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The effect of eicosapentaenoic and docosahexaenoic fatty acids on body composition and response to chemotherapy in patients with lung cancer

by

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Abstract

Patients with lung cancer are at high risk for malnutrition and even mild weight loss has been associated with decreased median survival and poor response to chemotherapy. The purpose of this research was to describe fatty acids status during chemotherapy and determine if supplementation with fish oil attenuates loss of weight, muscle and adipose tissue and improves chemotherapy efficacy in patients with lung cancer. Patients with non-small cell lung cancer who were chemotherapy naive were accrued to 1 of 2 contemporary studies; a descriptive study of fatty acids in patients receiving standard chemotherapy (standard of care, SOC; no intervention) or an open label study of fish oil supplementation during chemotherapy (~2.5g eicosapentaenoic acid; EPA + docosahexaenoic; DHA per day). Blood was collected at baseline and throughout chemotherapy treatment. Chemotherapy toxicities and response to chemotherapy were determined. Plasma phospholipid fatty acids were isolated and quantified using gas liquid chromatography. Plasma cytokines were quantified using Multi-Array Assay kits. Body composition was assessed using diagnostic computed tomography images when available. The majority of patients were over 60 years old, had advanced disease and heavy body weights. In the SOC group, low amounts of fatty acids were observed in patients with advanced and progressive disease. Depletion of EPA and DHA was prevalent and was associated with low muscle mass, accelerated loss of skeletal muscle and adipose tissue. Supplementation with fish oil provided a benefit over SOC on weight, and skeletal muscle; 69% of patients in the fish oil group maintained or gained weight and muscle compared to 29% of

patients in the SOC group. This effect did not appear to be mediated through catabolic cytokines, as overall amounts of plasma cytokines did not change with fish oil supplementation and was not different SOC. Supplementation with fish oil also resulted in a 2-fold improvement in chemotherapy efficacy compared to SOC: 60% of patients in the fish oil group had a reduction in tumour size and there was a trend towards greater 1-year survival. These results demonstrate the potential of fish oil to improve the care and treatment of patients with lung cancer.

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List of Abbreviations

AA	arachidonic acid (20:4n-6)
ALA	alpha-linolenic acid (18:3n-3)
AJCC	American Joint Committee on Cancer
ANOVA	analysis of variance
AT	adipose tissue
BIA	bioelectrical impedance
BMI	body mass index
CB	clinical benefit
cm^2	centimeter squared
CT	computed tomography
d	day(s)
DHA	docosahexaenoic acid (22:6n-3)
DXA	dual energy x-ray absorptiometry
ECOG	Eastern Cooperative Oncology
EFA	essential fatty acid(s)
EPA	eicosapentaenoic acid (20:5n-3)
ESAS	Edmonton Symptom Assessment System
FAACT	Functional Assessment of Anorexia/Cachexia Therapy
FFM	fat-free mass
FM	fat mass
FO	fish oil
HDL	high density lipoprotein

HSL	hormone sensitive lipase
HU	Hounsfield units
IFN-γ	interferon gamma
IL-1ß	interleukin 1 beta
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IL-12p70	interleukin 12
IL-15	interleukin 15
IMAT	intermuscular adipose tissue
kg	kilogram
L3	third lumbar vertebrae
L	liter
LA	linoleic acid (18:2n-6)
LBM	lean body mass
LDL	low density lipoprotein
LPL	lipoprotein lipase
m^2	meter(s) squared
µg	microgram
mL	milliliter
MRI	Magnetic resonance imaging
MUAC	mid-upper arm circumference
N_2	nitrogen gas

NSCLC	Non-small cell lung cancer
pg	picogram
PG-SGA	Patient Generated Subjective Global Assessment
PL	phospholipid
PPARγ	peroxisome proliferator-activated receptor gamma
PS	performance status
QOL	quality of life
REE	resting energy expenditure
RR	response rate
rpm	revolutions per minute
SAT	subcutaneous adipose tissue
SD	standard deviation
SE	standard error
SM	skeletal muscle
SOC	standard of care
SREBP-1c	sterol regulatory element binding protein-1c
TAT	total adipose tissue (sum of intermuscular, visceral and subcutaneous)
TNF-α	tumour necrosis factor alpha
TNM	tumour, nodes, metastasis
VAT	visceral adipose tissue
VLDL	very low density lipoprotein
WHO	World Health Organization
ZAG	zinc-α_2-glycoprotein

CHAPTER 1: Introduction and literature review

1.1 Purpose

The purpose of this chapter is to provide an overview of malnutrition, body weight, body composition and chemotherapy treatment in lung cancer and to describe how nutritional intervention may improve these factors.

1.2 Introduction

An estimated 40% of women and 45% of men in Canada will be diagnosed with cancer during their lifetime. Lung cancer is the second most common cancer and the leading cause of cancer death in both men and women (1). There are no effective screening processes for lung cancer, and approximately 75% of patients are diagnosed with advanced stage disease. The most recent survival data shows that only 15% of patients who were diagnosed with lung cancer between 2002-2004 survived 5 years (2). The trajectory of lung cancer generally begins with a gradual decline in health/function for several months followed by accelerated decline in the final months of life. Due to the progressive nature of lung cancer, malnutrition is prevalent (3,4) and may contribute to the poor prognosis that accompanies a diagnosis of lung cancer.

1.3 Malnutrition

Malnutrition can be defined as an excess, inadequate or unbalanced intake of nutrients which influences body size, body composition and function (5). Malnutrition is common in end-stage diseases and is associated with increased morbidity, increased length of hospital stay and shorter survival (6). Disease related malnutrition may occur from one or a combination of factors which alter

nutrient requirements, intake or loss and results in weight loss and specifically loss of muscle (7).

Factors affecting nutrition may be related to the disease or to treatment of the disease. For example, anorexia may result from gastrointestinal blockage, from taste and smell alterations or as a side effect of medications/therapy. Impaired absorption of nutrients from the gastrointestinal tract can result in loss of nutrients (8). Metabolism can also be altered due to catabolic factors and systemic inflammation, resulting in increased resting energy expenditure (the amount of calories expended at rest) (8).

Although weight loss is a central feature of malnutrition, malnutrition is not synonymous with low body weights. Obesity is a well-known risk factor for the development of many chronic diseases (9). Coupled with the high prevalence of obesity (10), malnutrition may occur concurrently with obesity. It is important for health care professionals to recognize that malnutrition can occur across a range of body weights as early detection and treatment of malnutrition is essential for improving outcomes.

1.3.1 Assessment of nutritional status

The goal of nutritional assessment is to identify people who are malnourished or at risk using factors associated with malnutrition. Malnutrition is primarily assessed using screening tools which involve grading nutritional status and identifying patients who should be referred to a nutritional professional for a full nutritional assessment.

There are several different nutritional screening tools including the Mini Nutritional Assessment, Malnutrition Universal Screening Tool, Nutritional Risk Screening and the Subjective Global Assessment. These tools vary in the criteria assessed and the thresholds used to define malnutrition which can result in variation in the prevalence of malnutrition depending on the tool used (11,12). The Patient Generated Subjective Global Assessment (PG-SGA) was adapted from the Subjective Global Assessment specifically for use in cancer patients (13) and is the standard for nutritional screening in oncology (14). The PG-SGA is designed as a check box format that patients complete which assesses BMI, weight loss history, symptoms, dietary intake and functional capacity.

1.4 Cancer related malnutrition

There are no formal criteria for identifying malnutrition but involuntary weight loss of ≥ 5% of pre-illness weight and low body mass index (BMI $<18.5 kg/m^2$ or $<20 kg/m^2$) are commonly used criteria. Weight loss may occur at any point during the disease trajectory but is most prevalent in advanced disease (15) and near the end of life (16). In the classic study by Dewys et al. (17), weight loss of 5-10% of body weight in the 6 months prior to chemotherapy was reported in 17% of patients with advanced cancer. A further 15% of patients had weight loss >10%. Weight loss was particularly prevalent in patients with solid tumours and 61% of patients with non-small cell lung cancer (NSCLC) reported some degree of weight loss (17). A more recent study of patients with advanced NSCLC reported weight loss in 59% of patients, however neither the % weight loss nor the amount of weight loss was reported (18).

Weight loss results from a deficit in energy due to an imbalance in metabolic needs and nutrient intake resulting in a draw on physiologic reserves for energy. Increased metabolic needs may result from tumour related changes in metabolism such as an increase in futile metabolic cycles like the Cori cycle (19). This can result in an elevated resting energy expenditure (REE), which has been observed in weight-losing cancer patients (20,21) and is particularly common in patients with an acute-phase protein response (21,22). Nutrient intake may also be affected by tumour related symptoms and side effects of anti-neoplastic treatment. Anorexia, dysphagia, malabsorption, mouth sores and alterations in taste and smell are associated with reduced nutrient intake, weight loss and decreased functional capacity (7,23,24). These factors may contribute to weight loss during chemotherapy which has been reported to affect upwards of 50% of patients (18,25).

Weight loss has been associated with several negative outcomes including poorer response to anti-neoplastic therapy, decreased quality of life and shorter median survival (17,18,26). In the study by Dewys et al. (17), the median survival of patients with NSCLC who did not report any weight loss was 6 weeks longer than patients who reported any degree of weight loss and 9 weeks longer than patients with weight loss >10%.

1.4.1 Cancer cachexia

Cachexia is a complex metabolic syndrome which is associated with many chronic or end-stage diseases such as cancer. The underlying causes of cachexia are not well understood but are likely the net result of several tumour-derived and

host-derived factors. Anorexia, inflammation, insulin resistance, elevated muscle and/or adipose tissue breakdown are features frequently observed with cachexia (27). Clinical manifestations of cachexia include reduced strength, fatigue, impaired function, poorer response to anti-neoplastic therapy and reduced quality of life (28).

Cachexia is particularly prevalent in advanced cancers of the gastrointestinal tract and lung and nearly half of all cancer patients experience some degree of weight loss (17). Although cachexia is characterized by unintentional weight loss, weight loss cannot be resolved with adequate nutrition and thus cachexia is distinct from starvation.

The prevalence of cachexia has been difficult to determine due to lack of a formal definition of cachexia. In the past, definitions of cachexia were based on weight and weight loss criteria of variously BMI $<18.5 kg/m^2$, or BMI $<20 kg/m^2$, weight loss of 2, 5, or 10% depending on the time frame (generally 1 to 6 months). Recently, an international panel of experts released a consensus definition of cachexia which defined cachexia as "loss of muscle with or without loss of fat mass" (29,30). Cachexia may be diagnosed under this new definition if patient has $\geq$ 5% weight loss or BMI $<20 kg/m^2$, and 3 or more of the following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass or abnormal biochemistry which indicates elevated inflammation, anemia or low albumin.

The re-definition of cachexia also includes a classification system which recognizes that cachexia occurs across a continuum, varying in severity and stage:

1) pre-cachexia: early clinical or metabolic signs of cachexia which may progress to cachexia, 2) cachexia: weight loss >5% in previous 6 months or a combination of >2% weight loss with low muscle or low BMI, and 3) the last stage of cachexia, refractory cachexia which occurs close to death due to rapidly progressing disease which is unresponsive to anti-cancer therapy (29).

1.4.2 Skeletal muscle

The new definition of cachexia recognizes that the central feature of cachexia is severe muscle depletion termed sarcopenia (30).Sarcopenia denotes muscle less than 2 standard deviations below that of typical healthy adults. In non-malignant disease, sarcopenia is associated with disability and functional impairment (31), increased risk of fractures (32), increased length of hospital stay (6) and shorter survival (33). Sarcopenia is prevalent in advanced cancer, affecting upwards of 50% of patients with advanced cancer newly referred to medical oncology (34) or a palliative care program (35). This is approaching the prevalence normally observed in healthy elderly well over the age of 80 (32). Sarcopenia may have additional implications for patients receiving chemotherapy as it has been associated with shorter time to tumour progression (36) and dose-limiting toxicities from several different types of chemotherapy resulting in dose-reduction or termination of treatment (36-38).

Over the course of the disease trajectory, muscle loss intensifies with exponential losses in the last months of life (39,40). While muscle loss is the predominant feature of advanced cancer, a proportion of patients exhibit stable muscle or muscle gain (40). The likelihood of stable muscle or muscle gain

decreases approaching death and within 1 month of death, only 12% of patients experienced muscle gain (40). This suggests that cachexia therapy should be focussed earlier in the disease trajectory when muscle stability and gain are more likely.

1.4.3 Adipose tissue

Conventionally, adipose tissue was considered a passive energy depot. With the discovery of adipose tissue derived pro-inflammatory and anti-inflammatory molecules, adipose tissue is now recognized as an endocrine organ which actively participates in regulating physiologic and pathologic processes.

1.4.3.1 Normal control of adipose tissue

Fatty acids stored as triacylglycerol in adipose tissue accounts for greater than 90% of energy reserves in adults. Lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) are key enzymes which regulate fatty acid turnover in adipocytes. LPL hydrolyzes fatty acids from lipoproteins which are transported into adipocytes and converted into TAG. HSL hydrolyzes intracellular triacylglycerol in adipocytes thus mobilizing adipose tissue and energy stores.

Adipocyte differentiation is controlled by a cascade of transcription factors, namely CCAAT enhancer binding protein (C/EBP) ß and δ which stimulate C/EBPα. This acts in concert with peroxisome proliferator-activated receptor gamma (PPARγ) to control adipocyte differentiation and is enhanced by sterol regulatory element binding protein-1c (SREBP-1c).

1.4.3.2 Adipose tissue in cancer cachexia

Loss of adipose tissue is common in cancer cachexia (40,41). Longitudinal assessments of body composition in advanced cancer have detailed progressive loss of adipose tissue during the disease trajectory (42,43). Similar to skeletal muscle, the prevalence and severity of adipose tissue loss increases approaching death (40). Near death, losses totaling 85% of body fat have been observed in patients with advanced lung cancer (41) and both body fat and adipose tissue loss have been shown to be predictive of survival (42,43).

Adipose tissue loss is associated with several morphological and molecular changes in tissue. In a mouse model of cachexia, examination of adipose tissue with light and electron microscopy revealed shrunken adipocytes, increased collagen-fibril tissue and smaller lipid droplets than adipose tissue from non-tumour bearing controls (44). Expression of adipogenic factors and lipogenic enzymes including C/EBPα and β, PPARγ, SREBP-1c, acetyl-COA, fatty acid synthase, stearoyl-COA desaturase-1 and glycerol-3-phosphate acyltransferase were also down regulated (44,45). In adipocytes from cachectic patients elevated mRNA expression of HSL has been observed which may contribute to mobilization of stored lipids (46).

Adipose derived factors may also contribute to altered adipose metabolism (47-49). A lipid mobilizing factor has been isolated from a cachexia-inducing adenocarcinoma and also from the urine of cancer patients with cachexia (50). The amino acid sequence of the lipid mobilizing factor is homologous to zinc-α_2-glycoprotein (ZAG); a naturally occurring adipocyte factor in white and brown adipose tissue. ZAG induces lipolysis in adipocytes via a cyclic AMP-mediated

process (51). In cancer cachexia, ZAG expression may be up-regulated, leading to adipose tissue mobilization. However, clinical studies of ZAG are limited and the extent to which ZAG contributes to adipose wasting in cachexia is unclear.

1.4.3.3 Lipid metabolism in cancer

Elevated lipolysis appears to be the main driver of adipose tissue loss in advanced cancer. Lipolytic activity has been reported to be 50% higher in cachectic patients compared to non-cachectic patients (52). Along with elevated lipolysis, weight-losing cancer patients have increased glycerol and fatty acid turnover compared to cancer patients without weight loss (53). Fasting levels of plasma triglycerides, non-esterified fatty acids and glycerol are also increased, reflecting elevated lipolysis (54,55). Furthermore, studies in advanced cancer patients have demonstrated reduced levels of high density lipoprotein (HDL) and low density lipoprotein (LDL), which are major constituents of total plasma PL (56). This suggests possible impairment of lipid absorption, chylomicron or very low density lipoprotein (VLDL) synthesis, which would affect HDL and LDL levels. This may be further complicated by the influence that some cancer drugs have on lipid metabolism (57).

1.4.4 Essential fatty acids

In humans, α-linolenic acid (ALA), 18:3n-3 and linoleic acid (LA), 18:2n-6 are essential fatty acids (EFAs) as they cannot be synthesized in the body and must be obtained from dietary sources. ALA and LA undergo series of elongation and desaturation to become longer, more unsaturated fatty acids (58). LA is converted to arachidonic acid (AA, 20:4n-6) and ALA is converted to

eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Although humans possess the ability to elongate ALA and LA to long chain fatty acids, there is evidence that the pathways are inefficient. In-vivo isotope studies of fatty acid metabolism estimate that the conversion rate of ALA to EPA is approximately 5% and ALA to DHA is less than 0.5% (59). This suggests that EPA and DHA may be considered to be dietary essential nutrients in certain conditions.

n-3 and n-6 fatty acids have numerous roles in the body: they act as structural phospholipids (PL) in cell membranes, moderate membrane fluidity, cell signaling and interaction (60,61), affect gene expression (62) and are immunomodulatory (63). The majority of EFAs in blood are found in phospholipids (PL) which reflect metabolism of endogenous and dietary fatty acids. Therefore the composition of fatty acids in PL may be used to detect aberrations in metabolism of EFA and have been used as indices of dietary intake and fatty acid status in a variety of conditions (64-66). However, the fatty acid status of patients with cancer is not well characterized.

1.4.4.1 Essential fatty acids in cancer

Plasma lipid profiles of cancer patients have been shown to differ from the lipid profiles of healthy subjects or those with benign disease (67-69). Low concentrations of n-3 and n-6 fatty acids and elevated n-6:n-3 ratios have been reported in patients with bladder (67), prostate cancer (69) pancreatic, NSCLC and stomach/esophageal cancer (70). Collectively these studies suggest that patients with cancer have alterations in n-3 and n-6 fatty acid metabolism. The

metabolic consequences of deficits in peripheral n-3 and n-6 fatty acids is unclear but may contribute to wasting of muscle and adipose tissue as concurrent loss of plasma fatty acids, muscle and adipose tissue has been observed in advanced cancer (43,71).

It is unclear what is causing the changes in fatty acid profiles and at what point in time these aberrations occur. One study of patients with advanced breast cancer suggested chemotherapy as a potential causation as n-6 and n-3 fatty acids in plasma PL were undetectable following high dose chemotherapy (68) but low fatty acids have also been observed in treatment naïve patients (72). It is also possible that fatty acids are influenced by factors unrelated to cancer. Cancer patients over the age of 70 have on average 3 comorbidities (nonmalignant diseases) (73), most commonly cardiovascular disease and hypertension (74). The medications for these comorbid conditions include statins and fibrates which are lower blood lipids and beta blockers which can contribute to dyslipidemia. Thus, it is possible that comorbidities and their corresponding medications play a role in altered lipid metabolism and PL fatty acid compositions that have been observed in cancer, but this has yet to be examined.

1.5 Body composition

Conventionally, it was held that a unit of body weight and a unit of weight change had a constant composition with muscle and adipose tissue acting in tandem. It is now recognized that a unit of weight does not have a constant composition and patients with the same weight may have very different proportions of lean and fat tissues (34). However, anthropometrics such as

weight, body mass index (BMI) and skinfold measures do not differentiate a unit of body weight into lean and fat tissues. Although these measurements are clinically practical and useful in large populations where cost and portability are issues, they are generally less accurate than other modalities which assess lean and fat tissue. Further, weight and weight change may be confounded by changes in hydration status, which are commonly reported in advanced cancer.

1.5.1 Body composition assessment

Bioelectrical impedance analysis (BIA) (75), dual energy X-ray absorptiometry (DXA) (42) and computerized tomography (CT) imaging analysis (76) are the most frequently used modalities of body composition assessment in cancer. DXA, CT and magnetic imaging resonance (MRI) are considered the gold standard for body composition assessment due to their high precision and accuracy (77). On the other hand, BIA is appealing because it is portable and cost effective but there are several methodological limitations associated with BIA. 1) BIA provides indirect measurements of skeletal muscle. Total body water is used to estimate lean tissue which consists of skeletal muscle, tumour and organs. 2) BIA also relies on predictive equations to generate an estimate of lean tissue. Few predictive equations are derived from cancer populations and do not account for changes in other organs or tumour. 3) BIA is not sensitive to small changes ($2.2 \pm 3.2\%$/100 days) in lean body mass (LBM) that were detected with DXA (76). In a comparison of BIA and DXA, discrepancies in LBM ranged from -9.3 to +7.3kg, with overestimation likely in patients with low LBM and underestimation likely in patients with high LBM (78). DXA is appealing due to

the low radiation exposure but is unable to distinguish different adipose tissue depots (intramuscular, visceral and subcutaneous) and can only partition skeletal muscle from total LBM in appendices. Additionally, outside of research environments the availability of DXA for clinical measurements of body composition may be limited.

CT imaging can discriminate muscle, adipose tissues depots, bone and organs (79,80). Fatty infiltration in muscle and organs can also be estimated with this technique and has been shown to relate to insulin resistance and muscle function (81-83). In healthy populations CT analysis is infrequent due to the associated radiation exposure. This issue is not relevant in patients with cancer as CT images are routinely taken for diagnostic and follow up purposes to assess tumour progression and response to therapy. As such, CT images are readily available in oncology settings and can be opportunistically used to precisely assess body composition and the evolution of body composition throughout the cancer trajectory.

1.5.2 Body composition and cancer

Despite the availability of CT images in oncology settings, CT based assessments of body composition in cancer are limited. The existing studies indicate wide variation in proportions of muscle and adipose tissues ranging from the traditional notion of an advanced cancer patient (underweight and sarcopenic) to the contemporary phenomenon of sarcopenic obesity; low muscle and high body weight (35,84). These studies have advanced our understanding of the diversity and range of lean and fat tissues that exists in cancer populations.

Longitudinal analysis of CT images have also provided detailed descriptions of the natural history of muscle, adipose tissue and visceral organ volume throughout the cancer trajectory (39,43).

Use of CT based assessment of body composition in clinical trials is almost non-existent. Trials have primarily used BIA (85,86) or rarely DXA (87) to assess body composition. The use of CT images to precisely identify dynamic changes in muscle and adipose tissue is a useful tool for body composition analysis in clinical trials and particularly so in trials of anti-cachexia therapy aimed specifically at attenuating skeletal muscle loss.

1.6 Anti-cachexia therapy

The search for effective anti-cachexia agents is an area of intense research. However, due to the complexity of cachexia, a definitive treatment for cachexia remains elusive. Anti-cachexia agents generally fall into one of 5 categories: appetite stimulants, anabolic hormones, immune modulators, autonomic system modulators and multimodality therapy which attempts to address the different mechanisms that are implicated in the pathogenesis of cachexia. Overviews of the various anti-cachexia agents are provided in several in-depth reviews (27,88) and also in a recently published textbook (89). Of the numerous anti-cachexia agents being investigated, fish oil has received a lot of focus due to its anti-inflammatory (90) and purported insulin sensitizing properties (91).

1.6.1 Fish oil as an anti-cachexia agent

Fish oil, which consists of EPA and DHA, has been the focus of many anti-cachexia trials over the past 2 decades. Early investigational trials of fish oil

supplementation in weight losing cancer patients yielded promising results. Benefits to patients included preservation of lean body mass (LBM), improvement in functional status, survival, appetite and weight gain (92-94). However, these studies were generally small, non-randomized and uncontrolled. Subsequent larger, randomized clinical trials failed to show a benefit of fish oil over placebo on LBM (75,85,95). A systematic review on the subject (96) further dampened enthusiasm for fish oil as an anti-cachexia therapy concluding that there is insufficient evidence that fish oil provides a benefit over placebo on cancer cachexia and related symptoms.

Differences in study designs may have contributed to the discrepant results across EPA studies in the last decade. Trials typically have included patients with >5% weight loss (85,95,97) to select patients who are likely to be cachectic and have excluded patients receiving anti-neoplastic therapy (75,95) to minimize potential confounding effects of anti-neoplastic therapy on weight and body composition. These criteria have resulted in selection of patients who likely have refractory cachexia (advanced wasting, progressive disease and short survival) for whom it is unlikely that any intervention would be successful. Another inherent challenge in fish oil trials is compliance. Failure to report compliance, poor compliance to placebo and to supplementation have been frequently reported (75,85,86,95). Additionally, the format of fish oil supplementation may be problematic. Many studies use fish oil enriched protein, energy dense oral supplements but there is some evidence that patients compensate for the supplement by decreasing their meal intake (75).

Recently several fish oil supplementation trials were conducted which utilized novel study designs. These trials included patients with early stage (curative) disease who were receiving active therapy thus enabling accrual of patients with better prognosis than previous trials. Additionally patients in the studies by Weed et al. (98) and Ryan et al. (99) received the supplement via tube feed, thus negating compliance problems. Collectively, these studies show a beneficial effect of EPA on LBM (25,98,99) and suggest that the reasons for discordance between trials may be due to heterogeneity in study design.

1.6.1.1 Potential mechanisms of fish oil on lean body mass

Fish oil appears to act on several direct and indirect pathways, which contribute to muscle wasting and muscle anabolism. Fish oil may support the anabolic potential of muscle through sensitizing skeletal muscle to insulin. Insensitivity to insulin has been observed in patients with cancer cachexia (100), and may contribute to the development of cachexia. In tumour-bearing mice, insulin insensitivity preceded weight loss and administration of Rosiglitazone, a drug used in the treatment of type 2 diabetes, improved insulin sensitivity and attenuated skeletal muscle proteolysis (101). In experimental models of diabetes, fish oil has been shown to improve glucose uptake and increase GLUT-4 expression in skeletal muscle (91). However, this relationship has not been explored in cancer cachexia and the precise points of fish oil interaction within the glucose-insulin signaling pathway in muscle remains unclear.

Conversely, fish oil has been shown to inhibit catabolic stimuli that would otherwise promote muscle degradation during the cachectic process. The acute-

phase protein response may contribute to muscle wasting as it is modulated in part by pro-inflammatory cytokines: IL-1, IL-6, TNF-α, IFN-γ, which, when administered in animal models have been shown to induce weight loss and increase proteolysis (102). Supplementation with fish oil may limit muscle degradation by down-regulating the acute-phase response. In weight-losing cancer patients, fish oil has been shown to reduce serum concentrations of C-reactive protein, an acute-phase protein, and suppress IL-6 production by peripheral blood mononuclear cells (90). A number of studies point to the ubiquitin-proteasome proteolytic pathway as the predominant contributor to muscle breakdown in cancer-cachexia. Fish oil may decrease muscle breakdown by decreasing the expression of proteasome subunits which are elevated in cancer-cachexia (27). Fish oil may also have a protective role in skeletal muscle differentiation. An *in vitro* study showed reduced necrosis and apoptosis of differentiating myotubes with addition of fish oil (103). In the same model, addition of fish oil completely abolished TNF-α induced necrosis and apoptosis. The effects of fish oil on catabolic stimuli are diverse and further research is required to determine under what conditions these pathways are activated and how to optimize the inhibitory effect of fish oil on muscle degradation.

Fish oil may also indirectly affect muscle mass by decreasing chemotherapy related side effects and enhancing chemotherapy response. Side effects from anti-neoplastic therapies may contribute to or exacerbate existing anorexia leading to negative energy balance and muscle wasting. In an animal model of colorectal cancer, providing fish oil reduced side effects from

chemotherapy: limiting weight loss and anorexia (104). Fish oil has also been reported to enhance tumour response to chemotherapy thereby reducing the disease burden (105). This may indirectly provide anabolic stimuli as improvements in functional activity, dyspnea, fatigue and physical function have all been observed in patients receiving chemotherapy (reviewed in ref 106). Thus, there are various experimental and observational studies in animals and humans that demonstrate potential positive results in the use of fish oil to attenuate symptoms of cachexia.

1.7 Chemotherapy in lung cancer

The majority of patients with NSCLC present with advanced stage disease for which the primary treatment consists of palliative chemotherapy aimed at palliating symptoms, improving quality of life and survival. The current standard treatment for advanced NSCLC is platinum based doublet chemotherapy. Typically cisplatin or carboplatin are given in combination with vinorelbine or gemcitabine. Chemotherapy is given on a three week cycle for 2 cycles followed by imaging to determine disease response (Figure 1-1). If there is disease progression then chemotherapy is discontinued. Otherwise, a further 2 cycles of chemotherapy is given followed by imaging. The dose of platinum based chemotherapy is limited by cardiac, gastrointestinal and haematological toxicities. Approximately 10-25% of patients experience sufficient toxicity to have their doses reduced and/or delayed or require hospitalization due to toxic side effects (107).

The efficacy of chemotherapy in NSCLC is low with trials reporting response rates (RR; number of patients with reduction in tumour/metastases divided by total number of patients) ranging from 25 to 35% (108-110). This leaves a large proportion of patients with stable or progressive disease. These patients have a poorer prognosis than patients who respond to treatment (111). Even if patients are well enough to receive additional lines of chemotherapy, less than 10% will respond to second line chemotherapy (112-114). Thus, enhancing response to first-line chemotherapy is an area of great interest.

1.7.1 Fish oil as an adjuvant to anti-neoplastic therapy

Interest in the use of fish oil as an agent to control cancer growth began after epidemiology studies linked diets rich in fish to decreased cancer risk (115-117). Experimental studies suggest that supplementation with fish oil slows tumour growth and may be a safe, non-toxic approach to improve chemotherapy efficacy (118-121). The ability of fish oil to enhance the cytotoxicity of chemotherapy has been demonstrated in a wide range of chemotherapy agents (anthracyclines, cisplatin, irinotecan and alkylating agents) and in several malignant cell lines and animal models including breast, colorectal, prostate and lung cancer (118,119,122-124).

