General introduction and outline of the thesis
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Cancer
The occurrence and burden of cancer is progressively increasing. Although worldwide cardiovascular diseases remain the primary cause of death, in the US and in some European countries (e.g. France, Denmark and the Netherlands) cancer has become the leading cause of death. In 2008, there were an estimated 12.7 million cancer cases worldwide, and this number is expected to be 21 million in 2030. Cancer occurs more frequently in men, in older people and in more developed countries. Lung cancer was the most common cancer, and breast cancer and colorectal cancer were the second and third most common types of cancer in 2008 (1).

Cancer survival depends on a number of factors. Most important are the type of cancer, the stage at which cancer is diagnosed and whether treatment is available. Groups with the lowest 5-year survival rates are patients with cancer of the pancreas (5.6%), liver (13.8%), lung, bronchus and trachea (15.8%) and oesophagus (17.0%) (1).

This thesis focuses on two groups of cancer patients who encounter many nutritional issues and complications during their treatment trajectory: patients with stage III non-small cell lung carcinoma (NSCLC) receiving concurrent chemoradiotherapy and patients with haematological malignancies, having Graft-versus-Host disease (GVHD) after allogeneic hematopoietic stem cell transplantation.

Cancer treatment
Treatment options for cancer include local treatments, such as radiation therapy and surgery, and systemic treatments, such as chemotherapy and targeted therapy. Cancer treatment has many side effects. Chemotherapy damages not only cancer cells, but also healthy fast-growing cells in the human body, such as blood-forming cells in the bone marrow, hair follicles and cells in the mouth, digestive tract and reproductive system. As a result, common side effects of chemotherapy are anaemia, fatigue, increased chance of bruising, bleeding and infection, hair loss, nausea and vomiting, constipation, diarrhoea, oral complications, nerve and muscle problems and skin and nail changes. Side effects of radiation therapy include fatigue, hair loss, anorexia and digestive problems. Radiation can also damage normal cells around the tumour side, and cause lymph oedema, bladder or bowel problems. Radiation to the chest area may affect the lungs or the heart.

Treatment modalities may be used alone or in combination, the latter of which appears to
be more effective, but increase the occurrence of treatment-related toxicities. Examples of multimodal treatments include (neo-)adjuvant chemotherapy with concurrent radiation therapy, followed by surgical resection of the tumour. There is increasing emphasis worldwide on the development of specialized cancer centres that apply evidence-based multimodal therapies and provide rehabilitation and palliative care.

For lung cancer, treatment options are determined by the type of lung cancer (small cell, non-small cell) and stage at diagnosis. Treatments include surgery, radiation therapy, chemotherapy and targeted therapies. For localized cancers, surgery is usually the treatment of choice. Recent studies indicate that survival with early stage NSCLC is improved by chemotherapy following surgery. In case of metastatic disease, radiation therapy and chemotherapy are often used, sometimes in combination with surgery (2).

For hematologic malignancies, treatment can occasionally consist of ‘watchful waiting’ or symptomatic treatment (e.g. blood transfusions). The more aggressive forms of disease require treatment with high-dose chemotherapy, immunotherapy, radiotherapy, and in some cases hematopoietic stem cell transplantations (HSCT). In autologous HSCT, stem cells of the patients are harvested, and given back to the patient after high dose chemotherapy and/or radiation therapy. Allogeneic HSCT uses stem cells of a matched donor to give to the patient after high dose chemotherapy and/or total body irradiation (Figure 1). After HSCT, in particular allogeneic HSCT, there is a high risk of graft failure, infections from the donor cells, and serious infections due to immunosuppression, often resulting in death. An important complication after allogeneic HSCT is GVHD, when T cells in the donor bone marrow graft recognize the host antigens and cause damage to the host’s tissues, most often the skin, liver, stomach and/or intestines (3-5).

Metabolic alterations in cancer

Cancer cells have different metabolic requirements from their normal counterparts and cause marked alterations in the carbohydrate, fat and protein metabolism of the host. Most cancer cells use glycolysis as the principal method to generate ATP. The high glucose uptake by the tumour leads to significantly higher rates of glucose production and recycling by the liver. Insulin resistance, which is probably related to inflammation, also occurs in cancer.

