Table 4-2: Changes in total adipose tissue and each depot at the time interval between two CT images (% change/100d)

Patient ID	Total AT Rate of Change/100d	IMAT Rate of Change/100d	VAT Rate of Change/100d	SAT Rate of Change/100d	N-6 (% of total)
Losing Fat					
1	-12.5	7.1	-85.2	-5.2	14.8
2	-11.6	10.0	-15.7	-4.6	14.4
3	-39.8	16.3	-111.0	-21.9	16.1
4	-22.9	-21.1	-50.9	-4.5	16.3
5	-7.2	-42.0	-5.9	-7.0	15.1
Gaining Fat					
6	4.2	24.2	6.7	2.8	13.1
7	6.3	13.8	1.2	7.0	13.8

Abbreviations: AT, adipose tissue; IMAT, intramuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 4-3: Mean changes in total adipose tissue and each depot at the time interval between two CT images (% change/100d)

	Losing Fat (n=5)	Gaining Fat (n=2)	P-value
Total AT Rate of Change/100d	-18.8 ±13.0	5.3 ± 1.5	0.06
IMAT Rate of Change/100d	-5.9 ± 24.7	19.0 ± 7.3	0.19
VAT Rate of Change/100d	-53.7 ± 44.7	4.0 ± 3.9	0.15
SAT Rate of Change/100d	-8.63 ± 7.5	4.9 ± 3.0	0.10
P-Value (VAT vs. SAT)	0.05	0.66	

Data are represented as Mean ± SD., n=7, $P<0.05$. Two sample t-test for normally distributed data. Mann Whitney test and Wilcoxon Signed Rank Test for non-normal distributed data. Abbreviations: AT, adipose tissue; IMAT, intramuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

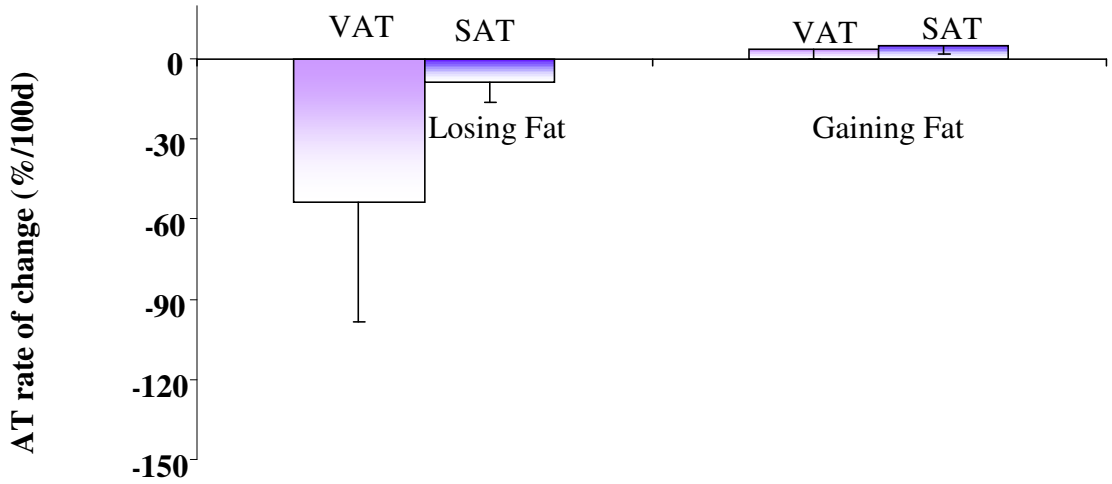
Table 4-4: Correlations between changes in fat mass and visceral adipose tissue triglyceride composition

	SFA		MUFA		N-6		N-3		N-6/N-3	
	r	P	r	P	r	P	r	P	r	P
Total AT Rate of Change/ 100d	0.28	0.53	0.23	0.61	-0.82	0.02	-0.9	0.17	0.64	0.12
IMAT Rate of Change/ 100d	0.5	0.23	-0.23	0.61	-0.58	0.18	-0.25	0.59	-0.07	0.88
VAT Rate of Change/ 100d	0.54	0.21	0.00	0.99	-0.75	0.05	-0.85	0.18	0.68	0.09
SAT Rate of Change/ 100d	0.60	0.15	-0.2	0.67	-0.6	0.15	-0.63	0.13	0.32	0.48

Spearman correlations, n=7, $P<0.05$. Abbreviations: AT, adipose tissue; IMAT, intramuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, poly unsaturated fatty acid.