Although fish oil has a chemo-sensitizing effect, this appears to be specific to tumour tissue (105,125). In fact, fish oil may have a protective effect on non-target tissue as attenuation of weight loss, gastrointestinal and haematological side-effects have been reported following supplementation (104,105,125-127). A

summary of clinical trials of fish oil supplementation during chemotherapy is provided in Table 1-1.

Several mechanisms which may contribute to the chemosensitizing effect of fish oil have been proposed (summarized in Table 1-2 and reviewed in references 128 and 129). Generally, it is believed that fish oil primarily acts by incorporating into tumour cell membranes and increasing membrane peroxidation as concomitant enrichment with anti-oxidant molecules abolishes chemosensitization (130). However, the role of fish oil is likely more complex as increased sensitivity has been shown with a variety of chemotherapy agents which have different characteristics and mechanisms of action. Fish oil may also effect tumor progression independent of an effect of chemotherapy via mechanisms involved broadly related to cell signaling, gene regulation, angiogenesis and metastasis (reviewed in reference 129).

While experimental studies are convincing, to date only a handful of clinical studies of fish oil supplementation exist. These studies report a positive correlation between chemotherapy efficacy and n-3 fatty acid concentration of breast tissue (131), as well as improved RR, milder haematological toxicity, greater time to tumour progression and longer survival with DHA supplementation (105)

1.8 Summary

From this review it is evident that health care for patients with advanced cancer is multi-faceted. Nutrition related research shows that malnutrition is prevalent in advanced cancer and indicative of poor prognosis. In the domains of

physiology and metabolism, research shows frequent depletion of skeletal muscle and adipose tissue as well as evidence of abnormal lipid metabolism and adipose tissue composition. From oncology, current chemotherapy regimens for advanced NSCLC are only effective in a small proportion of patients and novel approaches are needed. All of these research areas are intricately linked; thus demonstrating the importance of an interdisciplinary approach to understanding and managing care of patients with advanced cancer and developing effective interventions which have the potential to augment each of these areas.

Tables

Table 1-1: Summary of clinical studies of fish oil supplementation during chemotherapy

Study	Design	Population	Intervention	Outcome(s)	Results
van der Meij et al. 2010 (ref 25)	Randomized controlled, blinded	Stage III non-small cell lung cancer patients receiving cisplatin-based chemo-radiation. 14 in control (C) and 19 in intervention (I) group.	1. 2 cans ONS/d (2g EPA + 0.9g DHA) for 5 weeks. Mean intake:1.1 cans/d. 2. no EPA or DHA for 5 weeks. Mean intake: 1.0 cans/day.	Weight, IL-6, CRP, REE, LBM, fat mass, MUAC.	I: stable weight, increased MUAC, milder decrease of FFM then C. Lower IL-6 and CRP in patients with ≥ 1.5% increase in plasma PL EPA. Greater decrease of REE in I vs. C group.
Bougnoux et al. 2009 (ref 105)	Open label with post-hoc analysis of 2 sub-groups: high PL DHA vs. low PL DHA	Advanced breast cancer patients (n=12 high DHA; n=13 low DHA) receiving fluorouracil, cyclophosphamide and epirubicin.	9 capsules/d (1.8g) for 5 months. Mean intake not reported.	Chemotherapy response, time to tumour progression, survival, toxicity.	Response to chemotherapy similar to patients with less advanced breast cancer. Less haematological toxicities, increased time to tumour progression and survival in high DHA group.
Read et al. 2007 (ref 127)	Open label, single arm	Advanced colorectal cancer patients (n=15) receiving irinotecan with 5-fluorouracil	2 tetrapaks ONS (1.85g EPA + 0.78g DHA) for 9 weeks. Mean intake: 1.7 tetrapaks/d.	Dietary energy and protein, weight, LBM QOL, toxicity, CRP.	No change in energy or protein intake, LBM, QOL or CRP. Increased weight. Low incidence of toxicity
Bauer et al. 2005 (ref 132)	Open label, single arm	Advanced pancreatic (n=5) and lung cancer (n=2) patients receiving gemcitabine-based chemotherapy	At least 1 can ONS (1.36 g EPA/d) for 8 weeks. Mean 1.2 cans/d.	Energy, protein intake, LBM, nutritional status (PG-SGA), performance status, QOL.	Increased total energy and protein intake, no change in weight or LBM, improved nutritional status, performance and QOL

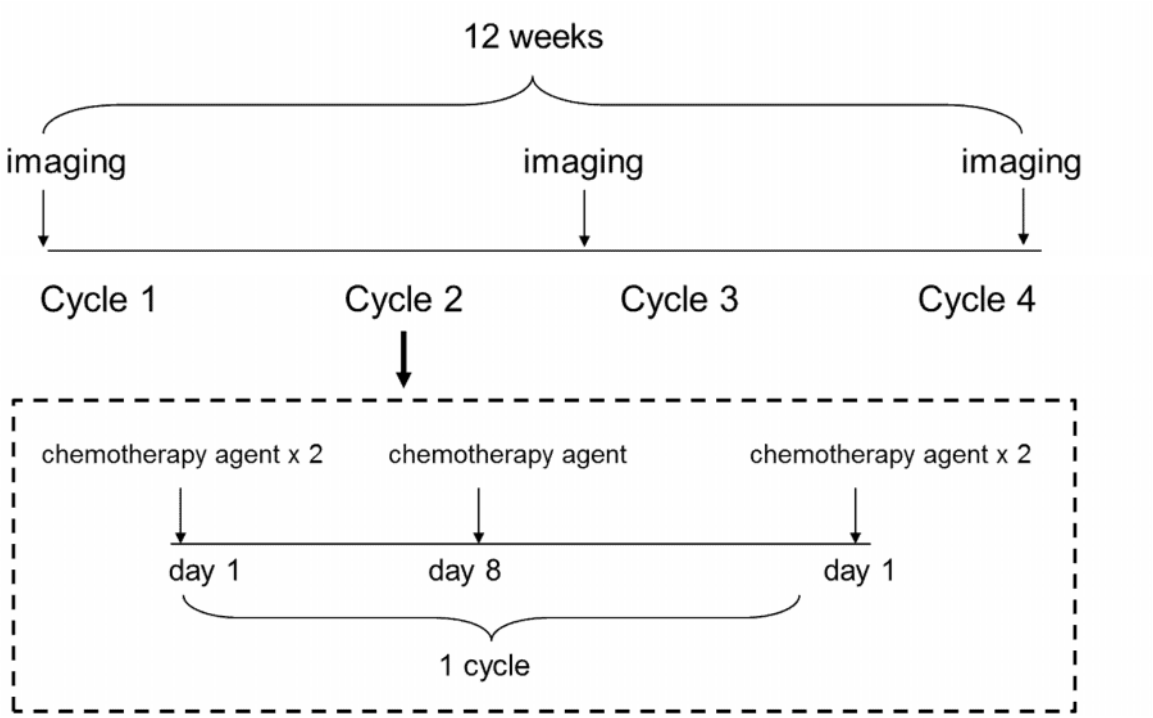
Abbreviations: LBM, lean body mass; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PL, phospholipid; MUAC, mid upper arm circumference; REE, resting energy expenditure, PG-SGA, patient generated subjective global assessment.

Table 1-2: Potential mechanisms which may contribute to the chemosensitizing effect of n-3 PUFA

Mechanism	References
Altered cellular signaling	(133,134)
Increased peroxidation	(118,135,136)
Suppressed NFκB activity	(137,138)
Increased intracellular drug accumulation	(139,140)
Increased apoptosis or inhibition of anti-apoptotic proteins	(141,142)

Figures

Figure 1-1: Timeline of typical first-line platinum based doublet chemotherapy regimen



On day 1 both chemotherapy agents are given, followed by 6 days of recovery after which 1 chemotherapy agent is given on day 8. This is followed by 2 weeks of recovery before the cycle is repeated.

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CHAPTER 2: Research plan

2.1 Rationale

Loss of weight, muscle and adipose tissue are commonly observed in patients with lung cancer and result in poor response to chemotherapy and shorter survival (1,2). There is also evidence of depletion of plasma PL EPA and DHA (3,4), which may further decline following chemotherapy (3). The consequences of deficits of EPA and DHA are poorly defined but may be associated with loss of muscle and adipose tissue (5,6). Thus, supplementation of fish oil containing EPA and DHA could be expected to attenuate loss of muscle and adipose tissue. Experimental studies have reported that fish oil may also improve chemotherapy efficacy (7,8), which could have a large impact in patients with lung cancer given the low efficacy of current chemotherapy regimens (9). However, research in animal models of lung cancer are limited and only 1 clinical trial assessing the effect of fish oil as an adjuvant to chemotherapy has been conducted (10).

2.2 Objectives and Hypotheses

Investigated in Chapter 3: Aberrations in plasma phospholipid fatty acids in newly diagnosed non-small cell lung cancer patients

The objective was to characterize the plasma PL fatty acid profile of NSCLC patients before, during and following first-line platinum based chemotherapy. The secondary objective was to identify potential causes of aberrations in plasma PL fatty acids. It was hypothesized that plasma PL EPA and DHA are low near

diagnosis, decrease further during chemotherapy and do not recover following completion of treatment.

Investigated in Chapter 4: Skeletal muscle depletion is associated with reduced plasma n-3 fatty acids in non-small cell lung cancer patients

The objective was to determine whether physiological concentrations of plasma PL fatty acids and particularly EPA and DHA are related to sarcopenia, change in muscle and adipose tissue in NSCLC patients. It was hypothesized that plasma PL EPA and DHA are reduced in patients with sarcopenia and associated with accelerated loss of muscle and adipose tissue.

Investigated in Chapter 5: Nutritional intervention with fish oil provides a benefit over standard of care on weight and skeletal muscle mass in non-small cell lung cancer patients receiving chemotherapy

The objective was to determine the capacity of supplementation with fish oil to attenuate loss of weight, skeletal muscle and adipose tissue during chemotherapy in NSCLC patients and compare this to NSCLC patients receiving standard of care (no intervention). The secondary objectives were to determine the effect of fish oil supplementation on nutritional status, symptoms and quality of life and identify cytokines which contribute to muscle and/or adipose tissue loss. It was hypothesized that supplementation with fish oil provides a benefit over standard of care; resulting in attenuation of weight, muscle and adipose tissue loss which is

mitigated in part via a decrease in catabolic cytokines. It is also expected that fish oil supplementation will improve nutritional status, symptoms and quality of life.

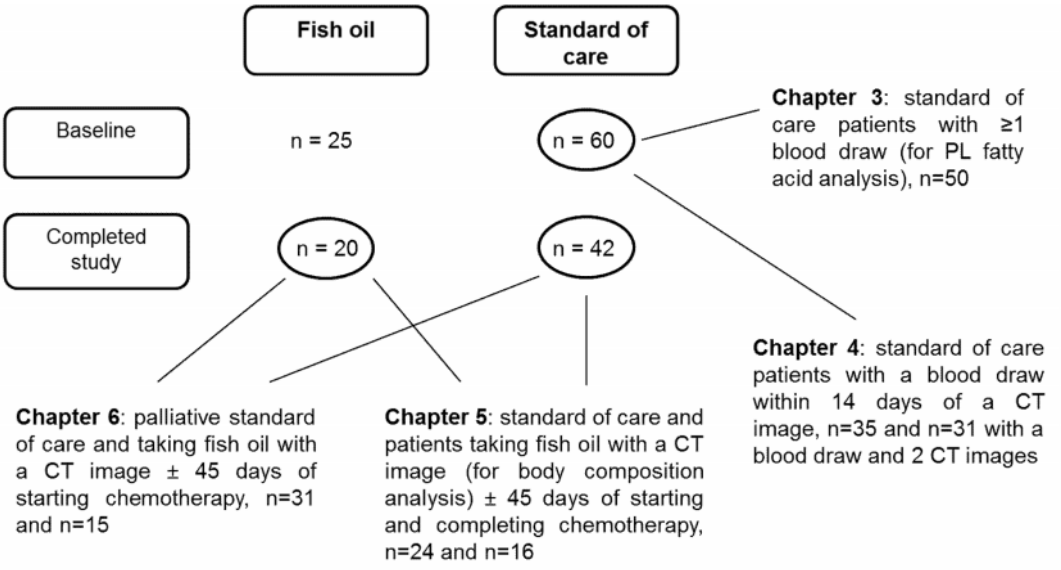
Investigated in Chapter 6: Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced non-small cell lung cancer

The objectives were to determine whether supplementation with fish oil in advanced NSCLC improves efficacy of first-line platinum based doublet chemotherapy (reduction in tumour/metastases) compared to standard of care and evaluate the effect of supplementation with fish oil on chemotherapy toxicity. The secondary objective was to determine the effect of supplementation with fish oil on survival. It was hypothesized that fish oil provides a benefit over standard of care; resulting in improved chemotherapy efficacy and survival without affecting chemotherapy toxicity.

The research presented in this thesis is from two contemporary trials: a descriptive study of fatty acids and body composition in patients receiving standard of care (SOC; no intervention) and an open label trial of fish oil supplementation. The studies had the same inclusion/exclusion criterion and the same study measures (with the exception of the PG-SGA, ESAS and FAACT which were only administered in the fish oil trial). Both studies began accrual in August 2007. However, the fish oil trial was closed several months later due to difficulties with patient accrual. The trial was re-designed and opened to accrual again in May 2008. Both studies closed to accrual in November 2009. Recruitment details are

provided in Chapter 3 and 5. Briefly, patients indicated interest in available nutritional studies and were approached about participation for studies of interest. If patients indicated interest in both studies, they were approached first for the fish oil trial. The patient cohorts presented in each chapter vary according to the objectives outlined in the chapter and are summarized in Figure 2-1.

Figure 2-1: Overview of patient cohorts presented in each thesis chapter



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CHAPTER 3: Aberrhations in plasma phospholipid fatty acids in non-small cell lung cancer patients

3.1 Introduction

In advanced cancer, metabolism of nutrients may differ from healthy individuals. Abnormalities in lipid metabolism, including increased lipolysis (1) and oxidation of free fatty acids (2) have been observed in advanced cancer. These abnormalities have been implicated in the pathogenesis of tissue wasting (3) and thus may contribute to the poor prognosis which accompanies weight loss (4). Plasma phospholipids (PL) reflect the metabolism of endogenous and dietary fatty acids and contain the majority of n-6 and n-3 fatty acids in blood. As such, fatty acid compositions of plasma PL have been used as indices of dietary intake and fatty acid status in a variety of conditions (5-7).

Several studies in cancer patients have reported alterations in PL n-3 and n-6 fatty acids compared to healthy individuals (8-11). In general, decreased arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) have been observed in parallel with elevated n6:n3 ratios. The observed differences were independent of total caloric intake and total fat intake (11). Total PL and phosphatidylcholine which is the main constituent of membrane PL, were also found to be reduced with advanced stage and aggressive cancers (12), likely due to higher phosphatidylcholine consumption and turnover by tumor cells (13). However, the relationship between advanced disease and individual plasma lipids has only been explored in one study which reported that EPA and DHA concentrations may be further reduced in advanced stage and aggressive forms of non-Hodgkin lymphoma (12).

The causes of the aberrations in n-6 and n-3 fatty acid metabolism are unclear. Chemotherapy may contribute to depletion of n-6 and n-3 (11), but low n-3 and n-6 fatty acids have also been observed in the absence of any type of anti-neoplastic therapy (8,12). The consequences of depletion of plasma n-3 and n-6 fatty acids are largely unknown but could be expected to affect the availability of these fatty acids which are delivered to tissues for immune function, tissue repair and other physiological processes. This notion is supported by observations of concurrent losses of plasma fatty acids (including n-3 and n-6 fatty acids), skeletal muscle and adipose tissue in advanced cancer patients (14).

Although the literature on fatty acids in cancer is limited, collectively it suggests the existence of a deficiency or imbalance of n-6 and n-3 fatty acids and possibly an increased requirement of n-3 fatty acids. Identification of the causes or the time in the course of disease progression and treatment that this evolves may be important for planning effective and timely nutritional intervention. The objectives of this study are to systematically assess fatty acids before, during and following chemotherapy treatment in patients with lung cancer, with a particular emphasis on n-3 and n-6 fatty acids. Factors which may potentially influence amounts of n-3 and n-6 fatty acids will also be determined.

3.2 Methods

3.2.1 Study design

This study was approved by the Alberta Cancer Research Ethics board as a minimal risk study (chart review, no intervention). All patients were recruited from the lung new patient clinic at the Cross Cancer Institute (Edmonton, Alberta,

Canada) between August 2007 and November 2009 and were followed for >1.5 years. The Cross Cancer Institute is the major cancer center for all of Northern Alberta (population 1.8 million) and sees >95% of referrals for consideration of treatment with chemotherapy or radiation therapy. Newly referred patients with lung cancer attend a medical oncology clinic. At clinic, patients are given a handout that briefly describes available nutritional studies and patients are asked to indicate interest (Figure 3-1). Patient response informs investigators who to approach regarding participation in research studies. Patients in this study were accrued with the purpose of detailing changes in fatty acids during chemotherapy and the potential this has on clinical outcomes. However, following amendments to the study design of a contemporary trial of fish oil supplementation (see Appendix 2) in the same patient population (lung cancer patients receiving chemotherapy), patients also served as a control group (standard of care, no intervention) for a study which examined the effect of fish oil supplementation on body composition (15) and chemotherapy efficacy (16).

3.2.2 Patient population, demographics and anthropometrics

Patients with a histologically confirmed diagnosis of non-small cell lung cancer were included in this study. This patient population was chosen because a high proportion of patients with lung cancer experience malnutrition (17). This study was open to all stages of lung cancer to facilitate enrollment and also because the complementary fish oil intervention study was focused on early intervention to prevent muscle loss and thus included patients with early stage disease. Additional inclusion criteria were: >18 years of age, no gastrointestinal

tumours, able to maintain oral intake, naive to chemotherapy and able to provide written, informed consent. Since this study was focused on change in fatty acids during chemotherapy, patients were only approached for study participation if they were referred for first-line chemotherapy treatment of either curative or palliative intent based on standard practice specifically agreed upon as Clinical Practice Guidelines by the Alberta Provincial Lung Tumour Group.

Stage of disease was based on the American Joint Committee on Cancer stage groupings, I, II, III, and IV (18). Height was measured by a stadiometer and weight was measured using a medical balance beam scale at baseline, throughout chemotherapy and following completion of chemotherapy (end of study period). Height and weight were used to compute body mass index (BMI) (kg/m^2). World Health Organization (19) categories were used to classify patients as: underweight, BMI <18.5; normal, BMI 18.5-24.9; overweight BMI 25-29.9; or obese BMI ≥ 30. Self-reported weight loss history was obtained from medical records or the Patient Generated Subjective Global Assessment (PG-SGA) (20) which is completed by patients at their first clinic visit. Eastern Cooperative Oncology Group Performance Status (ECOG PS) was assessed by an oncologist: 0=fully active, able to carry on all pre-disease performance without restriction; 1=restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work; 2=ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours; 3=capable of only limited self-

care, confined to bed or chair more than 50% of waking hours; 4=completely disabled, cannot carry on any self-care, totally confined to bed or chair (21).

Chemotherapy treatment

All patients were seen in consultation for treatment with standard first-line chemotherapy. At our cancer center, chemotherapy for NSCLC consists of platinum based doublet chemotherapy with cisplatin or carboplatin in combination with vinorelbine or gemcitabine. This chemotherapy regimen is typically administered on a 3-week cycle for 2 cycles, after which disease response (change in size of tumour or metastases) is assessed with x-ray or computed tomography (CT) imaging. If there is disease response (stable disease or partial response) chemotherapy is continued for 4 cycles, otherwise chemotherapy is stopped. The end of study was considered to be akin to completion/the end of chemotherapy as this coincided with the last study blood draw. For the group that had follow-up blood draws, the end of study was still considered to be the end of chemotherapy.

3.2.3 Lipid extraction and phospholipid separation

Blood (12mL) was drawn into a heparin tube by a Registered Nurse prior to starting chemotherapy (baseline), prior to each 3 week cycle of chemotherapy and 1 month following chemotherapy whenever patients continued to be followed at the Cross Cancer Institute. To minimize patient burden, blood draws were arranged to coincide with routine blood work that was ordered as part of the chemotherapy protocol.

Blood was centrifuged, plasma was isolated and immediately frozen at -80°C until analysis. Fatty acids were extracted from plasma using a modified Folch method (22), which is considered the standard for lipid extraction and is widely used in research (23-25). Plasma was thawed and 50µL from each sample was transferred to methylation tubes. Total lipid was extracted using 2mL chloroform/methanol (2:1, by volume) and 400µL calcium chloride (0.25%). Samples were then stored for 12 hours at 4°C to separate. The lower phase lipid was extracted and samples were further washed with 1mL chloroform/methanol/water (86:14:1, v/v) solution. The lower phase lipid was extracted again and pooled with the previous extract. Samples were dried with nitrogen gas (N_2) and re-suspended in 200µL of chloroform. Extracted lipid were applied in duplicate (100µL per lane) onto pre-coated silica gel "G" thin layer chromatography plates (Analtech, Newark, DE, USA) that had been heat activated at 110°C for 1 hour. Plates were then developed at room temperature in solvent tanks containing a petroleum ether/ethyl ether/acetic acid solution (80:20:1, by volume) for approximately 45 minutes to separate individual fatty acid classes: phospholipid (PL), cholesterol, triglyceride and cholesterol ester(26). Plates were sprayed with 0.1% aniline naphthalene sulfonic acid and the bands were identified under UV light. The triglyceride and cholesterol ester bands were scraped into test tubes, dried with $N_2$and stored at -80°C for future analysis. The PL band was scraped into test tubes, 50µL of C17:0 (10.1mg/100mL; Supelco, Bellefonte, PA, USA; Sigma Chemical, St. Louis, MO, USA) was added as an internal standard followed by 2mL of hexane and 1mL boron trifluoride. Samples

were methylated for 1 hour at 110°C after which tubes were cooled at room temperature for 1 hour. Once cool, 1mL of distilled water was added into each test tube and tubes were stored at 4°C for 12 hours. The top phase was transferred into gas chromatography vials, dried with $N_{2,}$ re-suspended in 50µL of pure hexane and transferred into a glass insert inside the gas chromatography vial. Samples were stored at -80°C until analysis by gas chromatography.

3.2.4 Quantification of fatty acid composition

Plasma PL fatty acid composition was determined using gas liquid chromatography (Varian 3900CX Gas Chromatograph) equipped with a flame ionization detector, autosampler and 30 meter BP-20 fused capillary column (SGE Instruments Australia).The flow rate of helium gas was 1.8 mL/min with oven, injector and detector temperatures of 150°C, 190°C and 220°C, respectively (27). Peaks of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids between 6 and 24 carbon chain lengths were identified by comparison with the C17:0 standard peak. PL Fatty acid amounts were calculated using the area peak of the internal standard and expressed as µg/mL (6). The lower limit of detection was ~0.20µg/mL or ~0.10% when expressed as a proportion of total PL fatty acids. An acceptable level of variation between duplicates was set at 5%. Mean fatty acid amounts were calculated from duplicates, otherwise individual samples were re-run using gas liquid chromatography (~5% of total samples) or failing that, lipid extraction and PL isolation was repeated from the original sample (<5% of total samples). A random

selection of 15 samples were run twice on the gas chromatograph on separate days and had a co-efficient of variation ~3%.

3.2.5 Statistical analysis

Data are reported as mean ± standard deviation (SD). Two sample t-tests, Chi-square or Fischer's exact test were used to compare groups as appropriate. All tests were two-sided. Repeated measures analysis of variance (ANOVA) with Bonferonni post-hoc comparisons was used to compare changes in fatty acids from baseline to end of study. Significance was reported at levels of $P<0.05$.Statistical analysis was completed using SPSS for Windows (version 18.0, SPSS, Chicago, IL).

3.3 Results

3.3.1 Patient population

Overall, 60 patients were accrued, 50 received at least a baseline blood draw and 42 patients had more than 1 blood draw. Patients who received at least a baseline blood draw were included in this analysis. The number of patients who consented but were subsequently withdrawn is a reflection of the patient population: the majority of our patients had advanced staged disease and symptoms which impacted upon performance status and their ability to tolerate chemotherapy.

The majority of patients (87.5%) received cisplatin or carboplatin with vinorelbine, 6.25% received adjuvant cisplatin/etoposide (stage IIIA) with concurrent radiation and 6.25% received carboplatin with gemcitabine. Patient demographics and anthropometrics at baseline are shown in Table 3-1. Despite

generally advanced disease, patients had heavy body weights and minimal history of weight loss. Patients had an average of 2 comorbid conditions. The most common were conditions were hypertension (44%), chronic obstructive pulmonary disease (20%) and hyperlipidemia/hypercholesterolemia (18%). Median survival was not calculated as only 2/3 of patients were deceased at date of censorship (April 2011).

3.3.2 Plasma phospholipid fatty acids at baseline

At baseline, total plasma PL ranged widely (655 ± 228μg/mL). To examine potential sources of this variation, patients were divided into groups based upon stage of disease and visceral metastases as these factors have been suggested to influence plasma PL fatty acids (12,24). Patients with advanced stage lung cancer (III and IV) were not different for age, gender, or BMI compared to patients with early stage (I and II) lung cancer but they had lower total fatty acids and alterations in the composition of fatty acids (Table 3-2). There were no differences in fatty acid amounts between patients with liver metastases and patients with advanced disease without liver metastases (Table 3-3).

3.3.3 Plasma phospholipid fatty acids during chemotherapy

During the course of the study two clear groups emerged: patients who received a full course of chemotherapy (≥ 4 cycles) and patients who stopped chemotherapy (<4 cycles) due to either disease progression or toxicities. The mean duration of chemotherapy was 80.1 ± 16.9d in the group that received ≥ 4 cycles and 50.1 ±20.5d in the group that received <4 cycles of chemotherapy.

The groups were similar for age, BMI and gender, however only 3 patients in the <4 group had early stage disease (13%) compared to 8 patients in the ≥ 4 (30%). Since our results suggest an effect of early vs. late stage disease on fatty acids, this comparison was limited to patients with advanced stage disease (n=13 in <4 group and n=19 ≥4 group).

There were no changes in fatty acids from baseline to end of chemotherapy in patients who received ≥ 4 cycles of chemotherapy (Table 3-4). Conversely, patients who received <4 cycles of chemotherapy had lower linoleic, n-6 fatty acids, PUFA and total fatty acids at the end of chemotherapy compared to baseline and a trend towards lower AA, DHA, n-3, SFA and MUFA (Table 3-4). Comparison of fatty acids at the end of chemotherapy between groups revealed no differences except for a trend towards lower EPA in the <4 cycles group (4.0 ± 2.8μg/mL versus 6.3 ± 4.1μg/mL; *P*=0.09).

3.3.4 Plasma phospholipid fatty acids following chemotherapy

Of the patients who received ≥ 4 cycles of chemotherapy, a subgroup of 17 patients had a follow-up blood draw 1 month following study and chemotherapy completion (38.1 ± 20.2d). In this group there were no significant differences in individual fatty acids, classes of fatty acids (n-3, n-6, SFA, MUFA and PUFA) or total fatty acids from baseline to follow-up or from end of study to follow-up. The mean amount of SFA, MUFA and PUFA at each time point is shown in Figure 3-2.

3.4 Discussion

The fatty acid profile of patients with cancer is not well characterized due to a limited number of studies in patients with heterogeneous tumour types. Here we provide a comprehensive assessment of plasma PL fatty acids in a major tumour group prior to, throughout and following chemotherapy treatment. Using this approach, we demonstrate that patients with advanced stage lung cancer have alterations in fatty acid composition near diagnosis. Patients had lower n-3 and n-6 fatty acids compared to patients with early stage disease, which is similar to what has been reported in neutrophil PL of patients with advanced cancer (11) and serum PL in non-Hodgkin lymphoma (12). Patients with advanced cancer also had lower SFA and total amounts of PL compared to patients with early stage disease. Alterations in plasma PL SFA and total fatty acids have been infrequently reported in the literature and the significance and etiology of these changes is unclear. Liver metastases did not appear to be a contributing factor. Medications taken for comorbid conditions could affect fatty acids as statins, fibrates and beta blockers affect lipid levels and are prescribed for the treatment of hypertension and hyperlipidemia which were prevalent in our patient population. However, we were not privy to medication details (manufacturer, dose and frequency) and our sample size limited sub group analyses. Proximity to death may also be in part responsible for the observed differences in fatty acids between early and advanced stage patients. Although, survival was not assessed, it is expected that advanced stage patients have shorter survival compared to

patients with stage I and II disease, and decreased amounts of fatty acids have been observed near death (14).

Plasma PL fatty acid composition reflects dietary fatty acids (28). Thus, it is possible that alterations in the amount of individual plasma PL fatty acids may be due to changes in dietary intake. As this study was descriptive in nature, we did not collect detailed information on dietary intake which limits our interpretation of our results. However, dietary intake alone cannot explain our results as intake would be expected to change the composition of fatty acids but not the amount of fatty acids. Put simply, the low amounts of total fatty acids that we observed in patients with advanced stage lung cancer cannot be attributed to diet.

Loss of fatty acids is progressive, with further decreases from baseline in patients who received <4 cycles of chemotherapy. Of interest, change in n-3 fatty acids during chemotherapy appears to be minimal, with only a trend towards lower n-3 fatty acids and DHA and no change in EPA. The fatty acid profile of advanced cancer patients from our cancer center which has been previously published was used to provide a frame of reference (14). EPA, DHA and n-3 fatty acids at the end of study in the <4 cycles group, were the only fatty acids that were comparable to amounts observed in patients <8 months from death (Table 3-5). The amounts of all other fatty acids were in the range of or appeared greater than fatty acids reported in patients >8 months from death. This may suggest that loss of n-3 fatty acids precedes depletion of other classes of fatty acids. However, this hypothesis needs to be tested in larger populations.