There is an increased turnover of both glycerol and free fatty acids: these changes lead to loss of body fat. An increased whole body protein turnover, caused by a depressed protein
synthesis in skeletal muscle, as well as an increase in protein degradation, leads to muscle wasting (6, 7).
Elevations in the resting energy expenditure (REE) in patients with cancer have been observed in patients with oesophageal, gastric, pancreatic and NSCLC patients (8-12), but not in patients with colorectal and haematological malignancies (8, 12, 13). Several studies showed that tumour localisation and the presence of a systemic inflammatory response contribute to the increased REE (10, 11, 13). Due to a decreased physical activity level of patients with cancer, total energy expenditure is not necessarily higher (14, 15).

Cancer-related malnutrition and cachexia
Involuntary weight loss is a common, well-known issue in patients with cancer, which (among others) can be caused by a diminished food intake, increased gastrointestinal losses, and increased energy needs. The terms weight loss, malnutrition and cachexia are often mixed up, in clinical practice as well as in the literature.
Malnutrition is defined as ‘a state of nutrition in which a deficiency of energy, protein and/or other nutrients causes measurable adverse effects on tissue / body form, composition, function or clinical outcome’ (16). Malnutrition can be further categorised into chronic starvation without inflammation and chronic conditions associated with a sustained inflammatory response (17).

Cachexia is derived from the Greek words ‘kakos’ and ‘hexis’, meaning ‘bad condition’. According to the dictionary, cachexia means ‘general ill health with emaciation, usually occurring in association with cancer or a chronic infectious disease’. Cachexia is characterised by a systemic inflammatory response induced by humoral pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF-alpha), C-reactive protein (CRP), leading to hypermetabolism and progressive weight loss (18-21). The tumour-derived proteolysis-inducing factor (PIF) also induces muscle wasting (18, 22, 23).

Recently, international experts proposed a universal definition for cancer cachexia: a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment (24). The experts also agreed upon criteria to diagnose cachexia and distinguished three stages of cachexia: precachexia, cachexia and refractory cachexia. Precachexia, the initial stage of cachexia, is characterized by early clinical and metabolic signs such as anorexia and inflammation. These signs precede substantial (≥ 5%) involuntary weight loss, and may hypothetically be halted by early multimodal intervention. In the next stage, the combination of weight loss greater than 5%, or weight loss greater than 2% in individuals already showing depletion (BMI < 20 or low muscle mass) indicates cachexia. For patients with cachexia, experts propose a multimodal treatment plan, which includes nutrition, physical exercise and anti-inflammatory strategies. Refractory cachexia is the result of advanced or rapidly progressive cancer, and is associated with active catabolism, a low performance status and a short life expectancy. In this stage, only symptom-control interventions are appropriate (24).

Apart from the degree of weight loss, anorexia and inflammation, other clinical parameters need to be evaluated to investigate the stage and severity of cachexia and to decide on clinical management, e.g. food intake, catabolic drive (inflammation, REE, insulin resistance, use of steroids), muscle mass and strength, functional and psychosocial impairment.

Many cross-sectional studies investigated nutritional status in cancer populations at a certain time point after diagnosis. These studies showed that weight loss, malnutrition and cachexia occur most often in patients with gastric, pancreatic and lung cancer, and
more frequently in patients with metastatic disease and anorexia (25-29).
Studies in patients with haematological malignancies showed that weight loss or
malnutrition was observed at diagnosis (43) and during chemotherapy (30, 33, 44) or HSCT
(30). Nutritional parameters do not always return to pre-treatment levels (30,31, 32). In
particular patients with complications showed significant weight loss, muscle wasting, and
an increase in body fat mass during follow-up after HSCT (31, 33, 34). In patients with
Graft-versus-Host disease, but also in other patients with cancer, the use of steroids is an
important cause of muscle atrophy.