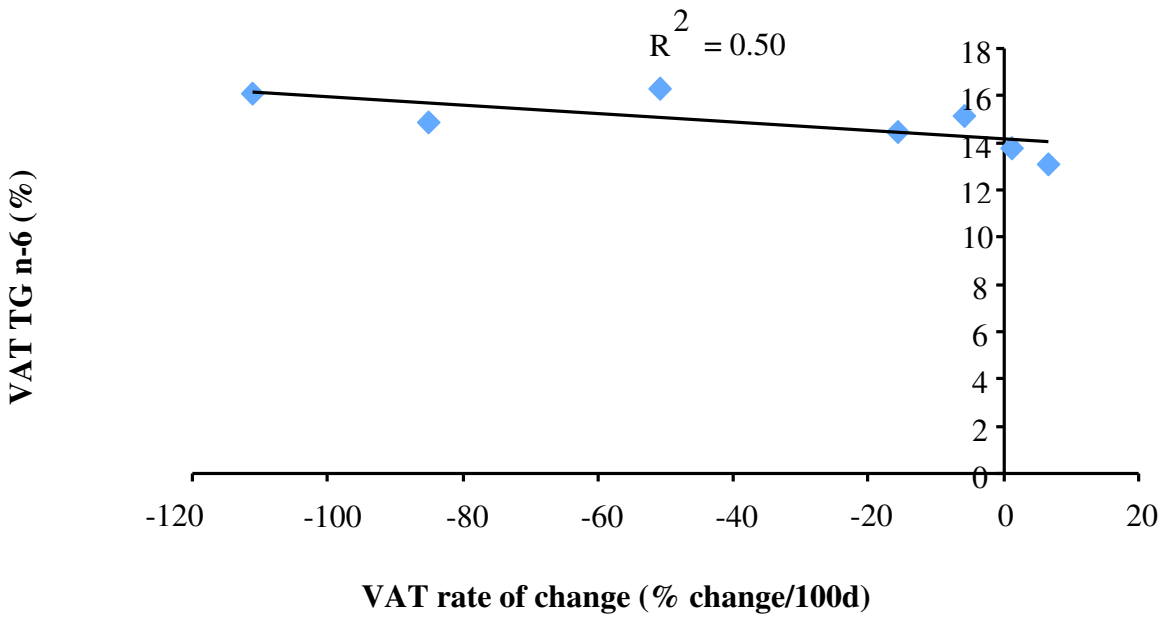
Figures

Figure 4-1: Mean rate of VAT and SAT change in fat losing and gaining patients as measured by computed tomography



Bars represent mean + SD, n=5 (Losing fat) and n=2 (Gaining fat). $P<0.05$. Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

Figure 4-2: Relationship between visceral adipose tissue rate of change (%/100d) and visceral adipose tissue triglyceride n-6 levels (%)



Linear regression, n=7; P=0.02. Abbreviations: VAT, visceral adipose tissue; TG, triglyceride.

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Chapter 5

5.1 Conclusion

The aim of this study was to explore the differences in adipokine levels and fatty acid composition between visceral adipose tissue (VAT) and subcutaneous (SAT) depots and relate this to change in fat mass (expressed as % change/100d) assessed using CT images obtained at 2 different timepoints in CRC patients. This discussion summarizes main points related to research results presented in the previous chapters and provides rationale for future research.

In chapter 3, it was hypothesized that the types of fatty acids and adipokine levels differ between these two depots. In this study we found no significant difference between VAT and SAT TG fatty acid composition. This was not consistent with our hypothesis where we expected higher levels of SFAs in VAT. However, we observed higher proportion of SFAs in SAT and higher PUFAs in VAT PLs which may be related to membrane associated functions. The protein levels of 5 adipokines levels were compared between depots and found that MCP-1 levels were higher in VAT and there was a tendency for SAT to have higher leptin levels. There was no significant difference between adiponectin, TNF-α and IL-6 amounts in these depots.

Chapter 4 tested the hypothesis that the pattern of fat mass changes was related to adipose tissue fatty acid composition. In this study we found that after surgery the majority of patients were losing fat and the patients with higher amounts of PUFAs, especially n-6 fatty acids in their TGs lost more fat.