It seems possible that depletion of fatty acids is related to either indirect or direct effects of chemotherapy treatment. Low levels of both n-6 and n-3 fatty acids in plasma PL have been observed in advanced breast cancer following induction chemotherapy, with further declines following high dose chemotherapy (11). Disturbances in the gastrointestinal tract and poor food intake resulting from chemotherapy may affect the availability, absorption and metabolism of fatty acids (29). This may explain why patients who stopped chemotherapy due to toxicity (<4 cycles group) had lower fatty acids following chemotherapy. Alternatively, the change in fatty acids may have been related to disease control. Patients who received a full course of chemotherapy and thus stable disease or reduced tumour burden, had little variation in amounts of fatty acids throughout chemotherapy. Conversely patients with progressive disease (<4 cycles) lost fatty acids. Given the short disease trajectory of advanced lung cancer, this hypothesis could be tested by following patients with stable disease until the point of disease progression and quantifying fatty acids throughout this time period.

This is the first study to document changes in fatty acids following completion of chemotherapy. This research shows that fatty acids continue to be stable in patients who received all planned chemotherapy. Unfortunately, we were unable to obtain follow-up blood samples from patients who received <4 cycles of chemotherapy, as the majority were lost to follow-up. Thus it is not known whether the loss of fatty acids observed in this group is an immediate or long lasting effect.

3.5 Conclusion

This research shows that loss of fatty acids is prevalent, progressive and possibly influenced by chemotherapy treatment, stage of disease and disease control (stable versus progressive). Larger studies which account for these variables and follow patients over a longer period of time would help to define these relationships further.

Tables

Table 3-1: Baseline patient demographics and anthropometrics

	Men	**Women**	***P***
Total, n (%)	19 (38.0)	31 (62.0)	
Age, years	67 ± 6.2	61 ± 9.0	0.03
Stage			
I & II, n (%)	4 (21.1)	7 (21.2)	1.00
III & IV, n (%)	15 (78.9)	26 (78.8)	
Histology			
Adenocarcinoma	13 (68.4)	22 (71.0)	
Squamous	3 (15.8)	3 (9.7)	
Adenosquamous	0	1 (3.2)	
Large cell	2 (10.5)	0	
Poorly differentiated	1 (5.3)	5 (16.1)	
Body mass index, kg/m^2	28.7 ± 5.3	26.5 ± 5.4	0.17
range	18.8-43.3	17.0-39.0	
[a]6 month weight loss history, %	-1.3 ± 3.2	-2.3 ± 3.8	0.39
ECOG PS, range	0-2	0-2	

Mean ± SD, two sample t-test, Chi-square or Fischer's exact test. [a]Data not available for 3 patients. Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 3-2: Baseline plasma PL fatty acids (µg/mL) in patients with early vs. advanced stage disease

	Stage I & II	**Stage III & IV**	***P***
Total, n	12	38	
LA (18:2n-6)	155 ± 38.4	120 ± 44.8	0.02
ALA (18:3n-3)	1.6 ± 1.6	1.3 ± 1.0	0.43
AA (20:4n-6)	76.1 ± 23.4	59.4 ± 25.9	0.05
EPA (20:5n-3)	10.3 ± 3.7	5.5 ± 4.8	0.002
DHA (22:6n-3)	18.1 ± 4.8	13.5 ± 7.0	0.04
Σn-6	268 ± 63.7	204 ± 72.8	0.009
Σn-3	29.7 ± 7.6	21.9 ± 12.3	0.05
ΣSFA	385 ± 68.0	294 ± 116	0.01
ΣMUFA	110 ± 19.4	94.5 ± 38.7	0.19
ΣPUFA	299 ± 68.8	233 ± 89.6	0.02
Total µg/mL	772 ± 166	600 ± 208	0.01

Mean ± SD, two sample t-test. Abbreviations: LA linoleic acid, ALA α-linolenic acid, AA arachidonic acid, EPA eicosapentaenoic acid, DHA docosahexaenoic acid, SFA saturated fatty acids; MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

Table 3-3: Baseline plasma PL fatty acids (µg/mL) in patients with liver metastases versus advanced stage disease without liver metastases

	No liver metastases	**Liver metastases**	***P***
Total, n	28	11	
LA	119 ± 40.2	123 ± 53.6	0.77
ALA	1.3 ± 1.0	1.2 ± 1.1	0.81
AA	57.7 ± 27.1	63.4 ± 22.5	0.53
EPA	5.7 ± 4.7	5.1 ± 5.0	0.71
DHA	13.2 ± 7.2	13.4 ± 7.5	0.94
Σn-6	202 ± 71.7	209 ± 75.2	0.78
Σn-3	21.8 ± 13.4	21.2 ± 10.8	0.89
ΣSFA	290 ± 103	304 ± 138	0.71
ΣMUFA	91.8 ± 37.4	102 ± 41.1	0.44
ΣPUFA	226 ± 82.5	249 ± 101	0.45
Total µg/mL	600± 207	598 ± 205	0.98

Mean ± SD, two sample t-test. Abbreviations are as in Table 3-2.

Table 3-4: Change in plasma PL fatty acids (µg/mL) from baseline to end of study

	<4 cycles chemotherapy			**≥ 4 cycles chemotherapy**		
Total, n	13			18		
	Baseline	End of study	***P***	Baseline	End of study	***P***
LA	127 ± 58.5	102 ± 63.2	0.04	121 ± 35.3	118 ± 46.4	0.71
ALA	1.0 ± 0.9	1.3 ± 1.0	0.31	1.6 ± 1.1	1.6 ± 0.9	0.99
AA	62.9 ± 33.0	51.2 ± 37.2	0.09	57.0 ± 21.7	54.7 ± 25.2	0.59
EPA	5.3 ± 4.9	4.0 ± 2.8	0.35	5.9 ± 5.1	6.3 ± 4.1	0.65
DHA	14.4 ± 9.0	11.5 ± 10.2	0.11	13.2 ± 5.9	12.8 ± 5.9	0.77
Σn-6	215 ± 99.4	173 ± 99.2	0.03	202 ± 53.4	198 ± 68.0	0.78
Σn-3	21.5 ± 13.1	16.9 ± 12.5	0.10	23.2 ± 12.9	22.2 ± 10.5	0.71
ΣSFA	298 ± 132	251 ± 142	0.10	277 ± 81.7	282 ± 103	0.79
ΣMUFA	91.8 ± 48.3	81.5 ± 53.1	0.16	93.0 ± 28.2	86.5 ± 27.3	0.37
ΣPUFA	237 ± 110	194 ± 117	0.05	227 ± 62.3	219 ± 77.0	0.63
Total µg/mL	623 ± 283	513 ± 285	0.05	593 ± 156	589 ± 184	0.88

Mean ± SD, repeated measures ANOVA with Bonferonni post-hoc comparisons. Abbreviations are as in Table 3-2.

Table 3-5: Plasma PL fatty acids (µg/mL) at the end of study in patients receiving <4 cycles of chemotherapy and in a previously published study of advanced cancer patients

	<4 cycles chemotherapy End of study	**<8 months to death**	**>8 months to death**
Total, n	16	36	36
LA	104 ± 58.1	82.0 ± 58.0	125 ± 63
ALA	1.1 ± 1.0	0.7 ± 0.8	1.6 ± 2.3
AA	55.1 ± 25.0	43.0 ± 35.0	60.0 ± 40.0
EPA	4.7 ± 4.3	4.4 ± 7.8	5.9 ± 5.2
DHA	13.3 ± 10.5	13.0 ± 12.0	21.0 ± 15.0
Σn-6	181 ± 93.3	142 ± 107	216 ± 117
Σn-3	19.6 ± 14.6	19.0 ± 17.0	29.0 ± 19.0
ΣSFA	264 ± 134	209 ± 150	329 ± 158
ΣMUFA	83.0 ± 48.3	69.0 ± 50.0	106 ± 61.0
ΣPUFA	202 ± 109	164 ± 123	249 ± 134
Total µg/mL	547 ± 278	442 ± 316	686 ± 336

Mean ± SD. Data from a previous study in advanced cancer patients (14) are shown as a reference and were not statistically compared to patients in this study. Abbreviations are as in Table 3-2.

Figures

Figure 3-1: Lung clinic recruitment handout

Cross Cancer Institute
Department of Pain & Symptom and Palliative Care

Research Studies for Patients visiting Lung Clinics

Please check the box by any of the research studies that interest you. A research assistant or research nurse will contact you to tell you more about the study and will let you know whether you are eligible for it. Please complete the form, and leave it with the clinic receptionist or nurse.

Your information is confidential and there is no commitment to participate.

Studies on Nutrition & Weight Loss

Your participation will involve clinical testing, the completion of questionnaires, and in some studies, the testing of a nutritional supplement or medication.

☐ **Testing of Taste and Smell Function in Cancer Patients**

☐ **Study to test how Chemotherapy affects Nutrients in the Body**

☐ **Trial of an Omega-3 Supplement to Maintain Levels of Essential Fats in the Body**

Studies on Cancer Symptoms & Quality of Life

Your participation will involve the completion of questionnaires, and an exercise test.

☐ **Tiredness, Fatigue, and Exhaustion: A Comprehensive Evaluation**

☐ **A Study to Test a Medication for Moderate to Severe Poorly Controlled Pain**

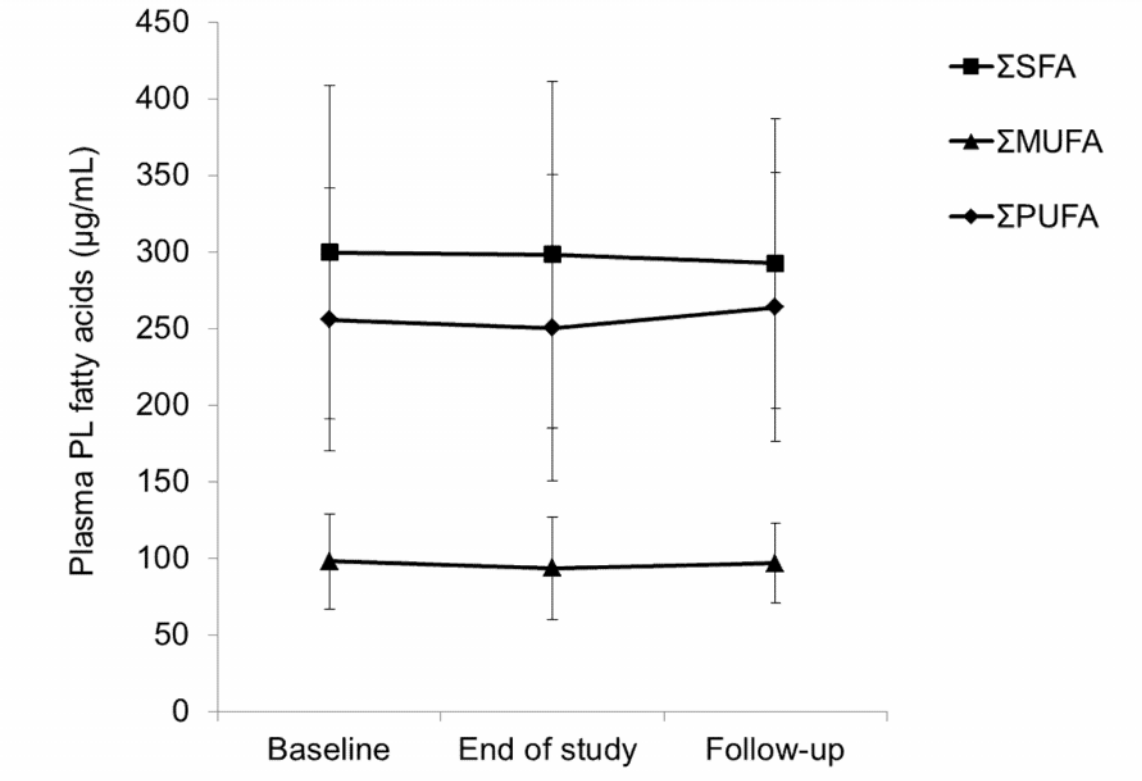
☐ **I am not interested in research studies at this time.**

Name:
_____

Telephone Number: _____

Best Time to Call: ☐ a.m. ☐ p.m.

Figure 3-2: Plasma PL fatty acids at baseline, end of study and follow-up 1 month post study completion



Symbols represent mean ± standard deviation, n=17 at each time point. Abbreviations: ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; ΣPUFA, sum of polyunsaturated fatty acids.

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CHAPTER 4: Skeletal muscle depletion is associated with reduced plasma n-3 fatty acids in non-small cell lung cancer patients

4.1 Introduction

Plasma phospholipids (PL) comprise the majority of n-6 and n-3 essential fatty acids in blood. The composition of fatty acids in PL reflects metabolism of endogenous and dietary fatty acids and have been used as indices of fatty acid status in a variety of populations (1,2). Abnormalities in plasma lipid profiles of cancer patients have been documented, including low essential n-3 and n-6 fatty acids (3,4). We recently reported the prognostic importance of plasma PL fatty acids in advanced cancer patients, with a 50% reduction in survival in patients with low quantities of n-3 fatty acids (5). Comparatively, supplementation with n-3 fatty acids has been reported to improve functional status and prolong survival (6,7).

Sarcopenia is defined as skeletal muscle >2 standard deviations below that of healthy adults (8). Little is known about the role of nutrition in the development of sarcopenia, however, a recent study reported an independent relationship between fatty fish consumption and grip strength (a clinical marker for muscle function) in an elderly population (9). In advanced cancer, supplementation with fish oil (eicosapentaenoic acid 20:5n-3, EPA; and docosahexaenoic acid 22:6n-3, DHA) has been shown to attenuate lean tissue and weight loss (10,11). Taken together, these findings suggest a relationship

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between n-3 fatty acids and body composition but the specific relation to muscle mass is unknown.

The potential interaction between n-3 fatty acids and adipose tissue in cancer is unclear. In healthy overweight individuals, supplementation with n-3 fatty acids has been associated with reduced adiposity (12,13). This effect may be due to the ability of n-3 fatty acids to promote hepatic fatty acid oxidation, leading to decreased availability of free-fatty acids for storage (14). Further, n-3 fatty acids may modulate lipoprotein lipase and hormone sensitive lipase in adipose tissue (15). In advanced cancer, fish oil supplementation has been shown to result in attenuation of adipose tissue loss and normalization of fat oxidation (16). EPA may also preserve adipose tissue by downregulating expression of a tumour derived lipid mobilizing factor (zinc-α2-glycoprotein) which has been identified as a potential cause of increased lipolysis in cancer patients (17-19).

Computed tomography (CT) is a precise method to quantify muscle and adipose tissue in vivo (20,21) with a precision error of approximately 2%. This makes CT image analysis an ideal method for detecting longitudinal changes in muscle and adipose tissue. Indices of sarcopenia have been established using dual-energy X-ray absorptiometry (8) and translated to CT imaging in cancer patients (21). In advanced cancer, sarcopenia is highly prevalent, affecting nearly 50% of newly diagnosed patients with non-small cell lung cancer (NSCLC) (22). Sarcopenia has also been associated with poor functional status (23), reduced survival (23,24) and excess toxicity from several common types of anti-neoplastic therapy: 5-fluorouracil (24), sorafenib (25) and capecitabine (26).

The relationship between fatty acids, muscle mass and adipose tissue in cancer has not been explored outside of fish oil supplementation trials. It is unclear what concentration of plasma n-3 fatty acids are required to maintain or increase muscle mass. This information is vital for determining effective interventions. The objective of this work was to determine if plasma PL n-3 fatty acid concentrations are related 1) to the amount of muscle and 2) to longitudinal changes in muscle and adipose tissue in a cohort of NSCLC patients.

4.2 Methods

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

4.2.1 Patient population, demographics and anthropometrics

The patient population is described in detail in Chapter 3. The present analysis is focused on the relationship between plasma fatty acids and body composition. To relate body composition to plasma fatty acids, it was important to ensure a short time period between the blood draw and CT image. Thus, only patients with a CT image within 14 days of a blood draw were selected from the standard of care population.

Stage of disease was based on American Joint Committee on Cancer stage groupings, I, II, III, and IV (27). Height was measured by a stadiometer and weight was measured using a medical balance beam scale at baseline and at each time point. Height and weight were used to compute body mass index (BMI) (kg/m^2). World Health Organization (28) categories were used to classify patients as: underweight, BMI <18.5; normal, BMI 18.5-24.9; overweight BMI 25-29.9;

or obese BMI ≥ 30. Self-reported weight loss history was obtained from medical records or the Patient Generated Subjective Global Assessment (PG-SGA) (29). Blood (12 mL) was drawn into a heparin tube by a Registered Nurse at the cancer center laboratory prior to chemotherapy initiation (baseline) and prior to each 3 week cycle of chemotherapy.

4.2.2 Body composition analysis

All images were taken for diagnosis, staging and follow up purposes; body composition was measured using secondary analysis of images that were retrieved from the patient clinical record. No images were ordered with the explicit purpose of body composition analysis. Skeletal muscle and adipose tissue were measured using two consecutive images extending from the third lumbar vertebrae (L3) to the iliac crest. L3 was chosen as a vertebral landmark because skeletal muscle and adipose tissue at L3 is strongly correlated to whole body muscle and adipose tissue (20,21,30). Images were analyzed using Slice-O-matic software (V4.3; TomoVision, Montreal, QC, Canada). Hounsfield unit thresholds were used to demarcate tissues: -29 to +50 for skeletal muscle (31), -150 to -50 for visceral adipose tissue (32) and -190 to -30 for subcutaneous and intermuscular adipose tissue (31). The L3 region contains the psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal obliques and rectus abdominus muscles. Cross-sectional areas (cm^2) were computed for the sum of the muscle groups and for each adipose tissue depot. Mean tissue area for the 2 consecutive images were determined and subsequently

normalized by height (cm^2/m^2). Total adipose tissue was calculated as the sum of visceral, subcutaneous and intermuscular adipose tissue.

4.2.2.1 Calculations

Threshold values for sarcopenia that were defined in an elderly population (8) and translated to an advanced cancer population (21) were used to classify patients as sarcopenic or non sarcopenic. Men with L3 cross-sectional muscle area <55.4 cm^2/m^2 were classified as sarcopenic and women with L3 cross-sectional muscle area <38.9cm^2/m^2 were classified as sarcopenic. There are no similar thresholds for adipose tissue. Therefore, plasma PL concentrations were related to cross-sectional muscle area but not cross-sectional adipose tissue area.

As CT images are taken for follow-up purposes, the timing of CT images is unique for each individual. To allow for comparison between individuals, change in muscle and adipose tissue were expressed as percent change from the initial CT image and divided by the number of days elapsed between the 2 CT images. The daily rate of change was multiplied by 100 to form a standard unit expressed as % change/100d.

To express muscle and adipose tissue in conventional units, whole body fat-free mass (FFM) and whole body fat mass (FM) were estimated from regression equations that have been applied in several different cancer populations (25,33):

Whole body FFM (kg) = 0.30 * (skeletal muscle at L3 (cm^2)) + 6.06; r^2=0.88 (21)

Whole body FM (kg) = 0.068 * (total adipose at L3 (cm^2)) + 4.142; r^2= 0.927 (20)

Whole body skeletal muscle volume = 0.166 * [skeletal muscle 5 cm above L4-L5 (cm^2)] + 2.142; r^2=0.855 (20).

A density of 1.04g/cm^3 was used to convert muscle volume to mass (34).

4.2.3 Plasma phospholipid fatty acid analysis

Plasma PL fatty acids were isolated and quantified as described in Chapter 3. ∑n-3 represents the sum of 18:3n-3, 20:3n-3, 20:5n-3 and 22:6n-3 fatty acids. Fatty acids were expressed as amounts (μg/mL) and as a proportion (%) of total PL.

4.2.4 Statistical analysis

Data are reported as mean ± standard error (SE) and significance reported at levels of $P<0.05$. All tests were two sided. Two-sample t-tests were used to determine differences between groups. Pearson correlations were used to determine potential relationships between fatty acids and rate of muscle and 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

Forty-one (n=41) patients had a blood draw and CT image <14 days apart and a mean of 5 ± 0.6d elapsed between blood draw and CT image. Following consultation for chemotherapy and baseline measurement, 3 patients had a decline in performance status and subsequently did not begin chemotherapy treatment. The remaining patients all received platinum based doublet chemotherapy: 30

patients received cisplatin or carboplatin with vinorelbine, 5 patients received cisplatin/etoposide with concurrent radiation (stage IIIA disease), 3 patients received carboplatin with gemcitabine and 3 patients received carboplatin with taxol. Medications in standard dosages (ondansetron, dexamethasone and lorazepam) were provided in conjunction with chemotherapy. The mean duration of chemotherapy was 3 cycles over 65 ± 5d.

Men and women are shown separately as there was an effect of gender on body composition (Table 4-1). Men had heavier body weights, more muscle and more adipose tissue than women. Only 2 patients were underweight (BMI <18.5kg/m^2) and nearly 50% of patients were overweight or obese.

4.3.2 Cross-sectional body composition and fatty acid analysis

A small number of patients (n=3) had subcutaneous adipose tissue outside of the CT image viewing field and were excluded from total adipose tissue analysis. There was high variability in lumbar skeletal muscle and adipose tissue within both genders. In men, skeletal muscle area ranged from 113 to 205cm^2 (21.8 to 37.6kg estimated whole body skeletal muscle) and adipose tissue area ranged from 102 to 823cm^2 (11.1 to 60.1kg estimated whole body fat mass). In women, skeletal muscle ranged from 86.7 to 129 cm^2 (17.1 to 24.9 kg estimated whole body skeletal muscle) and adipose tissue area ranged from 4.4 to 403cm^2 (4.4 to 31.5kg estimated whole body fat mass). Sarcopenia was not exclusive to patients with low adipose tissue. Accordingly, 62% of patients with BMI <25.0kg/m^2 and 55% of patients with BMI ≥ 25.0kg/m^2 met criteria for sarcopenia.

Patients with sarcopenia had lower plasma EPA (5.0 ± 0.6µg/mL versus 9.5 ± 1.3µg/mL; *P*=0.001), lower DHA (8.8 ± 2.8µg/mL versus 19.6 ± 1.8µg/mL; *P*=0.01) and lower ∑n-3 fatty acids (19.2 ± 1.8µg/mL versus 30.0 ± 2.4µg/mL; *P*=0.002) than non-sarcopenic patients (Figure 4-1A). Plasma linoleic (127 ± 11.4µg/mL versus 135 ± 10.4µg/mL) and α-linolenic acid (1.4 ± 0.2µg/mL versus 1.7 ± 0.2µg/mL) did not differ between sarcopenic and non-sarcopenic patients. Fatty acids were also expressed as a proportion of total PL (%) because the total amount of plasma PL fatty acids tended to be lower in sarcopenic patients (592 ± 48.7µg/mL versus 691 ± 45.0µg/mL; *P*=0.17). As a proportion of total fatty acids (Figure 4-1B), EPA, DHA and Σn-3 fatty acids were the only fatty acids that differed significantly between sarcopenic and non-sarcopenic patients.

4.3.3 Longitudinal body composition and fatty acid analysis

Longitudinal analysis focused on the relationship between skeletal muscle, adipose tissue and plasma EPA, DHA and Σn-3 fatty acids as these were the only fatty acids that differed in the cross-sectional analysis.

From the cross-sectional group of n=41, 3 patients were assessed at a second time point with a diagnostic test (x-ray) that did not permit body composition assessment and the 3 patients that did not receive planned chemotherapy were also excluded from longitudinal analysis. Therefore, change in muscle and adipose tissue were determined in a subgroup of patients (n= 35) who had an additional CT image taken within 200d of their initial CT image.

Longitudinal analysis revealed that sarcopenic patients lost muscle at a greater rate than non-sarcopenic patients (-12.6 ± 3.8%/100d versus 0.9 ±

1.9%/100d; *P*=0.01; Figure 4-2). Low concentrations of EPA, DHA and ∑n-3 were correlated to high rates of muscle loss. A scatter plot of EPA and DHA versus the rate of muscle change is depicted in Figure 4-3A). EPA decreased linearly (r= 0.47, *P*=0.04) from a maximum of 18.4µg/mL in patients gaining muscle to undetectable concentrations in patients losing muscle at the greatest rate (~3kg of skeletal muscle). DHA decreased linearly related (r= 0.70, *P*=0.001) from a maximum of 25.1µg/mL to undetectable concentrations in patients with accelerated loss of muscle. The ∑n-3 fatty acids also decreased linearly (r=0.61, *P*=0.05) from 46.9µg/mL to undetectable concentrations. Low concentrations of EPA, DHA, ∑n-3 fatty acids were also correlated to accelerated loss of adipose tissue (EPA; r=0.43, *P*=0.08, DHA; r=0.50, *P*=0.04, ∑n-3 fatty acids; r=0.53, *P*=0.03). The relationships between EPA, DHA and rate of change in adipose tissue are depicted in a scatter plot in Figure 4-3B.

4.4 Discussion

Loss of muscle, adipose tissue and plasma PL fatty acids are important predictors of treatment response and survival (5,26,33). This paper is the first to report a clear relationship between muscularity and plasma PL n-3 fatty acids. Concentrations of n-3 fatty acids were distinctly associated with sarcopenia and loss of muscle and adipose tissue. On the other hand, patients who gained muscle and adipose tissue had the highest concentrations of n-3 fatty acids, which suggests that physiological concentrations of EPA and DHA may play a role in modulating body composition. Although Chapter 3 showed aberrations in all classes of fatty acids, including PUFA, SFA, MUFA, n-3 and n-6 fatty acids, the

relationship between body composition and fatty acids appears to be specific to n-3 fatty acids, as only EPA, DHA and ∑n-3 fatty acids differed between sarcopenic patients and non-sarcopenic patients. The identification of the relationship between n-3 fatty acids and muscle and adipose tissue represents an important advance in our understanding of factors affecting body composition.

In general, patients were losing muscle, with loss of 1.1kg of skeletal muscle over the duration of chemotherapy treatment (~2.5 months) but the rates of muscle change ranged widely from intense loss (-62%/100d; -6.9kg skeletal muscle) to gain (14%/100d; +1.6kg skeletal muscle). Our data showed a relationship between plasma EPA, DHA and Σn-3 fatty acids and muscle rate of change. The observation that incremental changes in plasma EPA, DHA and Σn-3 fatty acids are reflected in the rate of muscle loss or gain is novel. This information could provide a basis for determining the concentration of plasma fatty acids levels required for maintenance of muscle in future supplementation studies. Of note, the relationship between sarcopenia, change in muscle and adipose was not exclusive to EPA. Several fish oil studies have used supplements containing purified EPA and no DHA (11,34), but there is limited evidence to suggest that EPA is more effective than DHA, or EPA and DHA combined. Based on our results, and the results from metabolic studies showing limited conversion of EPA to DHA (35), we suggest that trials with body composition endpoints should provide both EPA and DHA.

Despite the fact that the majority of patients were receiving palliative treatment, a portion of patients (20%) gained muscle. This quartile of patients

also had the highest mean plasma EPA, DHA and ∑n-3 fatty acids. Several biological mediators including cytokines and eicosanoids have been proposed as mediators of muscle catabolism in advanced cancer (36). The relationship between muscle mass and concentration of plasma n-3 fatty acids observed in this study may be partially explained by the anti-inflammatory effect observed when n-3 fatty acids derived from fish oil are provided (37,38). Additionally, supplementation with n-3 fatty acids has been shown to down-regulate the activity and expression of the proteolytic pathway (39), acute phase response (37,40) and enhance muscle sensitivity to insulin (41,42). Although there is an association between muscle depletion and n-3 fatty acids, this does not ensure causality and an important direction of our work will be to clarify the mechanisms involved in role of n-3 fatty acids in mediating these effects on muscle.

An effect of fish oil supplementation on muscle mass of cancer patients has not been consistently demonstrated. Previous studies used indirect measures of muscle such as mid-upper arm circumference (43) or bioelectrical impedance (11,34,44) which does not distinguish between skeletal muscle, organs and bone. An innovation of our study is the use of CT imaging which specifically quantifies skeletal muscle. Fearon et al. (44) showed a positive correlation between change in lean body mass of advanced cancer patients receiving fish oil supplementation and plasma EPA. Our findings confirm that this relationship is specific to skeletal muscle. The effect of plasma n-3 fatty acids on muscle appears to be robust as the study showing a relationship between lean body mass and EPA was carried out in

a different patient population than our study: pancreatic cancer patients not receiving anti-neoplastic therapy.

In healthy populations, adipose tissue gain is generally not desirable. However, the same may not be true in populations with chronic disease. The obesity paradox, a phenomenon where obese people have lower mortality than people with normal body weights has been observed in a variety of chronic conditions including cancer (45-47). Coupled with the prognostic significance of adipose tissue loss in advanced cancer (5,48), this suggests that slight gain or stabilization of adipose tissue in patients with the greatest amounts of n-3 fatty acids observed this study may be beneficial. Although weight gain in lung cancer has been associated with a survival benefit (49), the composition of this gain has not been defined and it is unclear whether this is due to an effect of muscle, adipose tissue or a combination of both. This is the first study to suggest a relationship between change in adipose tissue and n-3 fatty acids in cancer and thus the significance of both this relationship and of adipose tissue gain requires further investigation.