*Consequences of malnutrition and cachexia*
Weight loss, malnutrition and cachexia are associated with reduced survival, treatment
tolerability, performance status, quality of life and increased occurrence of complications
in patients with cancer (18, 20, 35-40). In patients with lung cancer, malnutrition
correlated with increased chemotherapy-induced toxicity (28) and decreased response to
first-line therapy (27), time to progression and survival (22, 27, 29). Furthermore, low
muscle mass in patients with lung cancer was related to toxicity and shorter time to
progression and shorter survival (41, 42).
In patients with hematologic malignancies, malnutrition during chemotherapy was
associated with impaired quality of life (30) and a higher risk of complications and relapses
(44). Malnutrition at diagnosis (43) or during treatment (45) was a prognostic factor in the
long-term outcome in paediatric patients with acute lymphoblastic leukaemia. Low muscle
mass was associated with low muscle strength, fatigue, and health-related quality of life in
patients before allogeneic HSCT (46).
Although it is hard to distinguish cachexia from malnutrition, typical features of cachexia,
such as systemic inflammation and anorexia, have been correlated with adverse prognosis
(18, 20, 27, 47, 48).
Thus, weight loss, cachexia, and muscle wasting are present in patients with lung cancer
and hematologic malignancies, and reduce quality of life, survival and increase the risk of
complications. It would be interesting to know whether nutritional support and
supplementation of fish oil could improve treatment and outcomes in these patient
populations.
Nutritional support in cancer

Nutritional support in cancer patients is expected to help patients to maintain nutritional intake and nutritional status in the presence of symptoms. Simple interventions consist of strategies to increase food intake through individual advice or by provision of additional foods such as snacks, the fortification of foods, and the prescription of energy- and protein dense oral nutritional supplements, containing high amounts (± 33% of RDA per 200 mL) of vitamins and minerals (49). The efficacy of nutritional interventions during cancer treatment, HSCT and palliative care has been examined in a number of systematic reviews (50-53).

The European society for clinical nutrition and metabolism and the American Society for Parenteral and Enteral Nutrition (ASPEN) have made recommendations for the management of nutritional issues in cancer patients (54-56). These guidelines concluded that there is only weak evidence suggesting that patients with cancer and those undergoing hematopoietic stem cell transplantations are nutritionally-at-risk and should undergo nutrition screening. There is fair evidence suggesting that nutrition support therapy is appropriate in patients receiving active anticancer treatment or undergoing hematopoietic stem cell transplantation who are malnourished and who are anticipated to be unable to ingest and/or absorb adequate nutrients for a prolonged period of time (55). However, there was no evidence for beneficial effects on clinical outcomes or quality of life using routine nutrition support therapy as an adjunct to chemotherapy. A recently published, well-designed study in patients with advanced gastrointestinal and lung cancer showed that simple nutritional interventions during chemotherapy did not improve clinical or nutritional outcomes or quality of life; weight gain predicted a longer survival, but occurred independently of nutritional intervention (57). The patients in this study did have a short life expectancy; so refractory cachexia may have been responsible for the lack of effect in this study. So far, there have been no large well-designed studies that have proven the opposite. Only small studies showed that routine dietary counselling and nutritional supplementation during cancer treatment improved energy and protein intake and quality of life in patients with colorectal and head and neck cancer (58-61).

Due to mucositis, thrombocytopenia and gastrointestinal toxicities, parenteral nutrition is often required around HSCT. It is recommended to discontinue TPN as soon as toxicities have resolved after stem cell engraftment, and to use enteral nutrition in patients with a functioning gastrointestinal tract but with insufficient oral intake (55). A retrospective study in 120 patients receiving autologous HSCT showed that multidisciplinary nutritional support (by a dietician, hemato-oncologist, nurse, and a pharmacist) reduced the duration of absence of oral food intake, TPN, hospitalization and therapeutic antibiotic usage, and
decreased the total cost of hospitalization (62). Only a few expert opinions on nutrition requirements and nutritional support in patients with Graft-versus-Host disease exist (63, 64). These guidelines focus on interventions recommended for various eating problems (63), indications for enteral and parenteral nutrition (63, 64), and the role of immunonutrition (64).

**Fish oil**

Since the late 1990s, fish oil supplementation has attracted attention as a new method to decrease protein degradation and anorexia in patients with cancer. Fish oil is rich in long-chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). PUFAs play a key role in inflammation, as very long chain PUFAs produce eicosanoids (prostaglandines, thromboxanes, leukotrienes and other oxidized derivatives), important mediators and regulators of inflammation (68-70).