Several limitations to this pilot study need to be acknowledged. The most important issue, and one that is commonly addressed, is the descriptive study design which limits our research. The major determinant of adipose tissue composition is dietary intake, but the retrospective study design did not allow for determination of fatty acids dietary intake. Moreover, dietary intake might have differed thorough the cancer trajectory which may affect on weight and fat mass changes. On the other hand, we had only one time measurement of adipokine levels in adipose tissue which limit our ability to assess the relationship between fat mass changes and adipokines

The small number is noteworthy. However, this pilot study on small number of CRC patients describes the differences between visceral and subcutaneous adipose tissue, we need to explore the differences in larger number of cancer patients, with matched group of female and male patients as gender is considered as one of the confounding factors in CRC studies. Moreover, in this study we examined the CRC patients with the majority of population being overweight or obese; therefore the results may not be generalized to patients with other types of cancers.

Among all 16 patients with both visceral and subcutaneous samples available, just 7 patients had two CT images available for analysis. Besides small number of patients, the time interval between two CT images varied among patients and was from different time points before and after surgery. So prospective studies with larger number of cancer patients with CT images at specific time intervals and at least two adipokines measurements are required for

better understanding of the relationship between fat mass changes, adipokines and fatty acid composition in various types of cancer patients.

The major strength of this study is that no studies to date have assessed the relationship between adipose tissue fatty acid composition and rate of fat loss in cancer neither in human nor in animals. It seems that a better understanding of fat loss in cancer is necessary for the development of effective treatments for wasting in cancer patients. Use of CT imaging to describe changes in adipose tissue in advanced cancer patients is another strength of this study. CT imaging, as a gold standard method, has the ability to precisely quantify adipose tissue.

The finding that the higher amounts of n-6 PUFAs in visceral adipose tissue may be associated with the greater fat loss in CRC patients after surgical resection is novel. This study yields data on expected baseline proportion for fat mass and adipose tissue composition in cancer patients. This could provide a basis for determining the concentration of both adipose tissue and plasma n-6 and n-3 fatty acids levels in a larger sample and to propose the necessity of supplementation for maintenance of fat in future studies. The significance of the association between higher amounts of n-6 fatty acids in VAT and greater fat loss and the importance of adipose tissue maintenance requires further investigation.

Tables

Table AP1: Fatty acid composition of Triglyceride in visceral adipose tissue of women and men groups

Fatty acid	Women (% of total)			Men (% of total)			P-value
C14:0	3.3	±	1.1	3.5	±	1.4	0.70
C16:0	21.6	±	3.1	22.9	±	2.4	0.36
C16:1	6.8	±	1.2	4.7	±	1.2	0.01
C18:0	3.5	±	0.9	4.6	±	1.3	0.13
C18:1(9)	49.5	±	5.2	48.0	±	2.9	0.42
C18:2(6)	12.3	±	2.2	12.8	±	2.8	0.69
C18:3(3)	0.9	±	0.3	0.9	±	0.3	0.85
C20:4(6)	0.3	±	0.1	0.3	±	0.1	0.63
C20:5(3)	0.1	±	0.1	0.1	±	0.1	0.70
C22:6(3)	ND			ND			
ΣSFA	28.8	±	5.1	31.6	±	4.0	0.24
ΣMUFA	57.0	±	3.7	53.6	±	2.2	0.03
ΣPUFA	14.2	±	2.0	15.4	±	2.4	0.36
Σn-6	13.0	±	2.1	14.1	±	2.3	0.37
Σn-3	1.2	±	0.4	1.2	±	0.4	0.93

Mean ± SD, unpaired student's t-test, n=16, P<0.01 Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; ND.

Table AP2: Fatty acid composition of Triglyceride in subcutaneous adipose tissue of women and men groups

Fatty acid	Women (% of total)	Men (% of total)	P-value
C14:0	2.5 ± 0.4	3.0 ± 1.0	0.31
C16:0	21.5 ± 1.8	23.1 ± 2.5	0.27
C16:1	5.0 ± 0.5	4.0 ± 1.0	0.09
C18:0	3.2 ± 0.8	4.7 ± 1.6	0.09
C18:1(9)	51.1 ± 2.3	48.4 ± 2.9	0.11
C18:2(6)	13.4 ± 1.0	13.0 ± 2.8	0.79
C18:3(3)	1.0 ± 0.1	0.9 ± 0.3	0.62
C20:4(6)	0.4 ± 0.1	0.3 ± 0.1	0.37
C20:5(3)	0.1 ± 0.0	0.1 ± 0.0	0.10
C22:6(3)	ND	ND	
ΣSFA	27.6 ± 2.9	31.4 ± 4.1	0.12
ΣMUFA	56.7 ± 2.6	53.1 ± 1.5	0.01
ΣPUFA	15.7 ± 1.0	15.6 ± 2.4	0.95
Σ n-6	14.3 ± 1.0	14.4 ± 2.3	0.98
Σ n-3	1.3 ± 0.2	1.1 ± 0.4	0.37