Our study was designed to capture the relationship between muscle, adipose tissue and plasma n-3 fatty acids over the course of treatment. The CT images in this study were from various time points throughout diagnosis and chemotherapy treatment, which suggests that the relationship between n-3 fatty acids, muscle and adipose tissue persists regardless of where a patient may be in the treatment trajectory. Nevertheless, we acknowledge that our findings should be confirmed in additional patient populations. A limitation of our study is that it

was not designed to detect an effect of n-3 fatty acids on muscle function. Prior studies have shown a beneficial effect of fish oil supplementation on muscle function via a reduction in fatigue (27) and increase in physical activity (33) and our future efforts are directed at delineating this relationship.

4.5 Conclusion

NSCLC patients with sarcopenia have pronounced alterations in fatty acids, specifically n-3 fatty acids. Low concentrations of n-3 fatty acids are present in patients losing muscle and adipose tissue at accelerated rates. Conversely, muscle and adipose tissue gain were greatest in patients with the greatest concentrations of plasma n-3 fatty acids. This work contributes toward defining optimal concentrations of n-3 fatty acids required for ameliorating tissue wasting in patients with cancer and provides strong support for intervention with n-3 fatty acids.

Tables

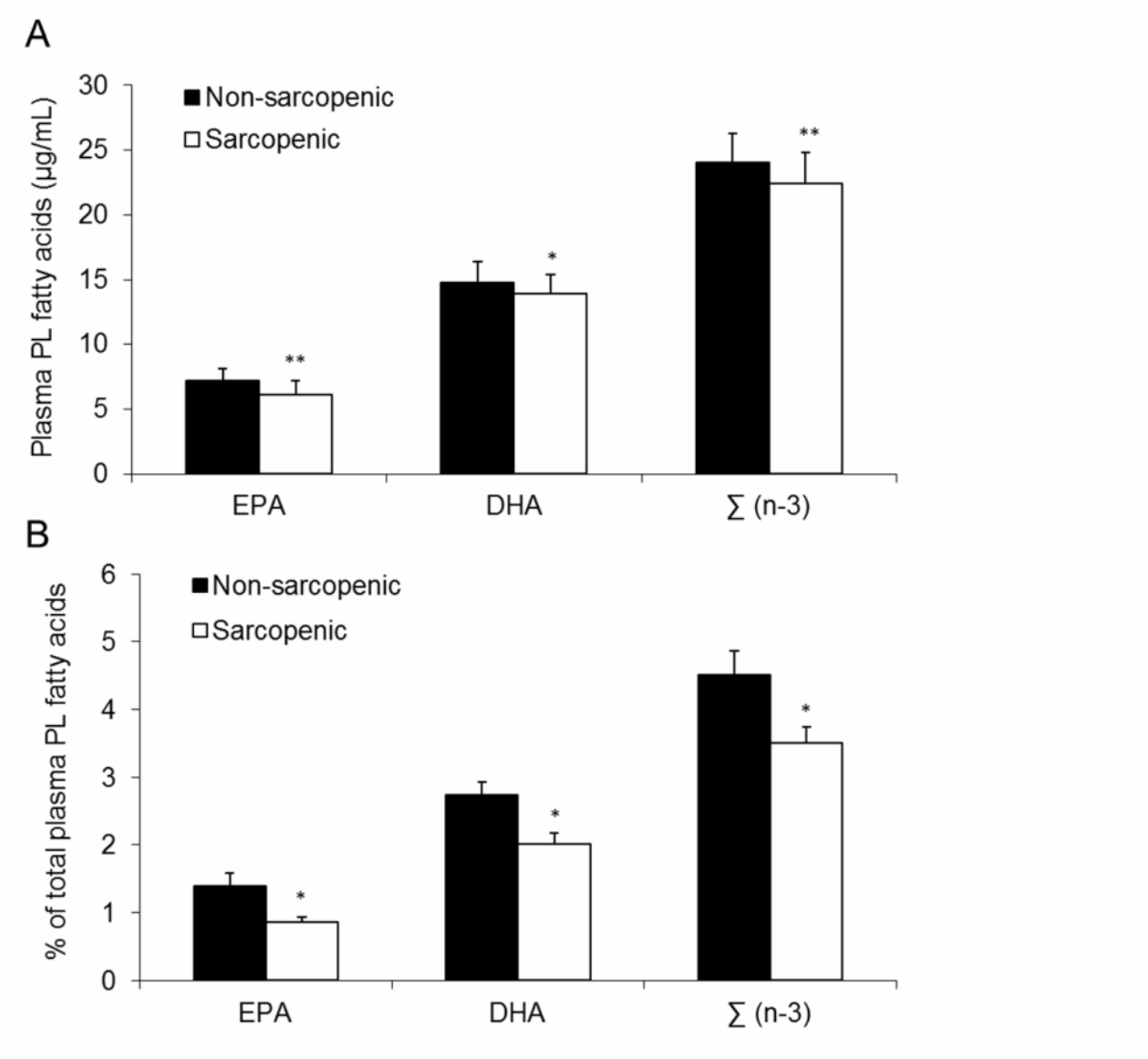
Table 4-1: Demographics and anthropometrics of patients with non-small cell lung cancer

	Men	Women
Total, n	19	22
Age, years	65 ± 1.6	60 ± 2.0*
TNM stage, n		
I	0	2
II	2	0
III	6	7
IV	11	13
BMI, kg/m^2	27.5 ± 1.2	23.4 ± 0.8*
BMI <18.5, n	1	1
BMI 18.5-24.9, n	5	14
BMI 25-29.9, n	8	7
BMI ≥ 30, n	5	0
Lumbar skeletal muscle area (cm^2)	157.8 ± 5	102.8 ± 4.2*
Lumbar skeletal muscle index (cm^2/m^2)	53.3 ± 1.7	39.8 ± 1.0*
Lumbar total adipose area (cm^2)	384 ± 53.5	194 ± 26.8*
Lumbar total adipose index (cm^2/m^2)	129 ± 18.0	74.5 ± 10.1*
[1]Sarcopenic (%)	63	59
[2]Estimated whole body skeletal muscle, kg	29.5 ± 0.7	19.9± 0.5*
[3]Estimated fat-free mass, kg	51.1 ± 1.1	37.2 ± 0.9 *
[2]Estimated whole body fat mass, kg	30.2 ± 3.6	17.3 ± 1.8*

Mean ± SE. *Different from men, $P< 0.05$. Abbreviations: SM, skeletal muscle.
[1]Based on cutpoints for muscularity (21). Derived regression equations from [2]Shen et al. (20) and [3]Mourtzakis et al. (21).

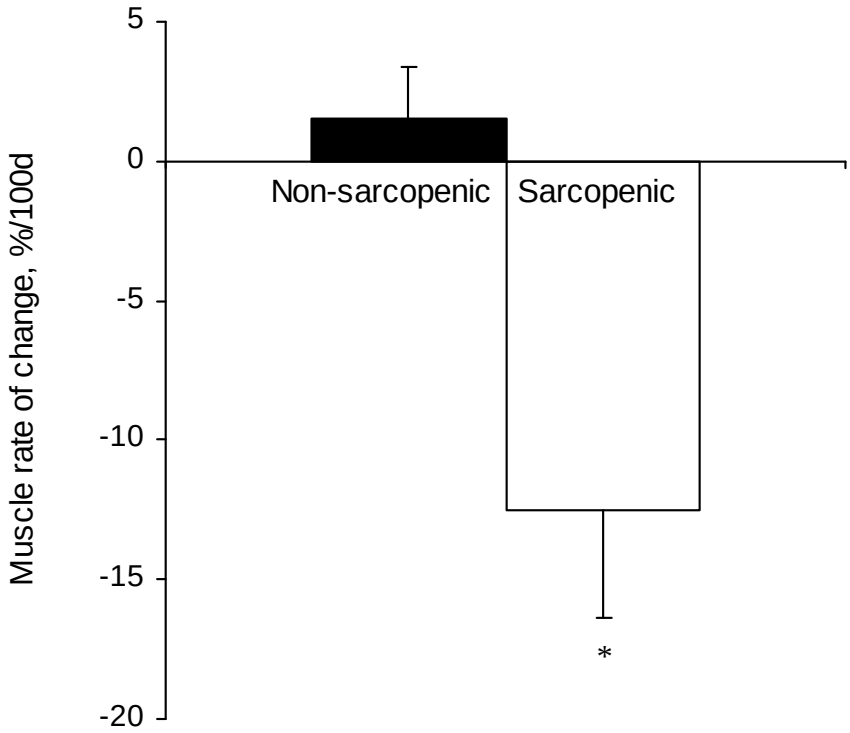
Figures

Figure 4-1: Relationship between EPA, DHA, Σn-3 fatty acids and sarcopenia



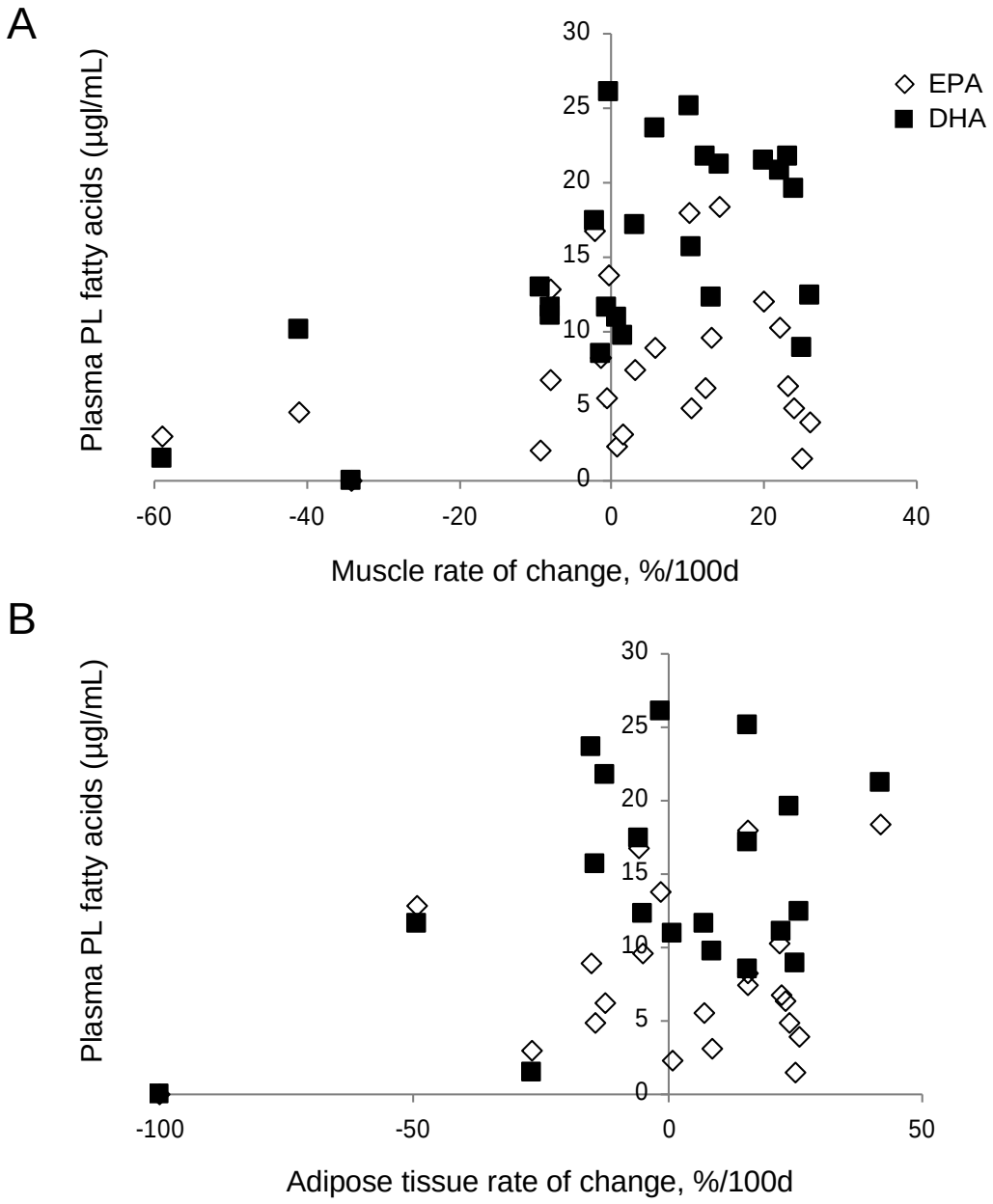
Amounts (A) and proportions (B) of plasma PL fatty acids in non-sarcopenic and sarcopenic patients. Bars represent mean + SE, n=16 (non-sarcopenic) or n=25 (sarcopenic). Different from non-sarcopenic patients, **P*<0.01,***P*<0.001.

Figure 4-2: Mean rate of muscle change in non-sarcopenic patients and sarcopenic patients as measured by computed tomography



Bars represent mean + SE, n=14 (non-sarcopenic) or n=21 (sarcopenic). Different from non-sarcopenic patients, *P<0.01.

Figure 4-3: Plasma PL EPA and DHA versus tissue rate of change as assessed by computed tomography



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CHAPTER 5: Nutritional intervention with fish oil provides a benefit over standard of care on weight and skeletal muscle mass in non-small cell lung cancer patients receiving chemotherapy

5.1 Introduction

Involuntary weight loss is common among advanced cancer patients, contributing to poor treatment response, functional decline and decreased survival (1). Supplementation with >2g per day of eicosapentaenoic acid (EPA) has been shown to stabilize weight loss (2) and attenuate lean tissue wasting (3) in patients with advanced cancer. It is hypothesized that this is due in part to the ability of EPA to down regulate the production of catabolic cytokines that promote muscle breakdown. EPA supplementation has been shown to decrease production of interleukin (IL)-1 and tumour necrosis factor alpha (TNF-α) in healthy subjects (4) and IL-6 production in weight losing cancer patients (5).

Although initial trials of EPA supplementation were promising, 3 large randomized trials have failed to demonstrate a clear benefit of EPA on body weight or lean tissue in cancer patients (6-8). Possible reasons for discordance are: time of initiation of intervention, contamination between treatment arms and indirect assessments of muscle mass. Studies have selected patient populations with advanced disease, who are often treatment refractory (6,7) and have median survival as short as 14 weeks Bruera et al. (9). The results of these trials are difficult to interpret due to several factors: advanced wasting at presentation of up to 56% of pre-illness weight (6), a large number of early deaths (9), poor

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compliance to both placebo and treatment (6,10) and failure to report study compliance (8). Additionally, studies used indirect measures of skeletal muscle such as estimation of lean body mass from body water (11), skinfolds (9) and bioelectrical impedance (2,6,7), none of which distinguish skeletal muscle from other lean soft tissue.

In advanced cancer patients, accelerated gain in lean tissue including the liver and spleen has been observed 3 months prior to death (12). Therefore, specific discrimination of skeletal muscle from other lean tissue is important. Computed tomography (CT) can precisely quantify skeletal muscle (13,14) but has not been previously used to assess the effect of EPA supplementation on skeletal muscle in advanced cancer. Adipose depots (visceral, subcutaneous and intermuscular) can also be differentiated using CT imaging. CT image analysis was recently utilized to describe changes in adipose tissue in advanced cancer patients, including accelerated loss of adipose tissue nearing time of death, with the exception of intermuscular adipose tissue (IMAT) which increased approaching death (15). Increased muscle fat content is also a negative predictor of muscle strength (16) and IMAT has been correlated with poor function (17) and increased incidence of mobility limitations (17).

This study uniquely used early intervention in newly referred patients with the goal of preventing weight and muscle loss rather than attempting to attenuate advanced wasting. The aim of this study was to examine the effect of nutritional intervention with fish oil on weight, and body composition against standard of care during the course of chemotherapy treatment.

5.2 Methods

This study was originally approved as a double blind, placebo controlled trial by the Alberta Cancer Research Ethics board. The trial was closed due to difficulties with implementing the study design (Appendix 2). The study was re-designed and approved as a new trial by the Alberta Cancer Research Ethics Board in the form that is presented in this chapter. Written informed consent was obtained from all patients (Appendix 3). Patients were accrued from the Cross Cancer Institute using handouts which describe nutritional studies. Details on the study design of the standard of care group (SOC) are provided in Chapter 3. Both studies were described as nutritional studies, thus minimizing potential bias and accrual of nutritionally motivated patients to the intervention group. In the event that patients indicated interest in both studies, patients were approached for participation in FO study as the rate of accrual in this study was lower. If patients indicated they were interested in the FO study but subsequently declined or were ineligible to participate, they were not approached for the SOC study.

5.2.1 Patient population

Patients with a histologically confirmed diagnosis of non-small cell lung cancer, who were naïve to chemotherapy were eligible for enrolment. We included patients receiving first line chemotherapy to maximize accrual of patients for whom better survival would be predicted. All patients consented to receive platinum based doublet chemotherapy of either curative or palliative intent based on standard practice specifically agreed upon as Clinical Practice Guidelines by the Alberta Provincial Lung Tumour Group. The duration of the

study was at least six weeks (2 cycles of chemotherapy). Because this study was aimed at preventing body composition changes, prior weight loss was not an inclusion criterion but self-reported weight loss in the 6 months preceding study enrolment was recorded from the Patient-Generated Subjective Global Assessment (18) or from medical charts. Performance status was assessed by a physician using the Eastern Cooperative Oncology Group (ECOG) scale at baseline. Stage of disease was based on American Joint Committee on Cancer stage groupings, I, II, III and IV (18). Response to treatment was evaluated by a radiologist and oncologist on the basis of clinical examination and imaging which consisted of CT, magnetic imaging resonance or x-ray. Patients with a CT image within 45d of starting chemotherapy and a second CT image within 45d of finishing chemotherapy were included in this analysis.

5.2.2 Study design

To avoid the challenges of previous studies (contamination between treatment arms and poor compliance), this study was designed as an open label study with a contemporary control group. To ensure patients recruited for this study are representative of the local advanced lung cancer population, we included a reference group to detail typical baseline characteristics of patients receiving first line chemotherapy treatment to provide information on the expected changes in body composition during chemotherapy. Our research group has prospectively followed over 600 patients with solid tumours of the lung who have longitudinal CT images from time of referral. Patient records were reviewed for the same inclusion criteria as patients in the SOC and FO group: NSCLC

diagnosis, naïve to chemotherapy and treatment with first-line platinum based doublet chemotherapy. One hundred and four patients met the inclusion criteria. Subsequently, weight information was recorded and CT images were analyzed. CT images were 95 ± 3.8d apart. A mean of 19.5 ± 1d elapsed between starting chemotherapy and CT1, 18 ± 1d elapsed between CT2 and the last day of chemotherapy.

As previous studies have reported a benefit of ≥ 2g EPA (3;16;17), patients were instructed to achieve an intake of at least 2g of EPA per day commencing on the first day of chemotherapy treatment and continuing for the duration of their chemotherapy treatment. Patients were given the choice of two formats of supplementation: 1) 4 gelatin capsules per day containing 1g of fish oil (2.2g EPA and 240mg DHA) or 2) 7.5mL liquid fish oil per day (2.2g EPA and 500mg DHA). Because poor compliance has been reported in previous studies (2;5;6), we chose this approach to increase study adherence. Capsules were provided in kind by Ocean Nutrition Canada (Ocean Nutrition Canada, Nova Scotia, Canada). Liquid fish oil was purchased from NutraSea (Ascenta Health, Nova Scotia, Canada). These companies did not have access to the study results or influence the conclusions of this study.

Compliance

The amount of supplement taken per day was recorded by the subjects and any unused capsules or liquid was returned at patient visits. An acceptable level of compliance was set at ≥ 80% of the prescribed dose (minimum 1.76g EPA per day).

5.2.3 Weight and body composition analysis

Height was measured by a stadiometer and weight was measured using a medical balance beam scale at baseline and before each cycle of chemotherapy. Height and weight were used to compute body mass index (BMI in kg/m^2). World Health Organization (19) categories were used to classify patients: underweight $<18.5kg/m^2$; normal 18.5 to $24.9kg/m^2$; overweight 25 to $29.9kg/m^2$; obese $\geq 30kg/m^2$.

Body composition was analyzed as described in Chapter 4. Skeletal muscle attenuation (muscle GLI), an indicator of muscle fat content (20) was recorded. A lower value indicates increased fat infiltration and is associated with lower muscle strength and lower strength per muscle size (poor muscle quality (16). Sex-specific cut-points for L3 skeletal muscle index previously defined in an advanced cancer population (14) were used to designate patients as sarcopenic: skeletal muscle index $<55.4\ cm^2/m^2$ in males and $<38.9cm^2/m^2$ in females.

Change in muscle and adipose tissue were expressed as percent change from the initial CT scan and divided by the number of days elapsed between the 2 CT images. The daily rate of change was multiplied by 100 to form a standard unit expressed as % change/100d to allow for comparison between individuals. As the precision error for muscle and adipose is ~1.5% (14), change between -2 and +2 was considered maintenance of tissue. Whole body fat mass and skeletal mass was estimated from lumbar cross-sectional areas as described in Shen et al. (13).

5.2.4 Nutrition, symptom and quality of life assessments

Patients in the FO group completed several assessments at set time points throughout the study to determine whether FO has an effect on nutritional status, disease related symptoms and quality of life. Patients in the SOC group did not undergo these assessments.

The PG-SGA is a validated nutritional screening tool that measures patient-reported weight change, dietary intake, gastrointestinal symptoms and PS (18,21). A PG-SGA score of 4-8 requires referral to a dietician, a score of ≥ 9 indicates a critical need for symptom management and/or nutrient intervention. Patients were asked to complete the PG-SGA at baseline and on the last day of chemotherapy.

The Edmonton Symptom Assessment Scale (ESAS) is used to assess common symptoms in advanced cancer patients (22). This tool assesses nine symptoms: pain, tiredness, nausea, depression, anxiety, drowsiness, appetite, well being and shortness of breath, and an additional line labeled “Other Problem”. The severity at the time of assessment of each symptom is rated on a scale of 0 to 10, 0 meaning the symptom is absent and 10 meaning the worst possible severity. The ESAS was administered at baseline, after each cycle of chemotherapy and on the last day of chemotherapy (last day of supplementation).

The Functional Assessment of Anorexia/Cachexia Therapy (FAACT) is a questionnaire designed to measure general aspects of quality of life as well as specific anorexia/cachexia-related concerns; higher scores indicate better QOL

(23). Patients were asked to complete the FAACT at baseline and on the last day of chemotherapy.

5.2.5 Plasma phospholipid fatty acid analysis

Blood was drawn by a Registered Nurse at the cancer centre laboratory prior to chemotherapy initiation (baseline), one day prior to each 3 week cycle of chemotherapy and on the last day of chemotherapy treatment. Plasma phospholipid (PL) fatty acids were isolated from plasma and individual fatty acids were quantified using methods described in Chapter 3. Fatty acids were expressed as a quantitative amount (μg/mL) and as a percentage of total PL. Plasma fatty acids were quantified throughout the trial (Chapter 3) but are only presented here from the first (baseline) and last (last day of the trial) blood draws.

5.2.6 Cytokine analysis

Plasma amounts of pro-inflammatory cytokines (interferon (IFN)-γ, IL-10, IL-12p70, IL-1β, IL-6, IL-8 and TNF-α) and IL-15; which has been proposed to have anabolic effects on skeletal muscle, were quantified at baseline and the last day of the trial using a human ultra-sensitive Pro-inflammatory 7-plex and a human ultra-sensitive IL-15 kit (Meso Scale Discovery, Gaithersburg, MD). The lower limits of detection were 0.80, 0.57, 0.77, 0.58, 0.18, 0.10, 0.28 and 0.46, respectively. The kits detect cytokines in a sandwich immunoassay format on plates pre-coated with capture antibodies. A diluent (25μL) was added into each well and the plate was incubated for 30 minutes with shaking (500rpm). Each sample was run in duplicate; 25μL of plasma or calibrator (for generating a standard curve) was pipetted into each well. The plate was incubated for 2 hours

with shaking (500rpm) then washed 4 times with a 0.05% phosphate buffered solution. The detection antibody solution (25µL) was added to each well followed by incubation for 2 hours with shaking (500rpm). The plate was washed a further 4 times with the phosphate buffered solution followed by addition of 150µL of read buffer to each well. The plate was read immediately on a SECTOR Imager 2400 (Meso Scale Discovery, Gaithersburg, MD) which measures the amount of emitted light. The concentration of cytokines was calculated using MSD DISCOVERY WORKBENCH® (Meso Scale Discovery, Gaithersburg, MD) analysis software.

5.2.7 Statistical analysis

Analyses were completed per-protocol. The per-protocol population included all patients with ≥ 80% compliance to fish oil supplementation. The primary endpoint was change in muscle between baseline and end of chemotherapy treatment. Adipose tissue, body weight and plasma EPA at baseline and end of chemotherapy treatment were secondary endpoints.

Data are presented as mean ± standard error (SE) and significance determined at $P<0.05$. All tests were two sided. Two-sample t tests and chi-square tests were used to determine differences between the SOC and FO groups. The reference data was collected during a different time period (2001 to 2007) than data from the SOC and FO groups. As such, data for the reference group is provided as a point of reference and was not compared statistically to the SOC and FO group. Repeated measures ANOVA with Bonferonni comparisons was used to compare changes in tissue mass and cytokines from baseline to end of

treatment in both groups and changes in symptoms, nutritional status and quality of life in the FO group. Linear regression was used to examine the relationship between muscle rate of change and change in plasma PL EPA. Cytokines in the SOC group were not normally distributed (Shapiro-Wilk test; $P<0.05$) and were log transformed. However, results did not differ from analyses using non-transformed data and thus all data and analyses are reported as non-transformed. Spearman correlations were used to relate change in cytokine levels to tissue rate of change and to change in plasma PL EPA. Statistical analyses were performed with SPSS (SPSS for Windows, version 18.0, SPSS, Chicago, IL).

5.3 Results

5.3.1 Patient demographics

Patients were recruited over 2 years (2007-2009). Figure 5-1 outlines details of patient accrual and study completion in both groups. The main reasons for exclusion included: ineligible for chemotherapy treatment and participation in a clinical trial (non-standard treatment). Sixty patients were recruited to the SOC group and twenty-five patients were recruited to the FO group. Fish oil was well tolerated. No serious adverse events related to the study intervention were reported. The adverse events in the FO group were related to chemotherapy treatment (n=2) and disease progression (n=1). In the SOC group, all adverse events were related to chemotherapy treatment. Other reasons for withdrawal were: deterioration of PS and subsequent ineligibility for chemotherapy (2 patients in the FO group and 8 in the SOC group), 1 patient in the SOC group received single agent chemotherapy, 1 patient in the SOC group moved and 1

patient missed several study appointments. Of the 19 FO and 42 SOC patients that completed the trial, 17 and 24 patients had CT images which permitted body composition analysis at baseline and end of study. One patient in the FO group was unable to achieve 80% compliance to the FO supplement (mean 1.3g EPA per day) and was subsequently excluded leaving 24 patients in the SOC and 16 patients in the FO group.

There were no significant differences in baseline demographics and anthropometric measures between the SOC and FO group (Table 5-1). Patients recruited to our study were representative of the local population as baseline characteristics were in the same range as the reference group. Although weight loss was not an inclusion criterion, a history of weight loss in the preceding 6 months was common. Despite this, over 50% of patients were overweight or obese, similar to the reference population (53%). The average number of comorbid conditions was 2 in the SOC group and 2 in the FO group. Hypertension, COPD and hyperlipidemia/hypercholesterolemia were the most common comorbidities in both groups. There was no difference in treatment intent (19% received adjuvant therapy in the FO group versus 17% in the SOC group). Average time on study was comparable between groups (FO 10.6 ± 0.8 weeks, SOC 9.8 ± 0.7 weeks; P=0.43) and thus the amount of chemotherapy received was comparable between groups.

5.3.2 Anthropometrics

Patients in the SOC group lost significantly more weight than the FO group (Table 5-2). In the SOC group, 29% of patients maintained or gained

weight (0 to 4.6kg) whereas 69% of patients receiving FO maintained or gained weight (0 to 6.7kg) during chemotherapy. The amount of weight lost by the SOC group was similar to the reference group (Table 5-2).

The first CT image was a mean of 25 ± 3d from the start of chemotherapy and the second CT image was a mean of 21 ± 5d after finishing chemotherapy. Sixty-nine percent of patients in the FO group maintained or gained muscle compared to 29% of patients in the SOC group. In the SOC group, 4 patients became sarcopenic over the course of chemotherapy (cross-sectional muscle area <55.4 $cm^2/m^2$for men and <38.9cm^2/m^2 for women), whereas no patients in the FO group became sarcopenic. SAT was outside the viewing field in 1 patient in the SOC group and 1 patient in the reference group who were not included in TAT analyses. Changes in muscle, IMAT and TAT over time are shown (Table 5-2). Tissue rates of change in the reference group fell within the same range as the rates observed in the SOC group.

Estimated whole-body skeletal and fat mass are shown in Table 5-3. Loss of skeletal mass is evident in patients in the SOC group with some patients losing up to 5.2kg of muscle from baseline to end of study. Loss of skeletal muscle occurred concurrently with increased muscle fat content in the SOC. Skeletal muscle attenuation (muscle GLI) decreased by ~3.5 units in the SOC group (Table 5-3). This change represents an approximate 3% increase in muscle fat content (20). Similar loss of muscle mass (-0.9 ± 0.1kg) and muscle attenuation (-2.8 ± 0.5) was observed in the reference group.

5.3.3 Nutrition, symptom and quality of life assessments

The mean PG-SGA score at the end of treatment did not differ from the mean score at baseline (8 ± 1.3 versus 7 ± 1.5; *P*=0.36). Compared to baseline, there were no changes in ESAS assessment of appetite or overall ESAS score after 1, 2, 3, or 4 cycles of chemotherapy (2 ± 0.5 versus 3 ± 0.6; *P*=0.16). QOL was maintained throughout the study period (100 ± 3.7 at baseline versus 102 ± 4.6 at the end of study; *P*=0.61).