Because inflammatory cells typically contain a high proportion of the n-6 PUFA arachidonic acid (20:4n-6) and low proportions of other 20-carbon PUFAs, arachidonic acid is usually the major substrate for eicosanoid synthesis. Increased consumption of long-chain n-3 PUFAs results in increased proportions of those fatty acids in inflammatory cell phospholipids at the expense of arachidonic acid. After fish oil supplementation, less substrate is available for the synthesis of eicosanoids from arachidonic acid, resulting in decreased production of mediators from AA, such as PGE2, thromboxane B2, and LTB4, and increased production of LTBS, LTE5, and 5-hydroxyeicosapentaenoic acid by inflammatory cells. The mediators formed from EPA are believed to be inflammatory than those formed from arachidonic acid (69, 70) (**Figure 2**).

In addition to long-chain n-3 PUFAs modulating the generation of eicosanoids, recent studies have identified a novel group of mediators, termed E-series resolvins, formed from EPA by COX-2, that appear to exert anti-inflammatory actions. In addition, DHA-derived mediators termed D-series resolvins, docosatrienes and neuroprotectins, also produced by COX-2, have been identified and also appear to be anti-inflammatory.

Clinical studies have suggested that EPA can promote weight gain and downregulate the acute phase protein response in patients with cancer (18, 71). Supplementation of the diet of healthy humans with fish oil providing > 2 g EPA + DHA per day was shown to decrease the production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-8)(70). Clinical studies showed beneficial effects of n-3 PUFAs on inflammation and disease activity in patients with rheumatoid arthritis, inflammatory bowel diseases and asthma (70, 72-74). EPA also prevented muscle wasting in mice by blocking PIF and possibly by inhibiting the production of prostaglandin E2 (23, 75).
The first clinical studies in cancer found promising effects of EPA and DHA on pro-inflammatory cytokine production, protein degradation and resting energy expenditure (71, 76-78). However, consecutive studies investigating oral (79-84), enteral (85-87) or parenteral (88-90) supplementation in cancer patients found inconsistent effects on clinical outcome parameters. As such, EPA is still not used as a standard nutritional therapy for patients with cancer. Oral fish oil around bone marrow transplantation appeared to have beneficial effects on survival and the severity of Graft-versus-Host disease (91, 92). Fish oil may have a therapeutic effect in Graft-versus-Host disease, but due to intestinal malabsorption, parenteral supplementation would be indicated. So far, no clinical studies on the supplementation of fish oil in patients with Graft-versus-Host disease have been published.

Figure 2: Pathway of the conversion of essential n-3 (alpha-linolenic acid) and n-6 (linoleic acid) PUFAs to their longer chain derivatives.
**Aim and outline of this thesis**

In summary, the occurrence of cancer is increasing worldwide, and cancer treatment can have many short- and long-term side effects. At any time through the treatment trajectory, or even before diagnosis, the nutritional status of patients with cancer can be affected. Deteriorations of nutritional status can range from a loss of body weight to (refractory) cachexia, and are associated with a worse outcome and quality of life. Conventional nutritional interventions appear to be ineffective on functional outcomes. Because of the immune-modulating effects of n-3 fatty acids from fish oil, we were interested to investigate effects of a supplementation of n-3 fatty acids on the clinical outcome in two high-risk patient populations: patients with lung cancer undergoing concurrent chemoradiotherapy, and patients with Graft-versus-Host Disease (Figure 3). Pre-clinical studies and time series point to immune-modulating and anti-cachectic effects of n-3 PUFAs from fish oil. However, clinical studies in patients with cancer have not consistently shown clinical benefits and it remains unclear when and to whom caregivers should supplement n-3 PUFAs.

The general aim of this thesis was to investigate the effects of enteral and parenteral supplementation of fish oil (omega-3 or n-3 fatty acids) on clinical outcomes in patients with NSCLC undergoing concurrent chemoradiotherapy, and those with Graft-versus-Host Disease after allogeneic HSCT.

**Chapter 2** describes a systematic literature review on the clinical effects of supplementation of omega-3 fatty acids in three patient populations with an inflammatory state; cancer, surgery and critical care patients. In this review, we summarised randomised controlled trials investigating the effects on clinical outcome of enteral and parenteral supplementation of n-3 fatty acids (by fish oil capsules, oral nutritional supplements or tube feeding). In addition, we described the incorporation and subsequent washout of n-3 fatty acids into phospholipids of plasma, blood cells, and mucosal tissue in response to enteral and parenteral supplementation of n-3 fatty acids.