Mean ± SD, unpaired student's t-test, n=16, $P<0.01$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table AP3: Fatty acid composition of phospholipids in visceral adipose tissue of women and men groups

Fatty acid	Women (% of total)			Men (% of total)			P-value
C14:0	2.7	±	0.9	2.5	±	1.1	0.79
C16:0	24.8	±	2.5	22.8	±	2.9	0.19
C16:1	2.6	±	0.9	0.5	±	0.9	0.001
C18:0	15.5	±	1.1	15.3	±	1.7	0.83
C18:1(9)	11.1	±	6.7	12.0	±	2.0	0.68
C18:2(6)	7.4	±	2.2	7.1	±	2.0	0.78
C20:0	3.7	±	1.0	5.9	±	1.6	0.02
C20:3(6)	2.9	±	1.2	5.9	±	1.4	0.001
C20:4(6)	4.9	±	1.6	4.8	±	1.1	0.87
C20:5(3)	3.2	±	0.6	5.2	±	1.4	0.01
C22:2(6)	3.0	±	0.7	4.1	±	2.0	0.28
C22:6(3)	ND			ND			
ΣSFA	56.1	±	5.3	58.5	±	4.3	0.36
ΣMUFA	14.6	±	5.8	12.5	±	2.4	0.32
ΣPUFA	22.2	±	5.2	28.0	±	3.2	0.02
Σn-6	18.3	±	4.3	21.9	±	3.3	0.09
Σn-3	3.9	±	1.4	6.1	±	1.8	0.03

Mean ± SD, unpaired student's t-test, n=16, P<0.01. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table AP4: Fatty acid composition of phospholipids in subcutaneous adipose tissue of women and men groups

Fatty acid	Women (% of total)			Men (% of total)			P-value
C14:0	3.8	±	0.8	3.6	±	1.2	0.85
C16:0	26.4	±	2.8	25.4	±	3.0	0.61
C16:1	0.7	±	1.1	0.4	±	1.1	0.76
C18:0	18.9	±	3.2	17.0	±	2.7	0.30
C18:1(9)	13.6	±	4.8	12.0	±	2.7	0.42
C18:2(6)	6.1	±	3.6	6.3	±	3.0	0.92
C20:0	4.1	±	2.0	2.9	±	2.5	0.43
C20:3(6)	3.5	±	2.2	3.0	±	2.1	0.71
C20:4(6)	4.2	±	0.4	3.0	±	2.0	0.35
C20:5(3)	2.8	±	2.6	2.3	±	1.8	0.69
C22:2(6)	1.8	±	2.4	1.1	±	1.5	0.53
C22:6(3)	3.8	±	0.8	3.6	±	1.2	0.85
ΣSFA	65.2	±	3.0	67.5	±	8.5	0.65
ΣMUFA	14.6	±	6.3	12.7	±	3.5	0.46
ΣPUFA	20.4	±	5.4	19.0	±	5.4	0.69
Σn-6	16.1	±	5.1	14.2	±	5.4	0.60
Σn-3	4.3	±	1.5	4.7	±	2.1	0.77

Mean ± SD, unpaired student's t-test, n=16, $P<0.01$. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

Table AP5: Adipokines protein levels in visceral and subcutaneous adipose tissue

Adipokines (ng/ml)	Men	Women	P-value
	Visceral adipose tissue		
MCP-1	148.4 ± 98	131.50 ± 40.3	0.69
TNF-α	NA	NA	NA
Leptin	1216 ± 1049	973 ± 75	0.15
IL-6	3.3 ± 1.9	3.46 ± 2.8	0.92
Adiponectin (pg/ml)	530. 3 ± 162.2	540.8 ± 235.6	0.41
	Subcutaneous adipose tissue		
MCP-1	81.8 ± 37.8	72.7 ± 36.3	0.75
TNF-α	NA	NA	NA
Leptin	1959.2 ± 1024.1	3253.3 ± 2211.3	0.68
IL-6	3.3 ± 1.6	3.5 ± 0.8	0.91
Adiponectin (pg/ml)	484.7 ± 178.9	571.1 ± 146.8	0.92

Mean ± SD, unpaired student's t-test, n=16, $P<0.01$. Abbreviations: MCP- 1 Monocyte chemotactic protein-1, TNF-α Tumor necrosis factor-alpha, IL-6 Interleukin-6.