5.3.4 Plasma phospholipid fatty acids

Mean plasma PL EPA and DHA in the SOC group at baseline were comparable to concentrations at the end of study period. Mean plasma PL EPA in the FO group was increased more than 2 fold from baseline (Table 5-4). Plasma PL DHA also increased significantly although by a lesser magnitude than EPA, which is reflective of the lower concentration of DHA in both the capsules and liquid. There was no change in PL AA concentration following fish oil supplementation. Despite reported compliance of over 95% (2.1 ± 0.6g EPA/day), variability in plasma EPA was evident with changes from baseline ranging from -0.3 to +3.8%. In view of this variation, regression analysis was used to examine the relationship between EPA incorporation and muscle rate of change in the FO group (Figure 5-2). There was a positive linear relationship between change in plasma EPA concentration and rate of muscle change from baseline to the end of the study (r^2= 0.55, *P*=0.002). This relationship also persisted in the control group (r^2=0.32, *P*<0.001). There were no similar

relationships between EPA concentration and rate of adipose tissue change or between DHA concentration and muscle or adipose tissue change.

5.3.5 Cytokine analysis

Coefficient of variation for all assays was <7%. The r^2 for all standard curves was >0.99. A representative curve is shown in Figure 5-3. Plasma cytokine amounts were variable and fluctuated throughout the trial but overall, only IL-15 changed significantly from baseline to end of trial in both groups (Table 5-5). There were also no differences in cytokine amounts between groups at either time point (Table 5-6).

Change in IL-8 and TNF-α during chemotherapy were modestly correlated with muscle rate of change, while change in IL-6 and IL-1β were modestly correlated with adipose tissue rate of change (Table 5-7). No significant correlations between change in plasma PL EPA and change in cytokine amounts were observed.

5.4 Discussion

This is the first study to use CT images to provide a direct measurement of the effect of FO on skeletal muscle and adipose tissue depots. Our results show that supplementation with FO ameliorates muscle and adipose tissue wasting in lung cancer patients and provides a benefit over SOC receiving first-line chemotherapy.

On average, patients receiving FO maintained muscle mass, but the rate of muscle change was variable. The concentration of plasma PL EPA following FO supplementation was also variable despite reported compliance. It is unlikely this

variation is solely due to misreporting of intake as a prior study in cancer patients also observed differential incorporation of n-3 fatty acids into plasma PL (24). Here we show that 55% of the variability in muscle change can be explained by plasma EPA and report a positive linear relationship between EPA concentrations and rate of muscle gain. Based on our results, we suggest that PL EPA concentrations upwards of 2.9% are required for maintenance of skeletal muscle, with even greater concentrations required to support muscle gain (mean PL EPA was 4.6% in patients with muscle gain). This relationship likely reaches a plateau as membranes and tissues become saturated with EPA, and it is important to identify at what concentration of EPA this occurs in future work to define the "optimal" EPA concentration.

With the exception of a trial conducted by Fearon et al. (7), previous studies have not related plasma EPA concentrations to body composition. Rather, plasma EPA was used as a measure of compliance (2,9,25) or was not reported (8,26). Since EPA must be incorporated into cells and tissues to exert physiological functions, we conjecture that failure to account for differential EPA incorporation limits interpretation of prior studies and explains why the efficacy of EPA to promote muscle gain has not been consistently demonstrated. Patients receiving FO maintained weight, muscle mass and adipose tissue throughout ~10 weeks of chemotherapy despite presenting with mean weight loss of 6.3% over the previous 6 months. This supports findings of earlier trials that showed a benefit of FO on weight and lean body mass (2,3). Improvements in weight have been reported in chemotherapy trials (27) and we acknowledge that maintenance

of weight and muscle mass reported here cannot be solely attributed to FO intervention. However, both the SOC group and FO group have similar chemotherapy response rates. Maintenance of body composition may have additional clinical implications as weight and tissue loss have been shown to influence performance status (1) and may dictate patients' eligibility for additional lines of treatment. Therefore, targeting patients receiving first-line chemotherapy represents an opportunity for timely intervention to prevent deterioration of body composition.

The relationship between IMAT and FO in patients with cancer has not been previously explored. The observation that FO supplementation results in loss of IMAT while maintaining muscle mass is novel and may have important functional implications given the associations between increased IMAT, decreased muscle strength (16) and impaired mobility (17). IMAT accumulation is also positively correlated with insulin resistance (28,29) which may contribute to the development of cancer cachexia (30). Increased fat infiltration of skeletal muscle has been observed with weight gain (20). Although over two-thirds of the FO group maintained or gained weight, this was not reflected in IMAT accumulation or muscle attenuation values. We hypothesize that EPA may function to decrease IMAT via its ability to suppress lipogenesis (31), thereby reducing deposition of lipids in muscle. Additionally, EPA supplementation has been shown to reduce fat accumulation via stimulation of lipid oxidation (32).

Overall, fish oil supplementation did not affect circulating amounts of cytokines even when differential incorporation of EPA was accounted for. It is

possible that variation in cytokine amounts or fluctuations in circulating cytokines (33) precluded our ability to detect an effect of supplementation. Assuming a difference in means of 3.5 and SD of 4.0, 44 patients would result in 80% power to detect a treatment difference at $P<0.05$, suggesting we may have been underpowered. However, previous studies have also reported no effect of fish oil on circulating amounts of cytokines (34,35). Further, the modest size of correlations observed between change in muscle, adipose tissue and cytokines indicates that other factors are influencing tissue change.

It is possible that differences in protein or energy intake may have may have contributed to the changes in weight and muscle that we observed between the FO and SOC group. The time period between referral to oncology and initiation of chemotherapy (when FO supplementation was started) was often a period of only a few days. This was not sufficient time for collection of pre-treatment dietary intake from all of our patients and it was unethical to delay treatment for the purpose of collecting study measures. In the future, it may be possible to use a 24 hour diet recall to elucidate the potential role of diet on tissue changes. It is also possible that medications for comorbid conditions could influence tissue change. Side effect of statins commonly used to treat hypercholesterolemia, include myopathy and muscle fatigue and recent research indicates statins may induce skeletal muscle apoptosis or alter the activity of the ubiquitin proteasome pathway (36). Although the medical records of patients contained information regarding medications, they did not consistently include details such as medication name, frequency and prescribed dose. Our small

sample size also precluded examination of subsets of patients according to comorbidities. This will be a focus of our future work, where larger sample sizes will provide the required power.

This study focused on intervention in newly referred patients, to prevent deterioration of body composition and weight loss. Previous studies have treated patients who were close to death and had severe weight loss (7-9) where intervention with a single agent may be limited. Selecting newly referred patients enabled accrual of patients with better survival prospects, thus avoiding patient morbidity which has complicated results of prior trials (6,8). Additionally, offering patients two formats of FO supplementation resulted in high compliance to the supplements, with only one out of 17 patients reporting compliance below 80%. This represents a 25% increase in compliance compared to prior studies (2,6), and may explain why our results show efficacy of fish oil on muscle mass while the results of other trials have been inconsistent (3,6,8,10). Providing patients with a choice of supplement format may be a potential approach to strengthen compliance in future trials.

At our cancer center, patients receiving treatment with palliative intent (who comprise over 80% of the patient population in this study) have expressed low interest in participation in randomized studies. The use of a non-randomized open label design resulted in timely study enrolment, improved compliance and no reports of contamination between treatment arms. Although this study was an open label design, we feel our results are strengthened by the inclusion of a larger reference group of NSCLC from our cancer center. The patients in the SOC and

FO group shared similar baseline characteristics as patients in the reference group and the changes in weight and body composition observed in the SOC group during chemotherapy were comparable to the reference group. However, we acknowledge that our results need to be replicated in additional larger studies.

5.5 Conclusion

Early intervention with FO during chemotherapy resulted in maintenance of weight and muscle compared to patients receiving SOC. Cytokines appear to play a limited role and other mechanisms may be responsible for the observed effect of EPA on muscle. As incorporation of EPA in PL varies greatly between individuals, future trials should stratify outcomes based on incorporation of EPA. Use of fish oil as a therapy to prevent body composition changes in patient populations who are at an increased risk of developing cancer cachexia merits further investigation.

Tables

Table 5-1: Baseline demographics and anthropometrics of standard of care, fish oil and a cohort of lung cancer patients from our cancer center

	Standard of care	Fish oil	Reference
Total, n	24	16	104
Women, n (%)	12 (50)	7 (44)	50 (48)
Men, n (%)	12 (50)	9 (56)	54 (52)
Age, years	64 ± 1.8	63 ± 2.1	62 ± 1.0
Stage			
I & II, n (%)	8 (33)	5 (31)	32 (31)
III & IV, n (%)	16 (67)	11 (69)	72 (69)
BMI, kg/m^2	27.3 ± 1.2	26.2 ± 1.1	25.9 ± 0.4
[1]6 month weight loss history, %	-4.2 ± 1.2	-6.3 ± 1.6	-
L3 SM area, cm^2	134 ± 6.1	135 ± 8.0	132 ± 3.1
[2]Sarcopenic patients, %	46	46	46
[3]Estimated whole-body SM, kg	24.4 ± 1.0	25.4 ± 1.4	24.0 ± 0.5
[4]L3 total AT area, cm^2	350 ± 42.7	281 ± 42.2	272 ± 14.6
[3,4]Estimated whole-body AT, kg	27.9 ± 2.9	23.2 ± 2.9	22.6 ± 1.0
ECOG PS	1	1	-
ECOG range	0-2	0-2	-

Mean ± SE. No significant differences between groups (Two sample t-test and chi-square test). [1]Data not available from n=3 in standard of care. [2]Based on cut-points for muscularity (14). [3]Derived from regression equations (13). [4]Data not available from n=1 in standard of care and n=1 in fish oil group. Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; SM, skeletal muscle; AT, adipose tissue.

Table 5-2: Weight and tissue changes quantified with computed tomography imaging from baseline to end of study in the standard of care, fish oil and reference groups

	Standard of care	Fish oil	Reference
Weight change, kg	-2.3 ± 0.9	0.5 ± 1.0*	1.9 ± 0.3
Muscle rate of change, %/100d	-6.8 ± 2.6	0.1 ± 1.6*	-6.0 ± 0.9
IMAT rate of change, %/100d	9.5 ± 5.2	-16.4 ± 13.9*	11.1 ± 3.5
[1]TAT rate of change, %/100d	-3.9 ± 5.0	-5.0 ± 6.5	-6.0 ± 4.6

Mean ± SE. *Significantly different from standard of care $P<0.05$. Two sample t-test. [1]Standard of care n=23, fish oil n=15. Abbreviations: IMAT; intermuscular adipose tissue, TAT; total adipose tissue.

Table 5-3: Body composition of patients receiving standard of care and fish oil supplementation using CT imaging

	Standard of care			Fish oil		
	Baseline	End of study	*P*	Baseline	End of study	*P*
[1]Whole-body skeletal muscle, kg	24.4 ± 1.0	23.5 ± 1.0	0.002	25.4 ± 1.4	25.4 ± 1.5	0.97
[1,2]Whole-body adipose tissue, kg	27.9 ± 2.9	27.6 ± 3.1	0.74	23.2 ± 2.9	22.9 ± 1.4	0.68
Muscle attenuation, HU	33.6 ± 1.7	30.1 ± 1.7	> 0.0001	33.2 ± 2.1	34.2 ± 1.9	0.45

Mean ± SE, repeated-measures ANOVA with Bonferroni pairwise comparisons. [1]Estimated from regression equations (13).
[2]Standard of care n=23, fish oil n=15. Abbreviations: HU, Hounsfield units.

Table 5-4: Plasma PL EPA and DHA in the standard of care and fish oil group at baseline and end of study

	Standard of care[1]			Fish oil[2]		
	Baseline	End of study	*P*	Baseline	End of study	*P*
Amount of EPA (µg/mL)	7.1 ± 0.8	6.5 ± 0.6	0.53	8.0 ± 0.9	26.4 ± 2.2	<0.0001
Proportion of EPA (%)	1.0 ± 0.1	1.0 ± 0.1	0.86	1.2 ± 0.1	3.7 ± 0.3	<0.0001
Amount of DHA (µg/mL)	14.6 ± 1.0	13.7 ± 1.2	0.78	15.0 ± 1.4	19.8 ± 1.9	0.05
Proportion of DHA (%)	2.3 ± 0.2	2.2 ± 0.1	0.79	2.2 ± 0.1	2.8 ± 0.2	0.02

Mean ± SE, two-sample t-test. Abbreviations: EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid.

Table 5-5: Plasma cytokines in the standard of care and fish oil group at baseline and end of study

	Standard of care			Fish oil		
Cytokine (pg/mL)	Baseline	End of study	*P*	Baseline	End of study	*P*
IFN-γ Range	2.0 ± 0.5 0.1-10.9	1.9 ± 0.3 0-6.9	0.74	1.9 ± 0.9 0-12.7	1.9 ± 0.4 0-6.7	0.72
IL-10 Range	13.5 ± 8.3 1.7-202	15.0 ± 8.9 2.2-218	0.26	7.7 ± 3.2 1.4-53.1	7.4 ± 2.9 1.8-47.7	0.62
IL-12p70 Range	16.5 ± 12.4 0.1-296	15.4 ± 13.0 0-312	0.48	6.0 ± 3.8 0-61.1	5.1 ± 3.1 0-49.5	0.29
IL-1β Range	0.6 ± 0.2 0-4.4	0.3 ± 0.1 0-1.1	0.18	0.7 ± 0.5 0.1-7.9	0.7 ± 0.3 0.1-5.4	0.62
IL-6 Range	6.7 ± 0.9 1.1-16.3	10.0 ± 0.9 0.9-54.7	0.18	5.7 ± 1.2 0.7-17.8	7.1 ± 2.1 1.0-33.6	0.54
IL-8 Range	7.9 ± 0.7 4.1-17.8	13.2 ± 3.9 3.4-95.4	0.14	9.0 ± 1.4 2.9-20.3	8.5 ± 1.7 2.9-30.6	0.60
TNF-α Range	9.2 ± 0.6 4.4-17.5	9.7 ± 1.0 4.3-23.0	0.44	14.0 ± 5.0 5.4-88.2	13.3 ± 5.5 5.2-94.6	0.29
IL-15 Range	1.4 ± 0.1 0.8-2.3	1.9 ± 0.2 0.8-4.0	>0.001	1.3 ± 0.2 0.5-2.8	1.9 ± 0.1 1.0-3.0	0.001

Mean ± SE, repeated-measures ANOVA with Bonferroni pairwise comparisons. Quantified using Multi-Array Assay kits.

Table 5-6: Plasma cytokines in the standard of care group compared to the fish oil group at baseline and end of study

	Baseline			End of study		
Cytokine (pg/mL)	Standard of care	Fish oil	*P*	Standard of care	Fish oil	*P*
IFN-γ	2.0 ± 0.5	1.9 ± 0.9	0.93	1.9 ± 0.3	1.7 ± 0.4	0.72
IL-10	13.5 ± 8.3	7.7 ± 3.2	0.59	15.0 ± 2.9	7.4 ± 2.9	0.50
IL-12p70	16.5 ± 12.4	6.0 ± 3.8	0.50	15.4 ± 13.0	5.1 ± 3.1	0.53
IL-1β	0.6 ± 0.2	0.7 ± 0.5	0.69	0.3 ± 0.1	0.7 ± 0.3	0.20
IL-6	6.7 ± 0.9	5.7 ± 1.2	0.50	10.0 ± 2.7	7.1 ± 2.1	0.44
IL-8	7.9 ± 0.7	9.0 ± 1.4	0.43	13.2 ± 3.9	8.5 ± 1.7	0.35
TNF-α	9.2 ± 0.6	14.0 ± 5.0	0.25	9.7 ± 1.0	13.3 ± 5.5	0.44
IL-15	1.4 ± 0.1	1.3 ± 0.2	0.69	1.9 ± 0.2	1.9 ± 0.1	0.83

Mean ± SE, two sample t-test. Quantified using Multi-Array Assay kits.

Table 5-7: Correlations between change in plasma cytokines and change in muscle or adipose tissue during the study period in the standard of care and fish oil group

Change in cytokines (pg/mL)	Muscle rate of change (%/100d)		Total adipose tissue rate of change (%/100d)	
	r	*P*	*r*	*P*
IFN-γ	0.06	0.70	-0.09	0.59
IL-10	-0.09	0.57	-0.08	0.66
IL-12p70	-0.06	0.71	-0.13	0.42
IL-1β	-0.08	0.62	-0.42	0.009
IL-6	-0.04	0.82	-0.37	0.02
IL-8	-0.40	0.01	-0.23	0.16
TNF-α	-0.36	0.02	-0.25	0.14
IL-15	0.07	0.67	0.04	0.82

Spearman correlations. Cytokines were quantified using Multi-Array Assay kits.

Figures

Figure 5-1: CONSORT diagram of patients in the standard of care and fish oil groups

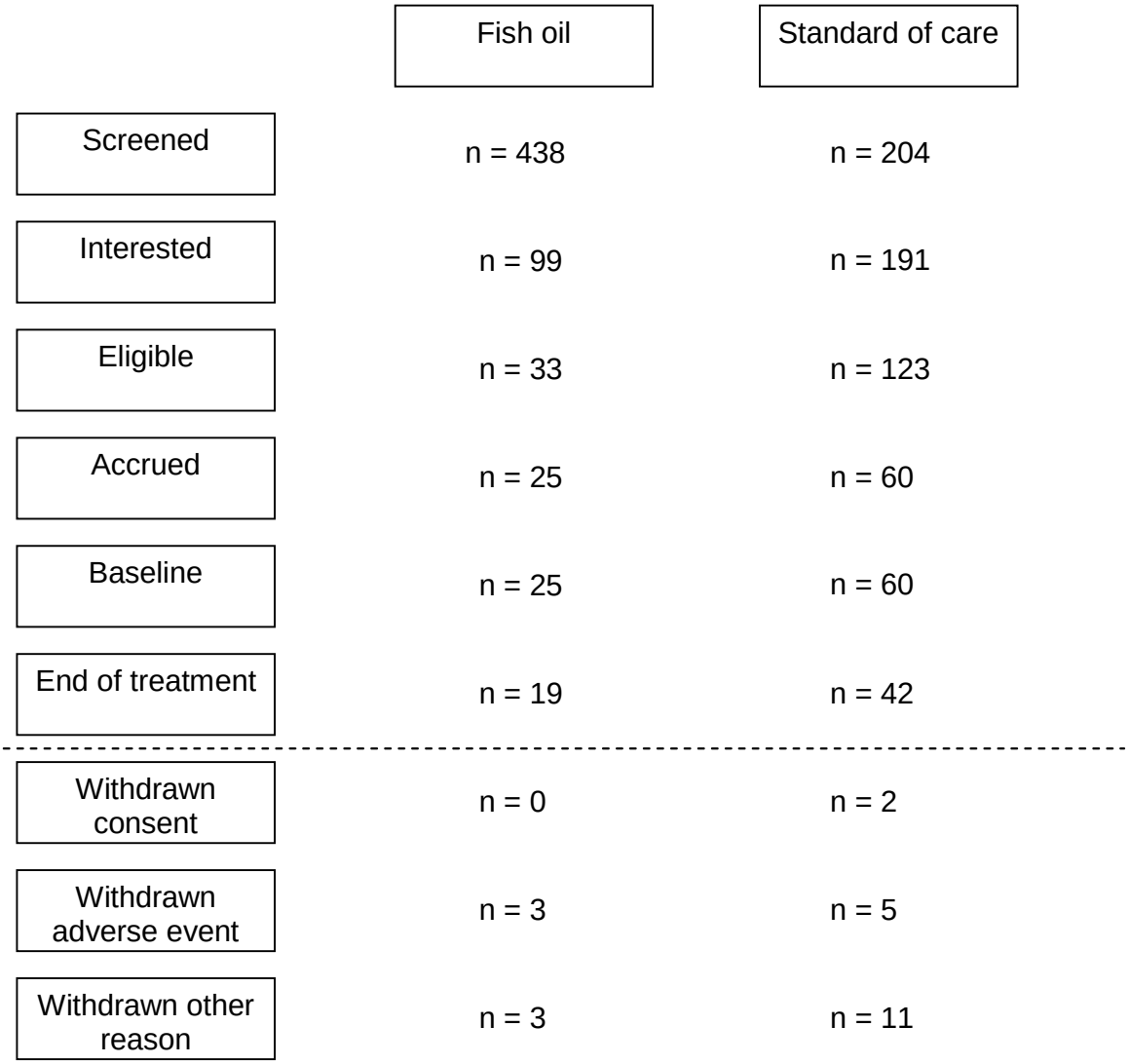
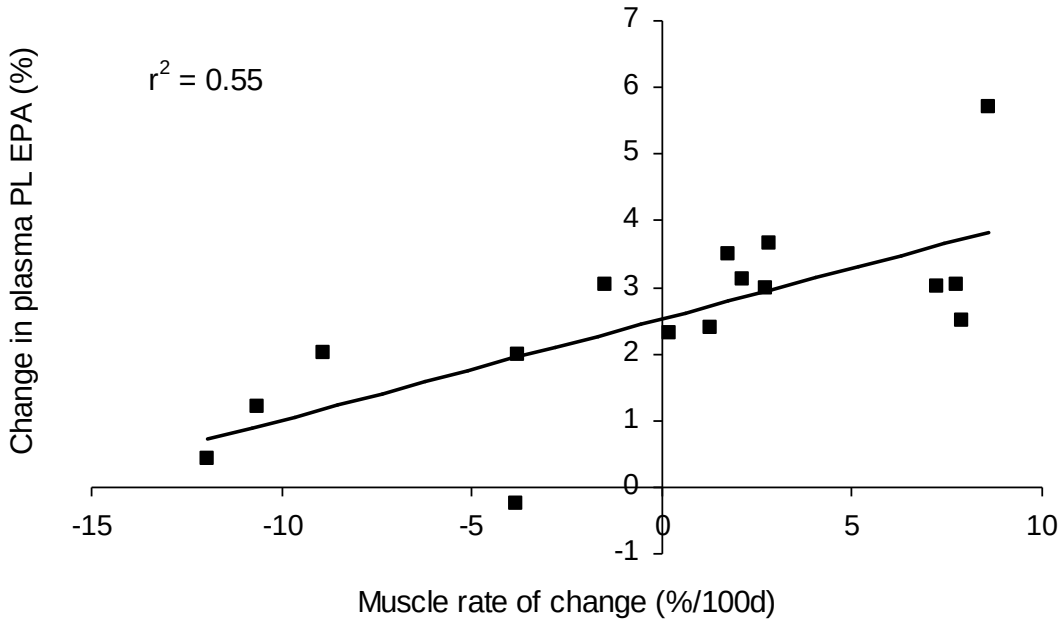
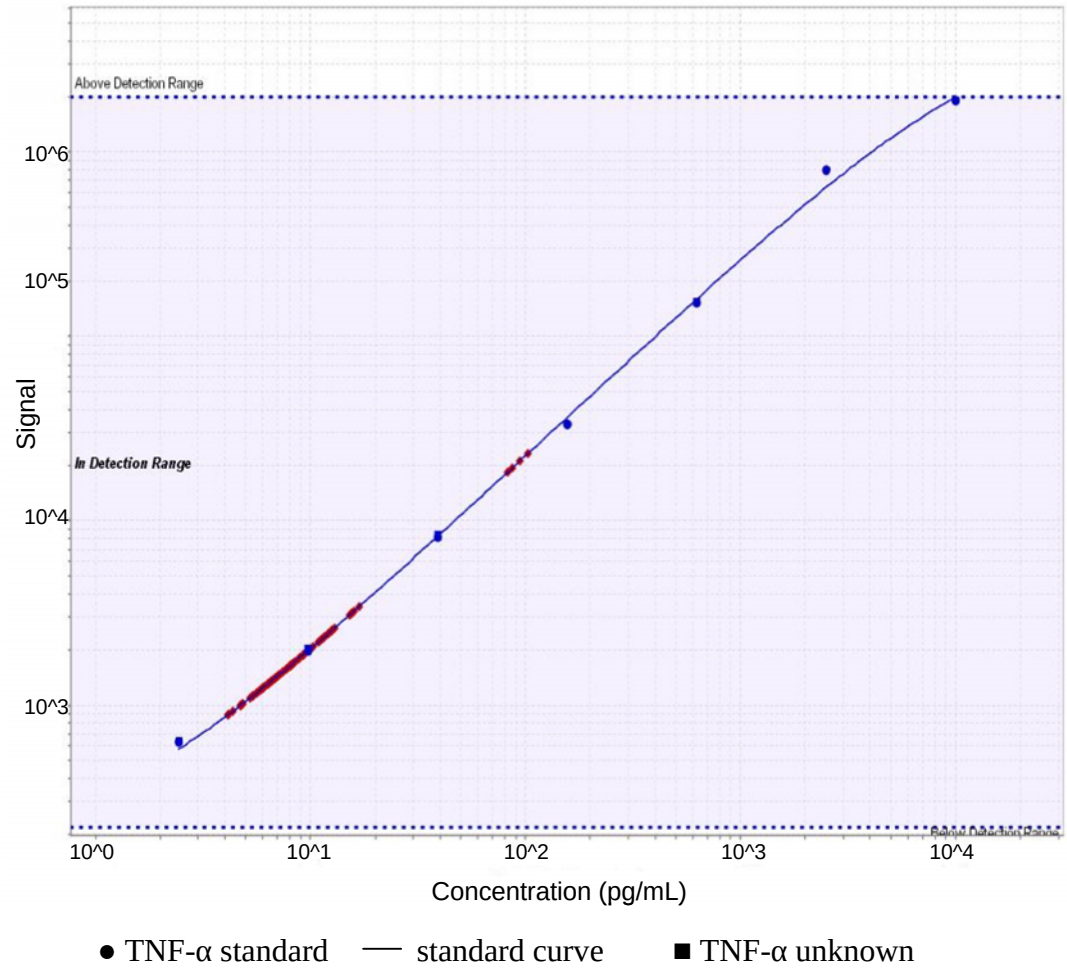


Figure 5-2: Relationship between change in plasma PL EPA and change in muscle from baseline to end of fish oil supplementation



Linear regression, n=16; *P*=0.002. Abbreviations: PL, phospholipid; EPA, eicosapentaenoic acid

Figure 5-3: Standard curve of TNF-α



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Chapter 6: Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced non-small cell lung cancer

6.1 Introduction

Lung cancer is the leading cause of cancer related deaths in Western countries (1). The majority of patients with lung cancers present with advanced-stage disease for which standard treatment consists of palliative chemotherapy or radiotherapy. Efficacy of chemotherapy for advanced non-small cell lung cancer (NSCLC) is low with large trials of different chemotherapy combinations reporting response rates (RR) below 30% (2-4). Chemotherapy efficacy in advanced NSCLC has reached a plateau, with little improvement in RR and it has been suggested that research should focus on new approaches and novel treatments rather than different combinations of chemotherapy drugs (5).

Experimental studies in a variety of tumour types using several different chemotherapy agents including anthracyclines, cisplatin, irinotecan and alkylating agents have reported greater efficacy of chemotherapy when fish oil (FO; eicosapentaenoic acid, EPA 20:5n-3 and docosahexaenoic acid, DHA 22:6n-3) is added to the diet or cell medium (6-10). The mechanisms of action of these anti-neoplastic agents vary, suggesting that fish oil modulates chemotherapy response via diverse mechanisms (see Biondo et al. (11) and Baracos et al. (12) for reviews). EPA and DHA may also have anti-tumour effects including inhibition of angiogenesis and metastasis (12); however, the specific mechanisms behind these effects have not been elucidated. Regardless of the exact mechanisms, these

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studies suggest that FO has potential as an effective adjuvant to chemotherapy treatment.

Current treatment of advanced NSCLC is non-specific and toxic, typically consisting of platinum-based regimens such as carboplatin in combination with vinorelbine or gemcitabine. Efficacy is dose dependant and limited by gastrointestinal, haematological and cardiac toxicities. Treatment approaches that enhance cytotoxicity of anti-cancer agents to tumour cells while minimizing toxicity to non-target tissue have the potential to increase benefits of chemotherapy including disease control and survival. Increased sensitivity to chemotherapy appears to be specific to tumour tissue as evidenced by lack of additional toxicity to non-tumour tissues following the addition of dietary EPA and DHA (13). There is also evidence that dietary FO enrichment may have a protective effect on non-tumour tissue including minimization of gastrointestinal and haematological toxicity (13;14). However, many experimental studies have used amounts of fish oil that are beyond concentrations achievable in humans, even with supplementation and only one study in humans has been conducted (15).

We aimed to expand upon the base of information on fish oil, chemotherapy efficacy and chemotherapy toxicity. This is the first study to evaluate the ability of fish oil to improve chemotherapy efficacy in patients with lung cancer. We hypothesized that supplementation with fish oil during first-line chemotherapy for lung cancer would increase response rates without significantly affecting chemotherapy toxicity.

6.2 Methods

6.2.1 Study design

This study is part of a larger open label trial that was focussed on nutritional status during chemotherapy in which 60 NSCLC patients receiving first-line chemotherapy consented to either 1) intervention with FO or 2) standard of care (SOC, no intervention). The subset described here was chosen on the basis of treatment intent (palliative versus adjuvant) to standardize the type of chemotherapy treatment patients received. Detailed rationale, study design and analysis of the effect of fish oil supplementation on body composition in a partly overlapping subset of these patients has been published (16) and is described in Chapter 5. In brief, patients with lung cancer who were newly referred to the Cross Cancer Institute (Edmonton, Alberta, Canada) were given an information sheet detailing available nutritional studies. Patients indicated their interest and were approached to participate accordingly. Written, informed consent was obtained from all patients.

Patients on the FO arm were offered a choice of two formats of supplementation: 1) 4 1g gelatin capsules per day containing 2.2g EPA and 240mg DHA or 2) 7.5mL liquid fish oil per day (2.2g EPA and 500mg DHA). The number of capsules or the amount of liquid remaining at the end of the study was measured to determine compliance. Capsules were provided in kind by Ocean Nutrition Canada (Ocean Nutrition Canada, Nova Scotia, Canada). Liquid fish oil was purchased from NutraSea (Ascenta Health, Nova Scotia, Canada).

6.2.2 Patient population

Inclusion/exclusion criteria are detailed in Chapter 5. For this analysis, patients with a clinical diagnosis of stage IIIB or IV NSCLC, who were chemotherapy-naïve were selected. Stage of disease was based on the American Joint Committee on Cancer stage groupings (17). Patients had consented to receive first-line platinum based doublet chemotherapy of palliative intent consisting of either carboplatin with vinorelbine or carboplatin with gemcitabine.

6.2.3 Weight and body composition analysis

Height and weight were measured using a stadiometer and a medical balance beam scale at baseline. Body mass index (BMI in kg/m^2) was calculated from height and weight. Self-reported weight loss in the 6 months preceding enrolment in the study was recorded using the patient-generated subjective global assessment (20) or from medical charts. Height and weight recorded by hospital staff on the same date was used for verification when available.

Lower than normal muscle mass (sarcopenia) has emerged as an important predictor of chemotherapy toxicity (21;22). Computed tomography (CT) image analysis can be used to precisely quantify skeletal muscle (23). To distinguish between the effect of FO and the effect of sarcopenia on toxicity, CT images that were taken for diagnostic purposes at the time of oncologic referral were analyzed to determine the prevalence of sarcopenia in the FO and SOC groups. Cross sectional muscle areas (cm^2) were determined for two consecutive images taken at the third lumbar vertebrae (L3) using Slice-O-matic software V4.3 (Tomovision, Montreal, Canada) as described in Mourtzakis et al. (23) and in Chapter 4. Mean

muscle area was calculated and subsequently normalized for stature (cm^2/m^2). To express the data in conventional units, a regression equation was used to estimate whole-body skeletal muscle (24) and a density of $1.04g/cm^3$ was used to convert muscle volume to mass (25). Sex specific cut points (males <55.4 $cm^2/m^2$and females <38.9cm^2/m^2) that were previously defined and utilized in advanced cancer populations (23;26) were used to classify patients as sarcopenic or non-sarcopenic.

6.2.4 Response to chemotherapy

Response to chemotherapy was evaluated by a radiologist and oncologist on the basis of clinical examination and imaging which consisted of computed tomography, magnetic imaging resonance or x-ray following 2 cycles of chemotherapy. If progressive disease was noted, chemotherapy was discontinued. Otherwise, patients received an additional 2 cycles of chemotherapy followed by imaging. As 4 cycles is standard practice specifically agreed upon as Clinical Practice Guidelines by the Alberta Provincial Lung Tumour Group, RR was defined as the sum of complete response and partial response following 4 cycles of chemotherapy divided by the number of patients. Clinical benefit (CB) was defined as the sum of complete response, partial response and stable disease following 4 cycles of chemotherapy divided by the number of patients.

6.2.5 Toxicity assessment

Toxicity was graded using the National Cancer Institute Common Toxicity Criteria, version 2.0. Patient toxicities were graded by a nurse prior to each cycle of chemotherapy. Dose was reduced or suspended if patients developed grade 3

or greater febrile neutropenia or thrombocytopenia or grade 3 or greater non-haematological toxicities excluding alopecia or inadequately treated nausea, vomiting, diarrhea.

6.2.6 Survival

Following completion of the trial, patients were followed for post-trial treatment and determination of time to death. Median follow-up was 18.5mo for all living patients. There were only 8 deaths in the FO group leaving 46.7% of patients censored, which coupled with our small sample size was too few to conduct a formal survival analysis. Instead, survival was expressed as % of patients surviving one year following enrolment in the trial (one-year survival).

6.2.7 Plasma phospholipid fatty acid analysis

The majority of plasma n-6 and n-3 fatty acids are carried in phospholipids (PL), which reflect the amount of EPA and DHA available to tissues including tumour tissue. Because previous trials of FO supplementation have reported non-compliance (18) or cross-contamination between control and intervention arms (19), concentrations of EPA and DHA in plasma PL were analyzed in both the SOC and FO groups as a secondary measure of compliance.

Blood was drawn by a Registered Nurse prior to chemotherapy initiation (baseline), one day prior to each 3 week cycle of chemotherapy and on the last day of chemotherapy treatment. Blood was collected in heparin tubes and centrifuged to isolate plasma, which was immediately frozen at -80°C until analysis. Plasma fatty acids were extracted using a modified Folch technique as detailed in Chapter 3. Fatty acid composition was determined using gas liquid

chromatography (Varian 3600CX Gas Chromatograph, Varian, Mississauga, Canada) as previously described in Park et al. (28) and Chapter 3. Plasma PL EPA and DHA were expressed as a proportion (%) of total PL fatty acids (29).

6.2.8 Statistical analysis

The primary endpoint was chemotherapy RR. CB, chemotherapy toxicity and survival were secondary endpoints. Data are presented as mean ± standard deviation. Significance was determined at $P<0.05$. Student t-test, Chi-square test or Fishers exact test were used to determine differences between SOC and FO groups where appropriate. All tests were two sided. Logistic regression was to determine predictors of response to treatment (partial response or complete response versus stable disease and progressive disease) and grade 3 or 4 toxicities in the FO and SOC groups and also in all patients combined. Statistical analyses were performed with SPSS (SPSS for Windows, version 18.0, SPSS, Chicago, IL).

6.3 Results

6.3.1 Patient demographics

Baseline characteristics and anthropometric measures of the FO and SOC group were well matched (Table 6-1). Additionally, characteristics of these groups are comparable to those observed in the NSCLC population at our cancer center (16;30). Although weight loss in the 6 months preceding oncology referral was common, no patients in either group were underweight (BMI <18.5 kg/m^2). Over 50% of patients in each group were overweight or obese. Sarcopenia was prevalent in both the FO and SOC groups (46.7% versus 51.6%; $P=0.75$).

Reported use of over the counter supplements did not differ between groups (P=0.49). The proportion of patients receiving carboplatin with vinorelbine versus carboplatin with gemcitabine did not differ between groups (*P*=0.75).

Compliance to the FO supplement was over 95%. Mean intake was 2.1 ± 0.25g EPA and 0.29 ± 0.04g DHA per day. No difference in plasma EPA or DHA concentration was observed between patients taking liquid fish oil or fish oil capsules, *P*=0.42 and *P*=0.62 respectively. In the FO group plasma PL EPA and DHA increased significantly following supplementation (Table 6-2). Plasma PL EPA and DHA post-supplementation was not available from 3 patients in the SOC group because their chemotherapy was discontinued due to toxicity and they were lost to follow-up. There were no changes in plasma PL EPA or DHA between baseline and end of trial in the SOC group (Table 6-2). Following supplementation, plasma EPA and DHA in the FO group were significantly higher than the SOC group (*P*<0.0001 and *P*=0.04, respectively).

6.3.2 Response to chemotherapy

The RR and CB were approximately 2 fold greater in the FO group compared to the SOC group (Table 6-3). The proportion of patients in the FO and SOC group who had a complete response, stable disease, partial response or progressive disease is shown in Table 6-3. Logistic regression demonstrated that plasma PL EPA concentration post supplementation was a significant predictor of response to chemotherapy independent of age, gender, BMI, presence of sarcopenia, PS and weight loss history (hazard ratio 1.8, CI 1.1 to 2.5; *P*=0.03). There were no other significant predictors of response to treatment.

Compared to the FO group, a greater percentage of patients in the SOC group had progressive disease after 2 cycles of chemotherapy. Consequently, more patients in the FO group completed all planned chemotherapy (86.7% versus 54.8%; *P*=0.03). On average, patients in the FO group completed an additional 3 weeks of chemotherapy (1 cycle) compared to patients in the SOC group (Table 6-3).

6.3.3 Toxicity

FO supplementation was well tolerated with no reported adverse events. The most commonly reported chemotherapy-related grade 1 and 2 toxicities were neutropenia followed by nausea, vomiting and thrombocytopenia. The overall incidence of any grade 3 or 4 toxicity was 22.6% in the SOC group versus 13.3% in the FO group (*P*=0.46). Grades 3 and 4 toxicities included nausea, vomiting, hand-foot syndrome, neutropenia, constipation and gastritis. One patient in each group discontinued treatment due to fatigue and increased symptom burden. Haematological toxicity resulted in treatment discontinuation in 2 patients in the SOC group. In the SOC group, a further 2 patients discontinued chemotherapy due to gastritis and hand-foot syndrome. The total incidence of grade 3 or 4 toxicity resulting in discontinuation of chemotherapy of planned chemotherapy did not differ between groups 16.1% versus 6.7%, *P*=0.52. Logistic regression did not reveal any significant predictors of grade 3 or 4 toxicity.

6.3.4 Survival

Despite the wide variation in overall survival (1-30 months), one-year survival in the FO group appeared to be greater than in the SOC group (Table 6-

3). The median number of post-trial lines of therapy was 1 in both cohorts. The most common post trial therapy was erlotonib (55% of SOC and 53% of FO group). Thirty-five percent of patients in the SOC group and 27% patients in the FO did not receive any further treatment (P=0.40).

6.4 Discussion

The goal of palliative chemotherapy is to improve symptoms and survival. However, over 65% of advanced NSCLC cases do not respond to first-line chemotherapy (2-4) and 1-year survival rates are low. This is the first study to show that the addition of approximately 2.5g of EPA + DHA per day significantly increases the RR to first-line chemotherapy compared to SOC without affecting the toxicity profile. Additionally, supplementation with FO has the potential to increase survival as a greater proportion of patients in the FO group were surviving at time of censorship.

The RR observed in this study represents an approximate 2 fold-increase over the SOC group and only 3 patients in the FO group did not experience CB from chemotherapy. Comparatively, one-third of patients in the SOC group responded to treatment, which falls within the range of RRs reported in large randomized trials of first-line combination chemotherapy regimens (2-4). Our finding is concordant with a previous study in an animal model of lung cancer which showed that the combination of cisplatin chemotherapy and dietary FO resulted in slower tumour growth and a lower metastatic load (10). In humans, a positive correlation between chemotherapy efficacy and breast tissue n-3 fatty acid concentration in patients with breast cancer has been reported (31). The

same researchers followed up this study with a trial of DHA supplementation in patients with breast cancer and progressive visceral metastases (15). Despite the poor prognosis of the patient population, they found that the RR was comparable to that reported in first-line chemotherapy trials for breast cancer.

Toxicities from carboplatin are cumulative with few patients receiving more than 4 cycles of chemotherapy. Although patients in the FO group received on average one additional cycle of chemotherapy versus the SOC group, the prevalence of grade 3 and 4 haematological and non-haematological toxicities did not differ. This is likely due to an effect of FO on toxicities rather than an effect of sarcopenia as the prevalence of sarcopenia was similar between groups at baseline. Our results are also consistent with animal studies which have demonstrated protective effects of fish oil on host tissue despite increased tumour response to chemotherapy (36-38). In addition, a protective effect of high plasma DHA concentrations on haematological toxicities was observed in patients with advanced breast cancer receiving anthracycline therapy (15). Taken together, this shows that supplementation with fish oil does not result in higher chemotherapy sensitivity in non-target tissue.

Only one-third of patients with non-operable stage III and IV NSCLC survive 1 year following first-line platinum based treatment (4;32;33). Comparably, approximately two-thirds of patients in the FO group were surviving 1 year post trial. Although the difference in 1 year survival only tended towards significance between the FO and SOC groups, this is likely a reflection of our small sample size. The trend towards increased survival may be due to the

increased RR and decreased tumour burden. Bougnoux et al. (15) reported a similar increase in survival in advanced breast cancer patients supplemented with DHA. Additionally, higher plasma PL EPA and DHA concentrations, as observed in the FO group have been shown to be predictive of increased survival in patients with advanced cancer (34). A further study by Gogos et al. (35) demonstrated increased survival in patients with generalized malignancy when provided with fish oil versus placebo.

Platinum based chemotherapy for NSCLC is the standard worldwide, and a 30% increase over the typically observed RR that we report in this study could have a large impact. In Canada alone, an estimated 24,000 people will be diagnosed with lung cancer each year (1), amongst which 75-80% are deemed incurable and two-thirds will receive palliative chemotherapy. Fish oil supplementation may represent a safe and non-toxic approach to improve current standard of care in patients with lung cancer. Nonetheless, we acknowledge that our results require verification in larger randomized controlled trials as our study was designed as a pilot study with a limited sample size. However, the observed difference in RR between the FO and SOC groups was striking even with a small number of patients.

Although this was an open label trial, our results are most likely due to an effect of FO and not due to group selection bias because the baseline characteristics of the SOC and FO group were well matched and representative of our local NSCLC population. As well, the RR and one-year survival in the SOC group were comparable to those typically observed in chemotherapy trials.

This study was a post-hoc analysis of a trial investigating the effect of fish oil on body composition. As mentioned throughout this discussion, it is likely that we did not have sufficient power to assess all of the potential effects of fish oil (improved chemotherapy efficacy, reduced toxicity and improved survival). The prevalence of grade 1 and 2 toxicities are difficult to determine in this population as toxicities such as dyspnea and cough are confounded by the fact that these are symptoms of lung cancer itself and without baseline measures of these symptoms, toxicity reports are difficult to interpret. While reporting of grade 3 and 4 toxicities are of value, they do not capture the true effect of chemotherapy as multiple low grade toxicities could be expected to influence quality of life and patient motivation to continue chemotherapy. It appears from the results reported here that the most likely benefits of fish oil are improved chemotherapy efficacy and increased survival. Future trials powered around these outcomes will help to elucidate the potential benefit of fish oil as an adjuvant to chemotherapy.

This study was not designed to investigate underlying mechanisms and we are aware that these results do not ensure causality. However, the association between FO and carboplatin-based chemotherapy holds promise for improving the treatment of lung cancer patients.

6.5 Conclusion

The addition of fish oil to standard first-line platinum based chemotherapy may increase RR and CB in patients with advanced NSCLC without affecting treatment toxicity. Additional randomized trials are warranted to confirm these findings.

Tables

Table 6-1:Baseline characteristics and anthropometric parameters of patients in the standard of care and fish oil groups

	Standard of care	Fish oil
Total, n	31	15
Women, %	51.6	40.0
Men, %	48.4	60.0
Age, years	64 ± 1.8	63 ± 2.1
[1]Stage		
III, %	25.8	20.0
IV, %	74.2	80.0
BMI, kg/m^2	26.5 ± 5.4	26.4 ± 4.9
[2]6 month weight loss history, %	-4.1 ± 5.8	-6.2 ± 7.8
Lumbar skeletal muscle area (cm^2)	129 ± 30.7	140 ± 34.8
Lumbar skeletal muscle index (cm^2/m^2)	46.3 ± 8.5	47.0 ± 9.3
[3]Sarcopenic, %	51.6	46.7
[4]Estimated whole-body skeletal muscle, kg	24.5 ± 5.3	26.4 ± 6.0
ECOG PS	1	1
ECOG range	0-2	0-2
Smoking status, %		
Never	6.5	13.3
Current	22.6	20.0

Mean ± SD. No significant differences between groups, two sample t-test and Chi-square test. [1]Based on staging from the American Joint Committee on Cancer Staging Manual, 6th edition (17). [2]Data not available from n=2 in standard of care group. [3]Based on cut-points for muscularity (23). [4]Derived from a regression equation (24). Abbreviations: BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 6-2: Plasma phospholipid EPA and DHA in the standard of care and fish oil groups

	[1]**Baseline**	[2]**Post-supplementation**	***P***
Standard of care			
EPA (%)	0.9 ± 0.6%	1.2 ± 0.6%	0.86
DHA (%)	2.3 ± 0.8%	2.2 ± 0.6%	0.80
Fish oil			
EPA (%)	1.0 ± 0.5%	3.6 ± 1.3%	<0.001
DHA (%)	2.3 ± 0.7%	2.7 ± 1.0%	0.05

Mean ± SD, two sample t-test. [1]n = 31 (SOC) and n=15 (FO). [2]n=28 (SOC) and n=15 (FO).

Table 6-3: Chemotherapy outcomes and survival in the standard of care and fish oil groups

	[1]**Standard of care**	[2]**Fish oil**	***P***
Response rate, n (%)	8 (25.8)	9 (60.0)	0.008
Clinical benefit, n (%)	13 (41.9)	12 (80.0)	0.02
Complete response, n (%) Partial response, n (%) Stable disease, n (%) Progressive disease, n (%)	1 (3.2) 7 (22.6) 5 (16.1) 18 (58.1)	1 (6.7) 9 (60.0) 2 (13.3) 3 (20.0)	
Number of chemotherapy cycles received	3.0 ± 1.4	3.9 ± 0.9	0.02
Time on chemotherapy, d	60.3 ± 31.1	78.9 ± 23.5	0.05
One-year survival	38.7	60.0	0.15

Mean ± SD, two sample t-test and Chi-square test. [1]n = 31, [2]n = 15.

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CHAPTER 7: Final Discussion

7.1 Introduction

The purpose of this research was to detail changes in fatty acid status during chemotherapy, define a relationship between EPA, DHA and tissue change and determine if supplementation with EPA and DHA results in attenuation of tissue loss and improved chemotherapy efficacy. A schematic which integrates the concepts presented in Chapters 3 through 6 is provided in Figure 7-1.

In Chapter 3, it was hypothesized that plasma PL EPA and DHA are depleted, decrease further during chemotherapy and do not recover following completion of treatment. Depletion of EPA and DHA were prevalent in patients with advanced disease as were alterations in SFA, PUFA and total amounts of PL fatty acids. However, EPA and DHA did not change markedly after 4 cycles of chemotherapy or at follow-up. Chapter 4 hypothesized and also demonstrated that physiological concentrations of plasma PL EPA and DHA are reduced in patients with sarcopenia and associated with accelerated loss of muscle and adipose tissue. Chapter 5 tested the hypothesis that supplementation with fish oil results in attenuation of weight, muscle and adipose tissue loss which is mitigated in part by decreased catabolic cytokines. The effect of fish oil on nutritional status, symptoms and quality of life was also investigated. Using an open label study design, Chapter 5 showed a benefit of fish oil over standard of care on weight, skeletal muscle and IMAT. There was no effect of fish oil on total adipose tissue, nutritional status, symptoms or quality of life. Contrary to our hypothesis, the effect of fish oil on skeletal muscle did not appear to be solely mediated through

catabolic cytokines, as there were modest correlations between muscle and cytokines but overall the amounts of circulating cytokines did not change with fish oil supplementation and were not different from standard of care. Finally, Chapter 6 hypothesized that supplementation with fish oil improves chemotherapy efficacy and survival without affecting chemotherapy toxicity. Analyses revealed a 2-fold higher response rate to chemotherapy in the fish oil group compared to the standard of care group and a greater proportion of patients completed all planned chemotherapy. There were no differences in toxicity between groups and a trend towards improved one-year survival in the fish oil group.

Collectively, these results have the potential to change the care and treatment of lung cancer patients which will lead to improved patient outcomes. This discussion outlines key points related to the research presented in the previous chapters and makes recommendations for future research.

7.2 Fatty acid metabolism

From Chapter 3, patients with advanced disease have lower n-3 and n-6 fatty acids as well as lower SFA and PUFA compared to patients with early stage disease, with continual loss of fatty acids in patients with progressive disease. Although Chapter 4 suggests that only EPA and DHA are specifically related to body composition, it is possible that deficits in the other classes of fatty acids could have important metabolic or physiologic consequences that have yet to be realized. It may be that correcting the altered compositions of plasma PL fatty acids is more complex then repleting EPA and DHA and understanding the basis of the impediments in lipid metabolism would help to clarify this. Stable isotope

tracers could be a powerful tool for increasing our understanding of whole body lipid metabolism in cancer. Levels of chylomicrons, VLDL, HDL and LDL or the fatty acid contents of these lipoprotein fractions have not been determined in cancer and would aid in determining where the impediments lie.

7.3 Considerations for future trials of fish oil supplementation

Much of the mechanistic framework regarding the effect of fish oil on body composition and chemotherapy efficacy presented in this thesis is speculative. The majority of the research on the effects of fish oil has been conducted in animal or cell models (1-3) which depending on the size of the tumour, dose of chemotherapy and dose of fish oil may or may not translate to clinical studies. Clinical research is usually limited to measuring circulating mediators due to the invasiveness of tissue biopsies. Additionally, our study did not specify that blood draws were to be fasted, and we were thus unable to determine whether insulin or triacylglycerol levels were impacted by fish oil or differed between groups. Although challenging, considering the limitations of animal models and clinical research, determination of a mechanistic framework is key to exploiting the benefits of fish oil.

Surgical patients represent a unique population whereby tissue biopsies could be obtained without increasing patient burden. Ongoing research in our laboratory using surgically obtained tissue and computed tomography image based assessment of body composition may help to determine what is driving changes in body composition locally i.e. we did not report an effect of fish oil on circulating cytokines but it is possible that cytokines exert their effects in a

paracrine manner. Several recent fish oil trials have been conducted in patients receiving surgery (4,5), but unfortunately, tissue biopsies were not obtained.

When designing future trials, several key points should be considered: what is the primary outcome i.e. amount of intermuscular adipose tissue, skeletal muscle mass or chemotherapy efficacy? What is the optimal dose? What concentration of EPA versus DHA should be used? These outcomes are likely related but may differ in the amount of fish oil or the composition of fish oil required for therapeutic benefit. This may especially be true for chemotherapy trials whereby different drugs have different mechanisms of action. It may be that for anthracyclines (which induce oxidative stress), DHA may be more effective than EPA due to the presence of an additional double bond and thus is more prone to oxidation. The composition and amount of fish oil that maximizes chemotherapy response is better suited to animal studies whereby multiple formulations of fish oil could be tested with a range of chemotherapy drugs. Ideally, dose response trials of fish oil supplementation would be conducted in patient populations with longer survival than lung and pancreatic i.e. breast or colorectal cancer and would allow for doses to be titrated upwards depending on interim results of computed tomography imaging.

Previous trials have generally had low patient accrual rates and high patient withdrawal (6-8). An open label trial of fish oil supplementation was a realistic approach in a patient population with short median survival where interest in placebo controlled trials is low. This study was designed to minimize patient burden and avoid the need for appointments solely for study purposes.

Patients had frequent CT scans taken for diagnostic and follow-up purposes and regular blood draws as part of chemotherapy which were coordinated with study draws. Study assessments (PG-SGA, FAACT, and ESAS) were arranged to coincide with chemotherapy administration. This resulted in high patient retention and may be an effective approach for future trials.

The common thread between this research and recent fish oil trials which showed an effect of fish oil on attenuation of lean body mass (4,5,9) is the timing of interventions; early in the disease trajectory when muscle anabolism is more likely (10). Many patients present with depleted EPA and DHA shortly after diagnosis which may contribute to or be a consequence of tissue loss (Chapter 4). This indicates a need for supplemental EPA and DHA prior to when supplementation trials have typically conducted. Future trials aimed at attenuating muscle wasting should be implemented early in the disease trajectory (shortly after diagnosis).

From this research and research from the lab of Bougnoux et al. (11), it is clear that there is variation in the incorporation of EPA and DHA into plasma PL. It is important to understand the cause of the variation in plasma PL EPA and DHA incorporation as this is an impediment for treating patients and for drawing conclusions from trials which do not account for varying degrees of incorporation within the study population. The sample size of this study limits our ability to draw inferences on potential sources of variation. Metastases in the liver could be expected to influence fat metabolism, proximity to death (12), or genetic differences in lipid metabolism may also play a role (13). An expanded

description of fatty acid composition throughout the disease trajectory as presented in Chapter 3 would help to identify possible sources of variation which could then be controlled for in clinical trials. Ideally, trials would also include a "pre-loading" period of approximately 1 week, whereby PL EPA and DHA concentrations are assessed and patients who did not show incorporation are excluded from the trial.

The dose of fish oil prescribed in interventions has ranged from 1 to 18 grams per day (14-16) but 2 grams of EPA is commonly prescribed (6,7,17). This dose is based on a study which demonstrated weight stabilization following 2 grams per day of EPA over an 8 week period (18). This assumes that weight stabilization equates to muscle stabilization as opposed to gains of adipose tissue, visceral organs, or fluid accumulation which have been observed in advanced cancer (19). The amount of EPA specifically required for maintenance or gain of skeletal muscle has not been defined. Further, it is assumed that all patients require the same dose but as discussed above and in Chapter 5 there is evidence of differential incorporation of EPA and DHA into PL. The results in Chapter 4 and Chapter 5 which relate plasma PL EPA concentrations to rates of muscle change could serve as a basis for determining the optimum dose of EPA and DHA. This dose should be dynamic; based upon reaching a pre-determined concentration of plasma PL EPA and DHA rather than prescribing the same dose for everyone.

Another consideration for future trials is the modality used to assess body composition. The primary outcome of fish oil trials is attenuation of skeletal muscle loss but this is typically assessed using BIA which provides an estimate of

total lean body mass. BIA cannot distinguish skeletal muscle from other lean soft tissue and is unable to detect small but clinically significant changes (2.2 ± 3.2%/100 days) in lean tissue (20). The research in Chapters 3 through 6 is the first to precisely assess skeletal muscle and adipose depots through the opportunistic use of diagnostic CT images. Future trials should include assessments of body composition using CT images as they are precise and expedient in an oncology setting.

CT image analysis is also able to quantify adipose tissue in skeletal muscle (21). The research presented in Chapter 5 was the first fish oil trial to assess IMAT and muscle attenuation values; lower values are a marker of greater muscle fat content (21). This showed that fish oil supplementation results in decreased IMAT and stable muscle attenuation values whereas standard of care results in gains in IMAT and lower muscle attenuation values. Greater muscle fat content is associated with lower muscle strength and lower strength per muscle size, termed "muscle quality" (22). Measures of muscle strength and function have been infrequently assessed in fish oil trials and are important outcomes that warrant inclusion in trials as decreased function can result in loss of independence which is a concern for oncologists, patients and their caregivers.

Although the fish oil supplementation trial presented here could be deemed a "success" in that it showed a positive effect of fish oil on skeletal muscle, the trial was not without challenges. Recruitment of this patient population is extremely challenging as there is often competition with trials of anti-neoplastic therapy. Understanding of the significance of body composition

by patients and oncologists is also lacking which can result in low interest in participation. On the other hand, supplementation to improve the efficacy of chemotherapy is a tangible, meaningful outcome for both patients and oncologists. This area holds promise for improving the treatment and outcome of a large number of patients while concurrently conveying benefits of fish oil on body composition. Clinical trials of fish oil should shift focus from primarily body composition related outcomes to include chemotherapy efficacy and chemotherapy related outcomes.

7.4 Considerations for nutrition and oncology practitioners

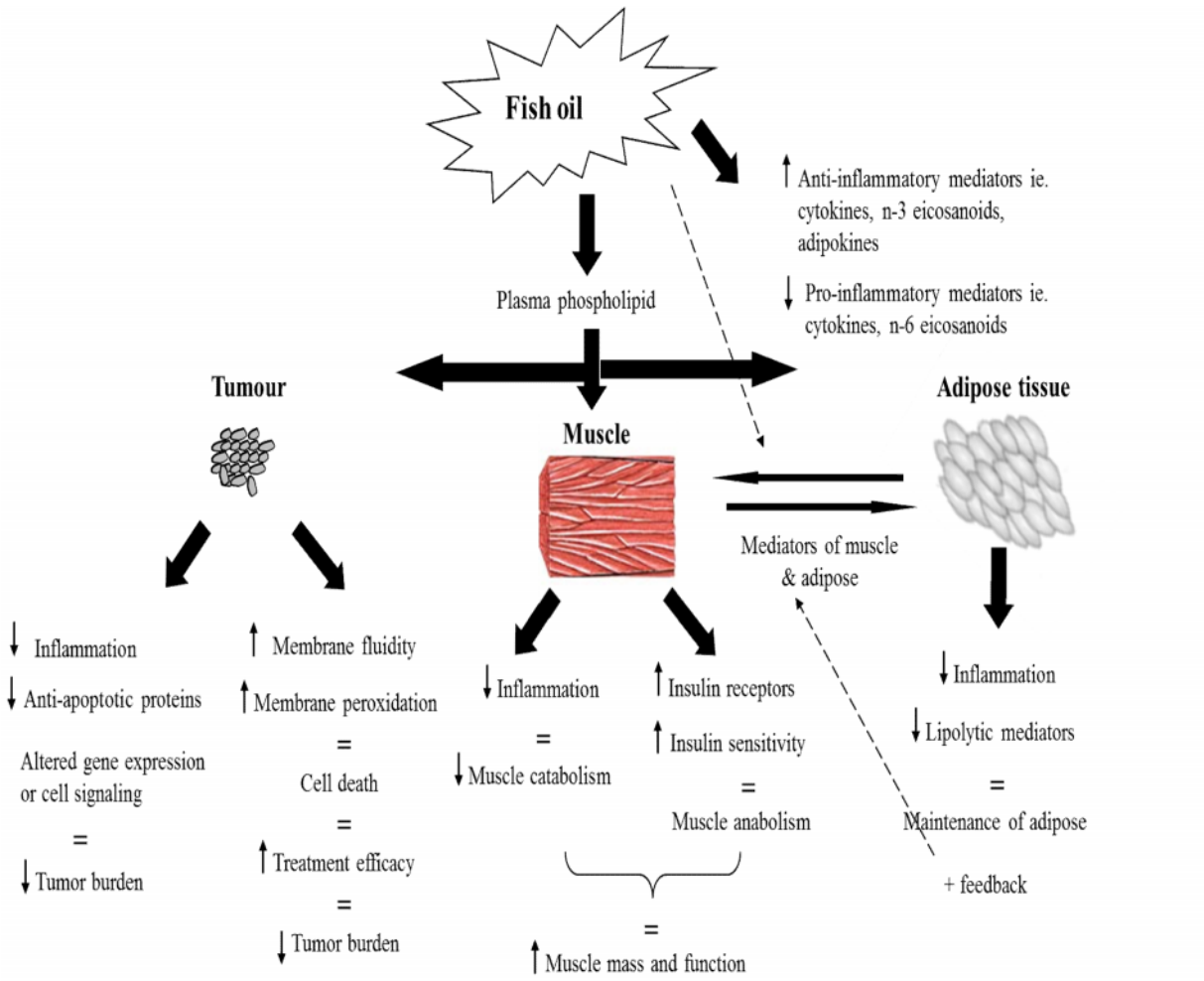
The need for an interdisciplinary approach for the care of patients with cancer cannot be overstated. Nutrition and patient outcomes are intricately linked. Interventions which bridge both nutrition and oncology are needed to improve patient outcomes. As depicted in Figure 7-1, the potential exists for a nutritional supplement to attenuate tissue loss and improve outcomes of chemotherapy. However, additional, larger trials are needed to confirm these relationships. This requires the cooperation of oncologists, dietitians and nutrition researchers. Unfortunately, nutrition is often overlooked by oncologists. Thus it is important for oncologists to develop an understanding of the impact that nutrition factors including, weight, body composition, and fatty acids have on chemotherapy efficacy (Chapter 6), chemotherapy toxicity (23,24) and survival (24,25).

7.5 Conclusions

This research shows that muscle wasting and loss of plasma PL EPA and DHA are prevalent, progressive and often severe in patients with lung cancer. Supplementation with fish oil increases plasma PL EPA and DHA and may be an effective agent to attenuate muscle loss and fatty infiltration of muscle when implemented shortly after diagnosis. Fish oil may also improve response to chemotherapy and prolong survival. Based on these results, fish oil may be an effective means to improve patient outcomes and warrants further research in lung cancer and additional cancer populations.

Figures

Figure 7-1: Proposed mechanisms for the effect of fish oil on muscle, adipose tissue and tumour tissue



This schematic depicts some of the potential mechanisms (reviewed in Chapter 1) through which fish oil may affect pathways and processes involved in muscle, adipose tissue and tumor metabolism.

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Appendix 1: Consent form for study of fatty acids during chemotherapy (standard of care)

Cross Cancer Institute
11560 University Avenue Edmonton, Alberta
T6G 1Z2 Tel 780.432.8771

The Effect of Chemotherapy on the Nutrient Status of Cancer Patients

CONSENT FORM

This form is part of the process of informed consent. It is designed to explain this research study and what will happen to you if you choose to be in this study.

If you would like to know more about something mentioned in this consent form, or have any questions at anytime regarding this research study, please be sure to ask your doctor or nurse. Read this consent form carefully to make sure you understand all the information it provides. You will get a copy of this consent form to keep. You do not have to take part in this study and your care does not depend on whether or not you take part.

Your doctor, who is one of the researchers, will discuss the study with you.

Your participation in this study is entirely voluntary. Please take your time to make your decision. It is recommended that you discuss with your friends and/or family about whether to participate in this study.

"WHY IS THIS STUDY BEING DONE?"

You are being asked to take part in this study because you have cancer and are seeking chemotherapy treatment for your cancer.

This study is being done because people with cancer are at risk for nutritional problems and we are trying to identify what these problems are and ways to prevent or treat these problems.

"WHAT DO WE HOPE TO LEARN?"

We hope to learn how certain nutrients in your body change throughout the course of chemotherapy.

The purpose of this study is to find out what effects (good and bad) chemotherapy has on your nutrient status.

"WHAT IS INVOLVED IN THIS STUDY?"

You will be followed to see what effect the treatment has on your health. Throughout your treatment you will have to come to the outpatient department at the Cross Cancer Institute. As part of your regular treatment you will be required to give blood samples. If you decide to participate in this study you will be asked to provide an additional vial (~10 ml) of blood each time a blood sample is taken as part of your regular care.

"HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?"

About 50 people will take part in this study at the CCI.

"WHAT WILL MY PARTICIPATION INVOLVE?"

If you take part in this study, you will have the following tests and procedures:

Procedure that is part of your regular care	Before Chemotherapy Starts	Before 2nd Round of Chemotherapy	Before 3rd Round of Chemotherapy	1 to 2 months After Chemotherapy Ends
Blood Test	X	X	X	X

Sample Banking for Future Research

You may also be asked whether your blood can be stored for future research. If so, you will be given another consent form asking for your permission.

"HOW LONG WILL I BE INVOLVED IN THE STUDY?"

You may be in this study for as long as you are receiving active chemotherapy treatment for your cancer.

"WHAT ARE THE SIDE EFFECTS?"

You may feel some discomfort from the needle when blood is drawn. There is also a small risk of fainting, swelling, bruising, bleeding or (rarely) local infections at the site of the needle punctures which will be used for taking blood samples.

If you have any side effects you should call the doctor or nurse in charge of the study. Their telephone numbers are on page 4 of this form.

"WHAT ARE MY ALTERNATIVES?"

You may choose not to participate in this study.

"**WHAT ARE MY RESPONSIBILITIES?"**

You must be willing to attend all scheduled study visits and undergo all of the procedures described above. It is very important that you inform the study doctor or study nurse of any side effects or health problems that you may be experiencing as well as any medications (prescribed or holistic) that you are taking while on this study.

"ARE THERE ANY BENEFITS TO PARTICIPATING IN THIS STUDY?"

Participation in this study may or may not be of personal benefit to you. However, based on the results of this study, it is hoped that, in the long-term, patient care can be improved.

"CAN I WITHDRAW FROM THIS STUDY?"

Taking part in this study is voluntary; you may withdraw from the study at any time if you wish to do so.

"ARE THERE COSTS TO ME FOR TAKING PART IN THIS STUDY?"

You will not have to pay for the extra blood tests performed in this study.

"WHAT ARE MY RIGHTS AS A PARTICIPANT?"

If you suffer an injury or become ill as a result of participating in this research, you will receive all medical treatments (or services) recommended by your doctors. No compensation will be provided beyond this point. However, it is important to note that nothing said in this consent form alters your legal rights to recover damages (e.g. legal action).

If new information becomes available or there are changes to the study that may affect your health or willingness to continue in the study, you will be told in a timely manner.

"WILL MY PERSONAL INFORMATION BE KEPT CONFIDENTIAL?"

Identifiable health information will be collected from you and from your Provincial Electronic Health Record (NetCare)during this study. This information may be used by the researchers who are carrying out this study, and may be disclosed to others as described below. Any research proposal to use information that identifies you for a purpose other than this study must be approved in advance by the Alberta Cancer Research Ethics Committee.

Direct access to your identifiable health information collected for this study will be restricted to the researchers who are directly involved in this study except in the following circumstances:

Your identifiable health information may need to be inspected or copied from time to time for quality assurance (to make sure the information being used in the study is accurate) and for data analysis (to do statistical analysis that will not identify you). The following organizations may do this inspection:

- Health Canada, the Canadian regulatory body.
- Alberta Cancer Research Ethics Committee, the institutional review board at this centre
- Members of the Regulatory/Audit team at the Cross Cancer Institute, for quality assurance purposes

Any disclosure of your identifiable health information will be in accordance with the Alberta Health Information Act. As well, any person from the organizations looking at your records on-site at the Cross Cancer Institute will follow the relevant Alberta Health Services - Alberta Cancer Research Ethics Committee policies and procedures that control these actions. Any disclosure of your identifiable health information to another individual or organization not listed here will need the approval of the Alberta Cancer Research Ethics Committee.

Your identifiable health information collected as part of this study, which includes records of your progress, your responses to the questionnaires, as well as related blood work will be kept confidential in a secure Alberta Health Services facility. Information that does not identify you will also be provided to the researchers where it will be kept confidential in a secure location.

The researchers who are directly involved in your study may share information about you with other researchers, but you will not be identified in that shared information except by a number. The key that indicates what number you have been assigned will be kept secure by the researchers directly involved with your study and will not be released.

Although absolute confidentiality can never be guaranteed, Alberta Health Services will make every effort to keep your identifiable health information confidential, and to follow the ethical and legal rules about collecting, using and disclosing this information in accordance with the Alberta Health Information Act and other regulatory requirements.

The information collected during this study will be used in analyses and will be published and/or presented to the scientific community at meetings and in journals, but your identity will remain confidential. This information may also be used as part of a submission to regulatory authorities around the world. It is expected that the study results will be published as soon as possible after

completion. Your study doctor will be informed of the results of the study once they are known.

“WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?”

For information about your disease and/or research related injury/illness, you may contact the Principal Investigator Dr. Quincy Chu, (780) 432-8248, or Co-Investigator Vera Mazurak, (780) 492-8048 to answer any questions you have about this study.

If you feel, at any time, that you have not been informed to your satisfaction about the risks, benefits, or alternatives of this study, or that you have been encouraged to continue in this study after you wanted to withdraw, you can call the Patient Representative at (780) 432-8585.

UNDERSTANDING OF PARTICIPANTS

I can refuse to take part or withdraw from this study at any time without jeopardizing my health care. If I continue to take part in the study, I will be kept informed of any important new developments and information learned after the time I gave my original consent.

I also give consent for the Principal Investigator and Alberta Health Services (the Custodian) to disclose identifiable health information, as per the Alberta Health Information Act, to the organizations mentioned on the previous pages.

I have read and understood all of the information in this consent form. I have asked questions, and received answers concerning areas I did not understand. I have had the opportunity to take this consent form home for review and discussion. My consent has not been forced or influenced in any way. I consent to participate in this research study. Upon signing this form I will receive a signed copy of the consent.

(PRINT NAMES CLEARLY)

_____	_____	_____
Name of Patient	Signature of Patient	Date
_____	_____	_____
Name of Person Obtaining Consent	Signature of Person Obtaining Consent	Date

Patient Study Number or Hospital Number: _____

Was the patient assisted during the consent process in one of the ways listed below?

□ Yes □ No

If yes, please check the relevant box and complete the signature space below:

□ The consent form was read to the patient, and the person signing below attests that the study was accurately explained to, and apparently understood by the patient.

□ The person signing below acted as a translator for the patient during the consent process.

_____	_____
Signature of person assisting In the consent discussion	Date

Appendix 2: Ethics amendment letter

January 16, 2008

RE: ETH-22521: A randomized phase II double blind trial, placebo controlled trial of a nutritional supplement for patients with advanced non-small cell lung cancer undergoing palliative chemotherapy

Several challenges have been encountered that necessitate changes in the protocol of this study. We are submitting, for approval, revisions to our study protocol and consent form. We propose to make the following amendments:

- **Remove the placebo control arm of the study thereby removing randomization and blinding**
  We would like to un-blind the study and forgo use of the placebo control arm because we are unable to meet requirements mandated by pharmacy due to the nature of the supplement we are providing. Pharmacy requires shipment directly from the manufacturer to the Cross Cancer Institute. However, this is not possible because the supplement is received at the Department of Agricultural, Food and Nutritional Sciences in bulk format from the manufacturer. Unfortunately, the manufacturer is unable to package the supplement into individual portions. Therefore, the bulk supplement is re-packaged it into individual portions at a certified food-grade HACCP controlled facility. These efforts were undertaken to ensure the supplement would be easy to use and store for patients. As such we are unable to randomize and distribute our supplement as intended.

  Additionally, difficulty with recruitment has been experienced. Unfortunately this study cannot accrue the sample size of 80 with 40 patients on the treatment arm and 40 patients receiving the placebo. Patients have been apprehensive about participating in our trial because they do not want to receive a placebo treatment with no apparent benefits. Patients who enroll in this study but who do not take the supplement will serve as a no treatment group.

- **Allow patients the option of participating in our trial with or without receiving the supplement but completing all study measures**
  We would like to provide patients with the choice of participating in our study even if they decline to take the supplement. The patients who choose not to receive the supplement will complete the same measures as the patients who choose to receive the supplement. These patients will serve as a control group. This information will be extremely valuable even without supplements to our ongoing research effort.

- **Change the form of supplementation from the MEG-3 microencapsulated fish oil powder to Webber Super Concentrate fish oil capsules, Ocean Nutrition concentrated fish oil and Ascent NutrSea fish oil.**
  We would like to change the format of the supplement from the MEG-3 microencapsulated powder to Webber Super Concentrate fish oil capsules and Ascent NutraSea fish oil. Patient response to the MEG-3 powder has been negative. The MEG-3 powder does not blend into foods as easily as we anticipated, and the powder has a fishy taste and odour that patients find hard to tolerate. We would like to give patients the option of taking either the fish oil, or the capsules or the option of taking them in combination. We feel this will increase interest in the study as well as compliance. The dose is comparable for both supplementation forms.

- **Include all non-small cell lung cancer patients who receive chemotherapy - adjuvant and palliative therapies.**
  We have been recruiting patients at lung new patient clinics but of the new patients only approximately 25% of them receive chemotherapy. Of this 25% only approximately 5% receive palliative chemotherapy versus adjuvant chemotherapy. Patient enrolment would be significantly enhanced by opening the study to all patients receiving chemotherapy.

- **Include additional questionnaires: Taste and Smell Assessment Survey and the Functional Assessment of Anorexia/Cachexia Therapy (FAACT)**
  The Taste and Smell Assessment Survey is used to determine how cancer affects taste and smell. This is important because cancer patients may experience chemosensory alterations as a result of chemotherapy. This tool will help us to assess the types of changes patients are experiencing and the impact of these changes on their quality of life.

  The FAACT is a questionnaire designed to measure general aspects of quality of life as well as specific anorexia/cachexia-related concerns. The FAACT will enable us to collect specific quality of life data as patients' progress through their treatments.

Proposed new title: **"ETH-22521: Trial of a nutritional supplement for patients with non-small cell lung cancer undergoing chemotherapy."**

Attached is the revised consent form. Thank you for your consideration.

Sincerely

Rachel Murphy

Appendix 3: Consent form for revised trial of fish oil during chemotherapy

Cross Cancer Institute
11560 University Avenue Edmonton, AlbertaT6G 1Z2 Tel 780.432.8771

Trial of a nutritional supplement for patients with non-small cell lung cancer undergoing chemotherapy
(A study to see whether nutritional supplementation is of benefit for people with lung cancer)

CONSENT FORM

This form is part of the process of informed consent. It is designed to explain this research study and what will happen to you if you choose to be in the study.

If you would like to know more about something mentioned in this consent form, or have any questions at anytime regarding this research study, please be sure to ask your doctor or nurse. Read this consent form carefully to make sure you understand all the information it provides. You will get a copy of this consent form to keep. You do not have to take part in this study and your care does not depend on whether or not you take part.

Your doctor, who is one of the researchers, will discuss the study with you.

Your participation in this study is entirely voluntary. Please take your time to make your decision. It is recommended that you discuss with your friends and/or family about whether to participate in this study.

"WHY IS THIS STUDY BEING DONE?"

You are being asked to take part in this study because you have lung cancer and are seeking chemotherapy treatment for your cancer. This study is being done because people with lung cancer are at risk for nutritional problems and we are trying to develop ways to prevent or treat these problems.

"WHAT DO WE HOPE TO LEARN?"

We hope to learn more about how the drugs that you take for your cancer impact your body's ability to use certain fats from your diet.

The purpose of this study is to assess how much and what types of fats you have available in your body and to see if nutritional supplementation is able to maintain the levels of essential fats in your body as you progress through your treatments. Secondly, we hope to gather information on the ability of supplementation with

nutrients to limit the side effects you may currently experience while on treatments.

“WHAT IS INVOLVED IN THIS STUDY?”

In this study, you will receive a nutritional supplement, which contains essential fats, and you will be followed to see what effect the nutritional supplements have on your health. You will be required to take the supplement from the time you enroll in the study until you have completed your chemotherapy treatments. The other option is you may participate in this study without receiving the supplement. For this option, you will not take the supplement but you will complete the same questionnaires and tests for the duration of your treatment. You will be asked to record all other supplements you take during the study but do not need to stop taking them.

“HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?”

About 45 people will take part in this study.

“WHAT WILL MY PARTICIPATION INVOLVE?”

If you take part in this study, you will have the following tests and procedures:

Procedures		
	Initial Visit	Before Each Round of Chemotherapy
[a] Toxicity Assessment	X	X
[b] Symptom Assessment	X	X
[c] Taste and Smell Assessment Survey	X	X
[d] FAACT Assessment	X	X
[e] Food Frequency Questionnaire	X	X
[f] Nutritional Assessment	X	X
Blood Test	X	X

a,b,c,d,e,f: Questionnaires: You will be asked to fill out several short questionnaires including toxicity assessment (~ 5 minutes to complete), symptom assessment (~5 minutes to complete), Taste and Smell Survey (~ 10 minutes to complete), FAACT quality of life survey (~15 minutes to complete), Food frequency questionnaire (~10 minutes to complete), and nutritional assessment (~5 minutes to complete) during your normal scheduled visits to the Cross Cancer Institute and will require approximately 45 minutes of your time.
Someone involved in the study will assist you in completing the questionnaires while you wait to see your doctor.

Blood Test: As part of this study you will be asked to provide and additional vial of blood (~10ml) each time a blood sample is taken as part of your regular care.

"HOW LONG WILL I BE INVOLVED IN THE STUDY?"

You may be in this study for as long as you are receiving active chemotherapy treatment for your cancer.

"WHAT ARE THE SIDE EFFECTS?"

The side effects of the nutritional supplement may include loose bowel movements and/or bloating. These side effects are temporary and reversible, meaning they will stop once supplementation is discontinued. 14% of participants in a previous study experienced these side effects when they consumed the nutritional supplement at levels well beyond what is being provided in this study. The chemotherapy that you will be receiving as part of your standard of care has side effects associated with it, which your doctor will explain.

If you have any side effects, either those above or others, or if you want more information on how the nutritional supplement could affect you, you should call the doctor or nurse in charge of the study. Their telephone numbers are on page 5 of this form. If we get any new information about the supplement and side effects in this study, you will be told about them so that you can continue to get the best care possible.

If we get any new information about the nutritional supplement or side effects in this study, you will be told about them so that you can continue to get the best care possible.

"**WHAT ARE MY RESPONSIBILITIES?"**

You must be willing to attend all scheduled study visits and undergo all of the procedures described above. It is very important that you inform the study doctor or study nurse of any side effects or health problems that you may be experiencing as well as any medications (prescribed or holistic) that you are taking while on this study.

"WHAT ARE MY ALTERNATIVES?"

You may choose not to participate in this study. At this time, nutritional intervention is not a standard part of your treatment, therefore, you would receive the standard treatments which your doctor has discussed with you with no nutritional intervention.

"ARE THERE ANY BENEFITS TO PARTICIPATING IN THIS STUDY?"

As a participant in this study, you will receive information about your nutritional status and diet. Based on the results of this study, it is hoped that, in the long-term, patient care can be improved.

"CAN I WITHDRAW FROM THIS STUDY?"

In discussion with you, your doctor at the Cross Cancer Institute may withdraw you from the study at any time if it is in your best interests. Taking part in this study is voluntary; you may withdraw from the study at any time if you wish to do so. If you decide to stop participating in the study, we encourage you to talk to your doctor first.

Your doctor can take you off the study treatment early for reasons such as:

- You are unable to tolerate the nutritional supplement.
- New information becomes available that indicates the nutritional supplement is no longer in your best interest.

If you stop treatment early, we would like to keep track of your medical condition for the rest of your life to look at the long-term effects of the study treatments.

"ARE THERE COSTS TO ME FOR TAKING PART IN THIS STUDY?"

You will not have to pay for the treatment you receive in this study.

"WHAT ARE MY RIGHTS AS A PARTICIPANT?"

If you suffer an injury or become ill as a result of participating in this research, you will receive all medical treatments (or services) recommended by your doctors that are not covered by health insurance. No compensation will be provided beyond this point. However, it is important to note that nothing said in this consent form alters your legal rights to recover damages.

If new information becomes available or there are changes to the study that may affect your health or willingness to continue in the study, you will be told in a timely manner.

"WILL MY PERSONAL INFORMATION BE KEPT CONFIDENTIAL?"

Identifiable health information will be collected from you and from your Provincial Electronic Health Record (NetCare) during this study. This information may be used by the researchers who are carrying out this study, and may be disclosed to others as described below. Any research proposal to use information that identifies you for a purpose other than this study must be approved in advance by the Alberta Cancer Research Ethics Committee.

Direct access to your identifiable health information collected for this study will be restricted to the researchers who are directly involved in this study except in the following circumstances.

Your identifiable health information may need to be inspected or copied from time to time for quality assurance (to make sure the information being used in the study is accurate) and for data analysis (to do statistical analysis that will not identify you). The following organizations may do this inspection:

- Health Canada, the Canadian regulatory body.
- Alberta Cancer Research Ethics Committee, the institutional review board at this centre
- Members of the Regulatory/Audit team at the Cross Cancer Institute, for quality assurance purposes

Any disclosure of your identifiable health information will be in accordance with the Alberta Health Information Act. As well, any person from the organizations looking at your records on-site at the Cross Cancer Institute will follow the relevant Alberta Health Services - Alberta Cancer Research Ethics Committee policies and procedures that control these actions. Any disclosure of your identifiable health information to another individual or organization not listed here will need the approval of the Alberta Cancer Research Ethics Committee.

Your identifiable health information collected as part of this study which includes your food records, responses to questionnaires and information from your blood will be kept confidential in a secure Alberta Health Services facility. If this study has a central office that is not in an Alberta Cancer Board facility, the information will be kept confidential in a secure location at the central office.

The researchers who are directly involved in your study may share information about you with other researchers, but you will not be identified in that shared information except by a number. The key that indicates what number you have been assigned will be kept secure by the researchers directly involved with you study and will not be released.

Although absolute confidentiality can never be guaranteed, the Alberta Health Services will make every effort to keep your identifiable health information confidential, and to follow the ethical and legal rules about collecting, using and disclosing this information in accordance with the Health Information Act and other regulatory requirements.

The information collected during this study will be used in analyses and will be published and/or presented to the scientific community at meetings and in journals, but your identity will remain confidential. This information may also be used as part of a submission to regulatory authorities around the world. It is expected that the study results will be published as soon as possible after

completion. Your study doctor will be informed of the results of the study once they are known.

“WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?”

For information about your disease and/or research related injury/illness, you may contact the Principal Investigator (Dr. Quincy Chu, (780) 432-8248) or page him through the Cross Cancer Institute Switchboard at (780) 432-8771 to answer any questions you have about this study. The Co-Investigator (Dr. Vera C Mazurak) may also be reached at (780) 492-8048 to answer any questions you have about this study. You may contact the research assistant (Rachel Murphy) at (780) 432-8233.

If you feel, at any time, that you have not been informed to your satisfaction about the risks, benefits, or alternatives of this study, or that you have been encouraged to continue in this study after you wanted to withdraw, you can call the Patient Representative at (780) 432-8585.

UNDERSTANDING OF PARTICIPANTS

I can refuse to take part or withdraw from this study at any time without jeopardizing my health care. If I continue to take part in the study, I will be kept informed of any important new developments and information learned after the time I gave my original consent.

I also give consent for the Principal Investigator and Alberta Health Services (the Custodian) to disclose identifiable health information, as per the Alberta Health Information Act, to the organizations mentioned on the previous pages.

I have read and understood all of the information in this consent form. I have asked questions, and received answers concerning areas I did not understand. I have had the opportunity to take this consent form home for review and discussion. My consent has not been forced or influenced in any way. I consent to participate in this research study. Upon signing this form I will receive a signed copy of the consent.

(PRINT NAMES CLEARLY)

_____	_____	_____
Name of Patient	Signature of Patient	Date

_____	_____	
Name of Person Obtaining Consent	Signature of Person Obtaining Consent	Date

Patient Study Number or Hospital Number: _____

Was the patient assisted during the consent process in one of the ways listed below?

□ Yes □ No

If yes, please check the relevant box and complete the signature space below:

□ The consent form was read to the patient, and the person signing below attests that the study was accurately explained to, and apparently understood by the patient.

□ The person signing below acted as a translator for the patient during the consent process.

_____	_____
Signature of person assisting in the consent discussion	Date

Appendix 4: Clinical Nutrition manuscript

Loss of adipose tissue and plasma phospholipids: Relationship to survival in advanced cancer patients

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Abstract

Background: Extensive loss of adipose tissue is a key feature of cancer cachexia. Advanced cancer patients also exhibit low plasma phospholipids (PL). It is not known whether these processes coincide across the cancer trajectory nor has their relationship with survival been defined. Changes in adipose tissue mass and plasma PL were characterized within 500 days prior to death and prognostic significance assessed.

Methods: Adipose tissue (AT) rate of change was determined in a retrospective cohort of patients who died of colorectal and lung cancers (n=108) and who underwent >2 computed tomography scans in the last 500 days of life. Plasma PL fatty acids were measured prospectively in a similar cohort of patients with metastatic cancer (n=72).

Results: Accelerated lossof AT begins at 7 months from death reaching an average loss of 29% of total AT 2 months from death. Plasma PL fatty acids were 35% lower in patients closest to death versus those surviving >8 months. Loss of PL fatty acids and adipose tissue occur in tandem and are predictive of survival.

Conclusion: Depletion of plasma PL likely indicates a deficit of essential fatty acids in the periphery which may contribute to loss of adipose tissue.

Introduction

Patients with advanced cancer often suffer from cachexia and exhibit extensive loss of muscle and adipose tissue (up to 85%). Cachexia is characterized by anorexia, early satiety, weakness, anaemia, reduced immune function and edema, and is estimated to be the direct cause of approximately 20% of cancer deaths (Tisdale, 2002). Loss of adipose tissue may be caused by alterations in lipid metabolism including elevated lipolysis (Ryden et al., 2008)associated with reduced activity of lipoprotein lipase and elevated hormone sensitive lipase activity (Agustsson et al., 2007). Impairment in lipid storage capacity of adipocytes in animal models of cachexia has been reported (Bing et al., 2006). However, the time course over which fat loss occurs in cancer patients has not been well-defined.

For the past several years, members of our team have been engaged in profiling the nutritional characteristics of advanced cancer patients (Prado et al., 2008; Lieffers et al., 2009). This work has focused primarily on patients with locally recurrent or metastatic cancers of the lung and gastrointestinal tract during the last 12 months of life. Patients participating in prospective studies of nutritional status have since died, allowing length of time from the measure of interest until death to be determined. Since diagnosis of cancer can occur one day or several years from death, time of diagnosis is not a useful criterion for selection of cancer patients for studies of nutritional status. Important relationships emerge from stratifying patients based on time to death for measures of fatty acid status and body composition.

Plasma phospholipids (PL) contain the majority of essential fatty acids in blood. Fatty acids in PL reflect overall metabolism of endogenous and dietary fatty acids and can be used to detect aberrations in n-6 and n-3 essential fatty acid metabolism. Our group has previously reported low plasma PL in a group of patients with mixed tumour types close to death (Pratt et al., 2002). Determining the cause and point in time that this occurs is important for understanding how deficits in peripheral fat availability, essential fatty acids and metabolites contribute to wasting of both adipose and muscle tissue.

Computed tomography (CT) is a precise method to quantify adipose and muscular tissues using single slice images (Ross, 2003), which can be used to estimate appendicular skeletal muscle, whole body lean and fat mass in cancer patients (Mourtzakis et al., 2008). Patients with cancer often undergo several CT scans over the course of their illness for diagnostic purposes and to assess tumour progression, which allows for a calculation of the rate of fat loss within each depot for a single patient. Reproducibility for CT image analysis of fat and muscle is ~2%, making it an ideal method for detecting small changes in body compartments in both cross sectional and longitudinal studies (Mourtzakis et al., 2008). Aligning retrospective CT images within the final 500 days of life of patients that are deceased has enabled quantification of fat loss in visceral (VAT), subcutaneous (SAT) and intramuscular (IMAT) adipose depots to be established throughout the trajectory of the disease.

The purpose of this study is to assess amounts of AT and PL fatty acids in 2 cohorts of advanced cancer patients with lung, colorectal, gastrointestinal or other

cancer types (kidney and prostate) over the last 500d of life to establish determinants of survival time. Within this objective we also aim to (1) assess rates of change in total AT and respective adipose depots; (2) assess amounts and types of fatty acids in plasma PL. This work represents a first step in defining fat loss over the course of the cancer trajectory and in determining the prognostic value of measures of fat metabolism. This information will be used to generate hypotheses and provide rationale for studies aimed at defining interventions to limit nutrient depletion in advanced cancer patients.

Materials and Methods

Participants

Data from two similar cohorts of patients with locally advanced or metastatic cancer of mixed diagnosis were included to characterize AT mass and plasma PL: non-small cell lung cancer (NSCLC), colorectal, gastrointestinal cancer and other cancer types (kidney and prostate). Data were collected prospectively and patients were followed until time of death. AT loss was evaluated in one cohort (AT cohort) and PL fatty acid composition and concentration was assessed in a second cohort (PL cohort); time to death was used to categorize all patients. Total AT and total PL data were tested for the presence of outliers and eliminated from all analyses (AT cohort, n=3). Protocols were reviewed and approved by the Research Ethics Board of Alberta Health Services (Edmonton, Alberta, Canada).

The AT cohort included 108 patients who had at least 2 CT images in the last 500 days of life in the interval between diagnosis of advanced cancer and death. The CT scans were required to be between 30 and 300 days apart, and averaged 82 ± 40 days apart. If patients had more than 2 valid CT images, the two images closest to death were used in the analyses. SAT from 6 obese patients was not fully visible in the image viewing field and therefore SAT and total AT from these patients was not included in the analyses.

The PL cohort consisted of 72 patients with plasma PL measurements only and included a range of patients who were newly diagnosed with cancer or newly admitted to the palliative care program. This cohort provided a broad range of values for plasma PL, and on average, was farther from death than the AT Cohort ($P<0.0001$, 136±71 versus 271±172 days, respectively). The characteristics of each cohort are shown (Table AP1).

Adipose Tissue Measurements

VAT, SAT, IMAT and total AT were measured using electronically stored CT scans previously taken for diagnostic purposes. The third lumbar vertebrae (L3) was chosen as a landmark as it is strongly correlated with whole body adipose tissue (Shen et al., 2004). Two consecutive images extending from L3 to the iliac crest were quantified and averaged for cross-sectional area of AT tissue in depots corresponding to SAT, VAT, and IMAT depots. Images were analyzed with Slice-O-Matic (V4.3; TomoVision, Montreal, QC, Canada) which permits specific tissue demarcation by using Hounsfield unit thresholds of -150 to -50 for VAT (Miller et al., 1998) and -190 to -30 for SAT and IMAT (Mitsiopoulos et al.,

1998). The sum of the tissue pixels were multiplied by the pixel surface area to derive cross sectional areas (cm^2). To estimate total AT areas, SAT, VAT and IMAT cross-sectional areas were summed. A regression equation derived from an advanced cancer patient cohort were used to estimate whole body fat mass (Mourtzakis et al., 2008):

Whole body fat mass (kg) = 0.042 x [total adipose tissue at L3(cm^2)] + 11.2; r^2=0.77.

The rate of AT change within a defined time interval was determined using two CT images from the last 500d of life. Given that CT images were analyzed retrospectively, the timing of each image was unique for each individual. Thus, tissue change for each scan interval was expressed as a percentage and divided by the number of days in each interval. The daily rate of loss or gain in fat was multiplied by 100 to form a standard unit expressed as % change/100d to allow for comparison between individuals. Details of CT image analysis have been previously described (Heymsfield et al., 1997; Mourtzakis et al., 2008) and validated for use in body composition analyses of advanced cancer patients (Mourtzakis et al., 2008).

Plasma Phospholipid Fatty Acid Analysis

Plasma was centrifuged, isolated from whole blood and immediately frozen at -80°C until analysis. A modified Folch method was used to extract lipids from plasma (Field et al., 1989) and plasma PL fatty acid composition was determined using thin layer chromatography as previously described (Pratt et al., 2001). Briefly, plasma lipids were extracted using chloroform/methanol followed by

isolation of PL fraction on G-plates (Layne et al., 1996). The PL band was scraped and C17:0 standard (50µL) was added for quantification, followed by direct methylation. Fatty acids were determined using gas liquid chromatography (Varian 3600CX Gas Chromatograph) equipped with a flame ionization detector and BP-20 fused capillary column (SGE, Australia). The flow rate of helium gas was 1.6 ml/min with oven, injector and detector temperatures of 200°C, 250°C and 250°C, respectively (Park et al., 2006). Peaks of saturated, monounsaturated and polyunsaturated fatty acids between 6 and 24 carbon chain lengths were identified by comparison with known standards (Supelco, Bellefonte, PA, USA; Sigma Chemical, St. Louis, MO, USA).

Statistical Analysis

Data are reported as mean ± SD. Levels of significance are P values ≤ 0.05. One way ANOVA was used to test differences when data were split into more than two groups. Significant outliers in the data were detected using Grubbs' test and excluded from all analyses. For plasma fatty acid profile analyses, the PL cohort was split into two groups based on mediantime to death (238d). Differences between two groups were evaluated by unpaired t-tests when data were normally distributed or otherwise by the Mann Whitney test. Correlations were done using Spearman's rank correlation tests for non-parametric data. All statistics were performed using SPSS (17.0) or GraphPad Prism (V5) software.

For survival analyses, optimum stratification was used (SAS version 9.1.3) to find the most significant P value by use of the log-rank Chi-square statistic to define the cut-points associated with patient mortality. Optimum stratification

solves the threshold value of the continuous variable (AT, IMAT, SAT, VAT and PL fatty acids) using log-rank statistics and separates patients with respect to time to an event outcome (mortality). These cut-points were then used to classify patients as higher versus lower risk of death. Survival was defined as number of days to death from the midpoint between two CT scans or the date of blood sample. The Kaplan-Meier method was used to establish the effect of AT, IMAT, SAT, VAT and amounts of PL fatty acids on survival. Log-rank tests were used to compare the survival curves of each variable. We focused on predictors known to influence nutrition and outcomes in advanced cancer including: sex, type of cancer (lung vs colorectal, lung vs other cancer types), BMI (≤18.5 or >18.5 kg/m^2) (data not available for entire AT cohort) and age (<65 or ≥65 y), while recognizing the limitations of not including additional predictors of outcome such as performance status that was not available for this data set. Variables were entered into a univariate Cox proportional hazards model with 95% confidence intervals. Significant univariate predictors were entered into a multivariate Cox proportional hazards model.

Results

Adipose Tissue Cohort

AT changes were significantly correlated with time to death for all depots (SAT P=0.004, VAT P=0.02, total AT P=0.005) except IMAT (P=0.12; data not shown). Scan intervals were divided according to time to death and the time course of mean AT loss or gain (% change /100 d) is shown (Figure AP1). Changes in each adipose depot followed a polynomial relation (r^2=0.99). IMAT

had a net loss of -4.3%/100 days from 230 until 98 days from death, to a gain of 9.41% at 66 days from death. Mean SAT and VAT also showed a polynomial relationship (r^2=0.98, 0.95), resulting in a mean decrease at 66 days from death of -29.34% and -24.77%, respectively. Total AT change followed a similar polynomial relationship (r^2=0.96), resulting in mean losses at 158 days and a total loss of -29.11% AT at 66 days from death. This equates to a net loss of ~10 kg of fat mass/100d in patients closest to death.

Median survival was ~2 times longer for patients with VAT, SAT and total AT rate of change above specific cut-points compared to patients below, however survival was not significantly affected by IMAT rate of change (Table AP2). Survival was not affected by gender (HR 0.90 [0.6-1.3] *P*=0.60), or age <65 or ≥65 y (HR 0.97 [0.7-1.4] *P*=0.90) at the univariate level. Patients with lung cancer lived longer than those with colorectal cancer (HR 0.344 [0.3-0.5] *P*<0.0001). It should be noted that the colorectal cancer patients within the AT cohort are closer to death because these patients were recruited for a study that focused on end of life measures. Type of cancer was modeled for IMAT, VAT, SAT and total AT in a multivariate Cox proportional hazards model. VAT, SAT and total AT remained significant predictors of survival (Table 2) and IMAT became a significant predictor of survival. Survival curves for total AT loss are shown (Fig AP2a) and results were also representative for SAT and VAT loss, respectively.

Phospholipid Cohort

The concentrations of all plasma PL fatty acids were significantly correlated with time to death ($P<0.05$) except arachidonic acid (AA) and α-linolenic acid (ALA) (data not shown). With the exception of EPA, these PL fatty acids also correlated with BMI ($P<0.005$), but not age. However, BMI was not significantly correlated with time to death ($P=0.09$). To compare PL fatty acid compositions and concentrations in patients closest versus those farthest from death, the cohort was divided into 2 groups based on median time to death (238d). Age and BMI were not significantly different between the 2 groups. The quantity of all PL fatty acids was significantly lower in the <238d group than the >238d group (Table AP3). Proportions (percent) of each fatty acid contributing to total PL were similar between groups.

Patients with plasma PL fatty acid concentrations above defined cut-points lived 1.4 to 2.3 fold longer than those below for all fatty acids with the exception of ALA (Table AP4). A representative survival curve for n-3 fatty acids is shown (Fig AP2b). Univariate analysis showed no difference in survival for sex (HR 0.9 [0.5-1.4] $P=0.59$), BMI ≤18.5 or >18.5 kg/m^2 (HR 0.96 [0.4-2.1] $P=0.91$) or age (HR 0.9 [0.6-1.5] $P=0.93$). Therefore PL fatty acids were analyzed using a univariate Cox proportional hazards model (Table AP4). As opposed to the AT cohort, type of cancer was not a significant predictor of survival in the PL cohort (lung vs colorectal or GI cancer: HR 1.0 [0.5-1.7] $P=0.92$, lung vs other: HR 0.6 [0.3-1.4] $P=0.26$).

Discussion

No studies to date have measured the rate of adipose tissue loss as it relates to changes in fatty acid status throughout disease progression in cancer patients. Therefore, the cause(s) or point in time that alterations occur as this metabolic situation evolves has not been identified. Data compiled on fatty acids in plasma PL and body composition spanning time of diagnosis to the last 60 days of life has enabled establishment of a relationship between fatty acid availability and adipose tissue loss throughout the cancer trajectory. It is evident from the current study that depletion of plasma PL essential fatty acids and loss of adipose tissue occur at approximately the same time (7 to 8 months prior to death) within the cancer trajectory.

Adipocytes from cancer patients are highly sensitized to catabolic signals stimulating lipolysis (Agustsson et al., 2007; Ryden et al., 2008). CT scans provide a unique opportunity to quantify and discriminate between adipose tissue depots which may differ in lipolytic rates and sensitivity to catabolic stimuli. Our results show that the rate of SAT, VAT and total AT loss follow a similar pattern and time-coursewith decreases in mean rate of AT gain beginning at approximately 7 months from death and within 6 months of death, accelerated losses occur. The functional implications of fat loss from specific depots require further investigation. The magnitude of change in plasma PL fatty acid levels and AT wasting observed in patients around this time is expected to be due to fundamental changes in metabolism. These changes are not likely to be evoked from altered dietary intake alone as a 10 kg loss of fat in 100 days would require a

caloric deficit >750 kcals/day. Work by our group in a similar group of patients suggests that changes in food intake occurs after accelerated loss of adipose tissue begins (unpublished data). Likewise, loss of adipose stores often precedes anorexia in animal models of cachexia (Bing et al., 2000). Furthermore, low intakes affect fatty acid composition but not amount of plasma PL (Decsi et al., 1998). The relationship between AT loss and peripheral fatty acid availability requires further exploration.

Plasma PL fatty acid levels were 35% lower in patients that were <7 months of death versus patients who survived >7 months from the time of assessment. This is noteworthy because about 80% of the essential fatty acids in plasma lipid are found in the plasma PL fraction. Pratt et al. (2002) reported that patients with advanced cancer in palliative care uniformly express levels of total plasma PL and essential fatty acids <30% of those seen in healthy individuals as well as other patient groups regardless of total caloric or total fat intake. Moreover, ALA and LA (linoleic acid) in plasma PL were 27% and 29% of the levels in healthy individuals. A measure of essential fatty acid deficiency, the ratio of mead acid (20:3n-9) to AA, was also elevated. Zuijdgeest-van Leeuwen et al. (2002), studied plasma fatty acid levels of patients with newly diagnosed pancreatic, NSCLC and stomach/esophageal cancer compared to normal healthy controls. They reported lower proportions of n-6 and n-3 fatty acids in cancer patients than those of healthy subjects (Zuijdgeest-van Leeuwen et al., 2002). We also report that declines in essential n-6 and n-3 fatty acids and AT depletion occur in tandem suggesting an important relationship between essential fatty acid availability and

maintenance of fat mass. Further work is directed at delineating this relationship more clearly. The n-3 series was particularly depleted prior to death in this cohort. ALA was 59% lower and the longer chain polyenes eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) were 26% and 40% lower in patients surviving less than 238 days versus those surviving longer. This coincides with evidence showing the existence of a progressive deficiency or imbalance in essential long chain n-6 and n-3 fatty acids in cancer patients (Gogos et al., 1998; Barber et al., 1999; Chaudry et al., 1991; McClinton et al., 1991). The merit of early intervention is emphasized in order to limit nutrient depletion, rather than attempting to treat the catabolic response at advanced stages.

In the classic study by DeWys et al. (1980), weight loss of >5% of body weight at time of diagnosis compared to no weight loss was associated with a significantly poorer median survival, chemotherapy response, and performance status. While several factors have been considered in prognostication for advanced cancer, emerging studies suggest that markers of metabolic impediments have a place in defining risk. The prognostic significance of fat versus lean tissue loss has not been explicitly established, although it is a widely held belief that loss of metabolically active lean tissue contributes to increased mortality, accelerated disease progression, as well as impairment of strength and functional status (DeWys et al., 1980). This paper is a first analysis of the prognostic significance of measures of fat metabolism in cancer patients. Both loss of AT and low plasma PL fatty acids are associated with ~ 2-fold shorter survival. It is likely that several metabolic factors work in concert to accelerate

fat wasting in cancer. AT loss may be an especially useful prognostic factor because it utilizes existing CT images and is minimally invasive in this vulnerable patient population. Additional research is required to mechanistically define and quantify underlying metabolic impairments that manifest as loss of AT and low plasma PL fatty acids.

In conclusion, we report that approximately 7 months from death, patients with advanced cancer begin to exhibit accelerated rates of AT loss. Decreasing plasma PL fatty acids accompany changes in AT, and both depletion of plasma PL fatty acids and AT hold prognostic value. Very little data collected on human cancer patients exists to define the status of individual fat stores, how fatty acids are metabolized, or whether there are impediments to fatty acid synthesis and utilization. This study more clearly defines the metabolic changes that lead to marked AT loss in advanced cancer and is a first step in establishing relationships between measures of fatty acids and death. This work provides the rationale for examining mechanisms underlying the evolution of cachexia with regards to fat metabolism in order to design interventions aimed at maintaining fat mass in advanced cancer patients. Mechanisms underlying depletion of essential fatty acids and identification of possible constraints to normal utilization of dietary lipid must be elucidated to ameliorate wasting and improve nutritional support for cancer patients.

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Tables

Table AP1: Characteristics of patients

	Adipose Tissue Cohort		Phospholipid Cohort	
Characteristic	Males	Females	Males	Females
N	47	61	42	30
Age (y)	62 ± 8	61 ± 9	66 ± 9	60 ± 10
BMI (kg/m²)[b]	NA	NA	23.6 ± 4.1	23.0 ± 3.8
Distribution of cancer type (*n*)				
Lung	33	36	23	24
Colorectal or GI	25	14	13	3
Other[a]	0	0	6	3

Values are mean ±SD. Measurements coincide to the closest available data for CT scan date (AT cohort) or blood draw (PL cohort). [a]Other cancers included kidney and prostate. [b]BMI not available for AT cohort.

Table AP2: Median survival times and hazard ratios for univariate and multivariate proportional hazards model to assess the effect of AT rate of change on survival for patients in the AT cohort

					Cox proportional hazards model analysis[c]					
		Survival Time (days)			Univariate			Multivariate		
AT depot	Cut-point[a] (%ΔAT /100d)	< Cut-point Median (CI)	>Cut-point Median (CI)	*P*[b]	Coefficient (SE)	HR (95%CI)	*P*	Coefficient (SE)	HR (95% CI)	*P*
IMAT	-2.1	70 (49-91)	88 (70-106)	0.1	0.31 (0.20)	1.4 (0.9-2.0)	0.11	0.40 (0.20)	1.5 (1.01-2.2)	0.05
VAT	-30.6	41 (28-54)	88 (77-99)	0.001	0.76 (0.22)	2.1 (1.4-3.4)	0.001	0.69 (0.23)	2.0 (1.3-3.1)	0.003
SAT	-32.8	46 (31-61)	88 (75-101)	0.002	0.80 (0.24)	2.2 (1.4-3.5)	0.001	0.66 (0.26)	1.9 (1.1-3.2)	0.01
TAT	-29.2	46 (31-61)	88 (73-103)	0.001	0.87 (0.22)	2.4 (1.5-3.7)	0.001	0.76 (0.24)	2.1 (1.3-3.4)	0.001

AT adipose tissue, IMAT intramuscular AT, VAT visceral AT, SAT subcutaneous AT, TAT total AT, HR hazard ratio. [a]Cut-points most significantly associated with patient mortality were defined by optimum stratification. [b]Some p-values were log-ranked. [c]Survival was not affected by sex or age. When type of cancer was included in the model, lung cancer (vs colorectal or GI) was an independent predictor of survival (HR 0.3 [0.2-0.5] p<0.001). Lung cancer (vs. colorectal) was included in the multivariate Cox proportional hazards model analysis and remained significant for IMAT (HR 0.3 [0.2-0.5] p<0.001), VAT and SAT (HR 0.3 [0.2-0.5] p<0.001) and total AT (HR 0.3 [0.2-0.5] p<0.001).

Table AP3: Amount of plasma PL fatty acids in patients with advanced cancer

Fatty acid	>238d before death (n = 36)	<238d before death (n = 36)	P^a
	(µg per ml plasma)		
LA (18:2n-6)	125 ± 63	82 ± 58	0.003
ALA (18:3n-3)	1.6 ± 2.3	0.7 ± 0.8	0.03
AA (20:4n-6)	60 ± 40	43 ± 35	0.03
EPA (20:5n-3)	5.9 ± 5.2	4.4 ± 7.8	0.01
DHA (22:6n-3)	21 ± 15	13 ± 12	0.008
Σn-6	216 ± 117	142 ± 107	0.003
Σn-3	29 ± 19	19 ± 17	0.006
ΣSFA	329 ± 158	209 ± 150	0.002
ΣMUFA	106 ± 61	69 ± 50	0.006
ΣPUFA	249 ± 134	164 ± 123	0.002
Total	686 ± 336	442 ± 316	0.002

LA linoleic acid, ALAα-linolenic acid, AA arachidonic acid, EPA eicosapentanoic acid, DHA docosahexanoic acid, ΣSFA saturated fatty acids, ΣMUFA monounsaturated fatty acids, ΣPUFA polyunsaturated fatty acids, Σn-6 omega-6 PUFA, Σn-3 omega-3 PUFA. Results are mean ± SD. Patients with phospholipid data were divided based on median time to death from time of blood sampling. [a]P-value is based on the comparison between >238d and <238d before death evaluated by unpaired t-tests when data were normally distributed and otherwise by the Mann Whitney test.

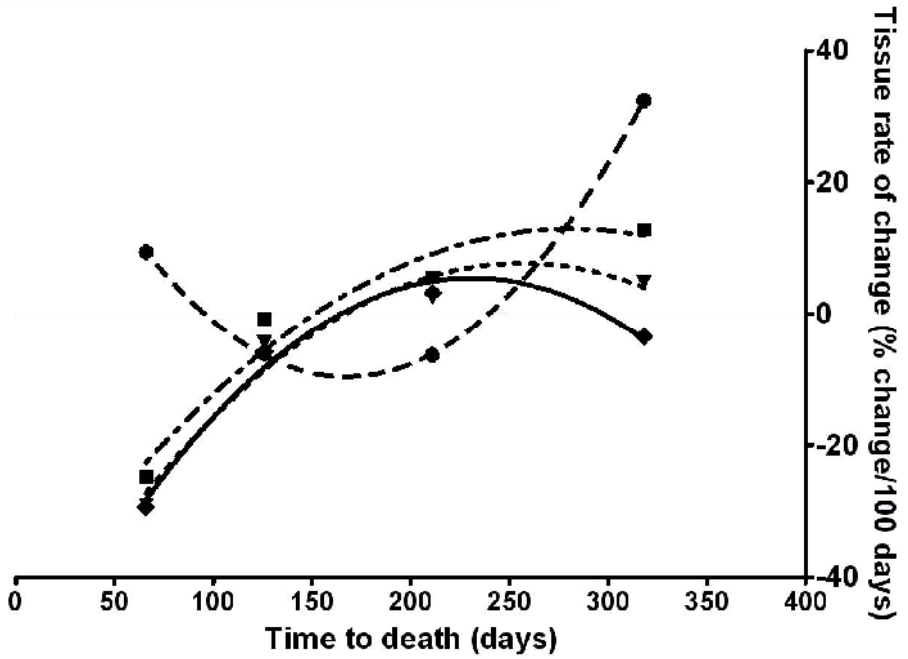
Table AP4: Median survival times and hazard ratios for univariate proportional hazards model to assess the effect of plasma PL fatty acids on survival for patients in the PL cohort

Fatty acid	Cut-point (µg/mL)	Survival Time (days)			Cox univariate[c] proportional hazards model		
		<Cut-Point Median (CI)	>Cut-point Median (CI)	*P*[a]	Coefficient (SE)	HR (95% CI)	*P*
LA	74	206 (142-271)	321 (224-418)	0.007	0.68 (0.26)	2.0 (1.2-3.2)	0.008
ALA	0.6	206 (128-285)	321(217-425)	0.35	0.52 (0.27)	1.7 (1.0-2.8)	0.05
AA	27.2	210 (92-328)	293 (206-380)	0.05	0.35 (0.24)	1.4 (0.9-2.3)	0.15
EPA	2.6	199 (121-277)	342 (302-382)	0.01	0.59 (0.24)	1.8 (1.1-2.9)	0.01
DHA	11.7	210 (134-286)	323 (260-386)	0.006	0.68 (0.25)	2.0 (1.2-3.3)	0.007
Σn-6	146.6	177 (81-273)	323 (272-374)	0.02	0.58 (0.24)	1.8 (1.1-2.9)	0.02
Σn-3	11.7	177 (93-261)	313 (228-399)	0.004	0.75 (0.27)	2.1 (1.3-3.6)	0.005
ΣSFA	154.9	141 (45-237)	321 (226-416)	0.003	0.76 (0.26)	2.1 (1.3-3.6)	0.004
ΣMUFA	77.7	210 (154-266)	323 (260-386)	0.04	0.49 (0.24)	1.6 (1.0-2.6)	0.04
ΣPUFA	170	177 (84-270)	323 (267-379)	0.02	0.55 (0.25)	1.7 (1.1-2.8)	0.02
Total	321.3	141 (45-237)	321 (226-416)	0.003	0.76 (0.26)	2.1 (1.3-3.6)	0.004

Abbreviations are as Table 2 and 3. [a]Cut-points most significantly associated with patient mortality were defined by optimum stratification. [b]Some p-values were log-ranked. [c]Univariate Cox regression model used, no multivariate model was required as sex, type of cancer, BMI and age were not significant predictors of survival.

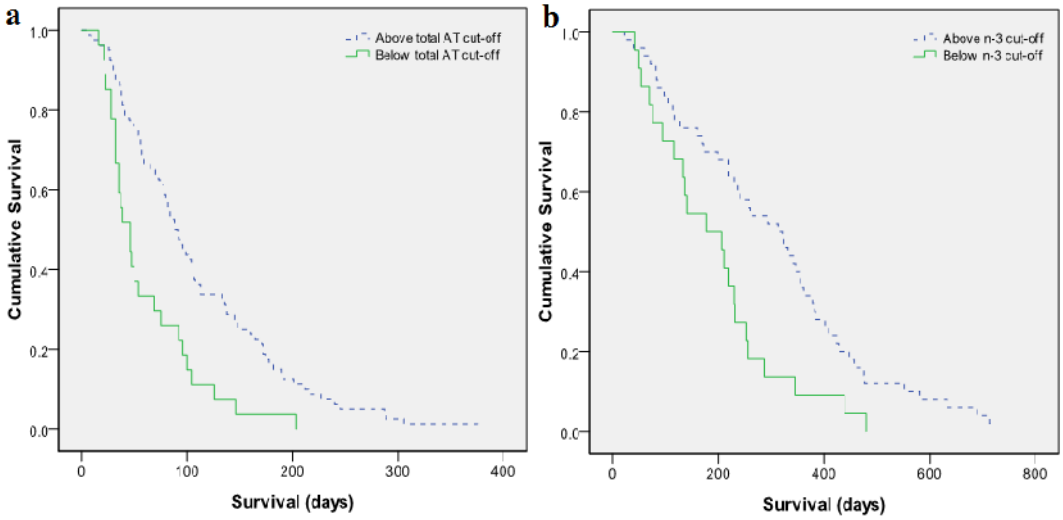
Figures

Figure AP1: Time course rates of change in AT depots (n=104)



Scan intervals were categorized for IMAT (● dashed curve), VAT (■ dashed curve), SAT (♦ solid curve) and total AT (▲ dotted curve) relative to the time of death and divided into 4 categories. For each time point, mean rates of change were calculated normalized to %/100 d. Best-fit regression lines were polynomial and were used to approximate the trajectory of AT loss until death. The rate of changes are: IMAT %change/100 d =0.0019(time to death, in d)^2 – 0.6213(time to death, in d) +42.52, VAT %change/100 d = -0.0008(time to death, in d)^2 + 0.4339(time to death in d) -47.996, SAT %change/100 d = -0.0012(time to death, in d)^2+ 0.5752(time to death in d) -60.919 and total AT %change/100 d = -0.001(time to death, in d)^2 + 0.5752(time to death in d) -60.919.

Figure AP2: Kaplan Meier survival curves for patients in the a) AT cohort (n=108) and b) PL cohort (n=71)



Patients in the AT cohort with total adipose tissue above the level associated with an increased risk of mortality obtained by optimum stratification (29.2% total AT/100 days). Patients in PL cohort with n-3 fatty acids above or below the level associated with an increased risk of mortality obtained by optimum stratification (11.7μg/ml).

Appendix 5: Health Science Inquiry manuscript

Advancing cancer treatment: a move towards individualized therapy

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In Canada, cancer has surpassed heart disease as the leading cause of death.[1]The incidence of cancer and cancer-related deaths is increasing.[1] This is likely to continue to increase in the coming years given our aging population and the fact that cancer primarily affects people over the age of 50.[1] Each year, the government and voluntary sectors spend over $400 million on cancer research in Canada.[2] Although progress has been made in the treatment of certain types of cancer, we are still far from being able to offer all patients effective treatment.

The primary goal of an oncologist is to recommend the most effective cancer treatment available. However, it is difficult to predict patient response or resistance to therapeutic agents. In order to maximize benefits from treatment, specifically to improve quality of life and to prolong survival, we must understand and address variability in treatment response. Molecular differences in malignant tissue may explain some of the heterogeneity in treatment response and provide novel treatment targets.

Currently, patients receive standardized anti-neoplastic therapy according to tumour histology and disease stage. Advancements in molecular profiling and drug development have led to the possibility of individualizing treatment according to the molecular characteristics of a patient's tumour. These new therapies target specific cellular features that are essential for tumour growth or

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survival. Due to the specific nature of these therapies, the side effects from targeted therapy are often milder than conventional anti-neoplastic treatments.[3] Together, molecular profiling and targeted therapy may improve upon standardized treatment by identifying molecular characteristics associated with response or resistance to therapeutic agents.

Targeted therapy in lung cancer is an area of intense research due to low efficacy of standard chemotherapy. The drugs erlotinib and gefitinib, which inhibit tyrosine kinase activity in the epidermal growth factor receptor (EGFR), are examples of targeted therapy. Response to these therapies has been associated with somatic mutations in the tyrosine kinase region of EGFR which are particularly prevalent in Asian women with no smoking history who develop adenocarcinoma of the lung.[4,5] Thus, erlotinib and gefitinibare most effective in this particular population.[6] Molecular profiling may also be useful for selecting the most effective treatment for patients without EGFR mutations as these patients have been shown to benefit from standard chemotherapy compared to gefitinib.[7]

Similarly, targeted therapies have been successful in improving treatment for breast cancer. Approximately 20% of patients with breast cancer overexpress a growth factor receptor gene, human epidermal growth factor receptor (HER2), which is associated with aggressive disease and higher risk of cancer recurrence.[8] The development of trastuzumab, a monocolonal antibody which interferes with the HER2 receptor, has resulted in longer progression-free survival and significant improvements in survival in HER2-positive breast cancer patients.[9]

This represents a major advancement in treatment of breast cancer and has contributed to a 25% decline in mortality from breast cancer in Canadian women over the last two decades.[1]

Although targeted therapy seems promising, there are concerns about the feasibility of an individualized approach to cancer treatment. These concerns are centered on obtaining and characterizing tumour biopsies in a timely manner. However, these concerns may be unfounded as a recent study in advanced cancer patients obtained tumour biopsies for all study patients (n=86) from 9 different cancer centers.[10] Molecular profiling was then used to identify treatment targets and to select treatment regimens. All patients had refractory disease, having previously failed to respond to chemotherapy. Despite this, 27% of patients had longer progression-free survival with individualized treatment compared to their previous treatment regimens. Marked differences between therapies that would have been recommended by the patients' oncologist in the absence of molecular profiling were also reported. This study not only demonstrates that individualized cancer therapy is feasible but that it may also represent an improvement over standard treatment.

Current knowledge of the complex interactions between specific gene expression and targeted treatment is evolving, as is molecular profiling technology. Great advancements in treatment have already been made with the advent of targeted molecular agents such as trastuzumab, gefitinib and erlotinib. These agents have fewer side effects and provide more effective disease control than standardized therapy in subgroups of patients. Although thus far, molecular

agents are most effective in well-defined subsets of patients, further development of targeted therapies will open new avenues in treatment for broader populations. Continued research and development of novel molecular targets and treatments are needed, but the encouraging results to date suggest that individualized anti-neoplastic therapy holds promise for advancing the treatment of cancer.

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