In **Chapter 3** we studied the presence of precachexia and cachexia in patients at diagnosis of stage III NSCLC, and described the relationship with quality of life and survival. In **Chapter 4** and **Chapter 5** we describe the outcomes of a double-blinded RCT on an intervention with oral nutritional supplements containing n-3 fatty acids on nutritional status, functional status and quality of life in patients with stage III NSCLC during multimodality treatment. In **Chapter 6** all randomized controlled trials and meta-analyses comparing effects of oral or enteral supplementation of n-3 fatty acids were used to describe the latest evidence for supplementation of n-3 polyunsaturated fatty acids in
patients with cancer receiving palliative care, anticancer treatment or surgery.

The predictive value of nutritional parameters on postoperative morbidity, mortality, and survival in patients with stage III NSCLC receiving multimodality treatment was studied in Chapter 7. Graft-versus-Host disease of the digestive tract, a severe complication of allogeneic HSCT, has a major impact on nutritional status, quality of life, morbidity and mortality. Knowledge of the management of these patients is limited. Chapter 8 describes a critical appraisal of the literature on nutritional assessment, nutritional status and nutritional support for patients with Graft-versus-Host disease of the digestive tract, concluding with clinical recommendations. Chapter 9 describes a pilot study on feasibility and safety of intermittent fish oil infusions in outpatients with chronic Graft-versus-Host disease of the digestive tract, and the dose-response effects on fatty acid composition of plasma lipids and white blood cells.

In Chapter 10 the main findings of our studies are summarised, methodological issues are discussed, and implications for future research are described.
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General introduction

n-3 PUFAs in cancer, surgery, and critical care: a systematic review on clinical effects, incorporation, and washout of oral or enteral compared with parenteral supplementation

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MA van Bokhorst-de van der Schueren
JAE Langius
IA Brouwer
PAM van Leeuwen

Abstract

Background
n–3 (omega-3) Fatty acids (FAs) may have beneficial effects in patients with cancer or in patients who undergo surgery or critical care.

Objective
Our aim was to systematically review the effects of oral or enteral and parenteral n–3 FA supplementation on clinical outcomes and to describe the incorporation of n–3 FAs into phospholipids of plasma, blood cells, and mucosal tissue and the subsequent washout in these patients.

Design
We investigated the supplementation of n–3 FAs in these patients by using a systematic literature review.

Results
In cancer, the oral or enteral supplementation of n–3 FAs contributed to the maintenance of body weight and quality of life but not to survival. We did not find any studies on parenteral supplementation of n–3 FAs in cancer. In surgical oncology, we did not find any studies on enteral supplementation of n–3 FAs. However, postoperative parenteral supplementation in surgical oncology may reduce the length of a hospital stay. For general surgery, we did not find any studies on enteral supplementation of n–3 FAs, and evidence on parenteral supplementation was insufficient. In critical care, enteral supplementation of n–3 FAs had beneficial effects on clinical outcomes; evidence on parenteral supplementation in critical care was inconsistent. The incorporation of n–3 FAs in plasma and blood cells was slower with enteral supplementation (4–7 d) than with parenteral supplementation (1–3 d). The washout was 5–7 d.

Conclusions
This review shows the beneficial effects of n–3 FA supplementation in cancer, surgical oncology, and critical care patients. Supplementation in these specific patient populations could be considered with the route of administration taken into account.
Introduction

n-3 (omega-3) FAs frequently attract the attention of medical experts, scientists, and consumers. The main n-3 FAs are EPA (20:5 n-3), DHA (22:6 n-3), and α-linolenic acid (18:3 n-3). EPA and DHA are mainly in fish and seafood, and α-linolenic acid in flaxseed, canola, soy, perilla, and walnut oils and is an essential FA for humans. In several animal and human studies, an increased consumption of dietary n-3 FAs resulted in a decreased proportion of AA and an increased proportion of EPA in membrane phospholipids of immune cells (1, 2), which caused immunomodulation, less inflammation, and attenuation of cachexia (3, 4). In healthy volunteers, the immunomodulation of effects continued for ~10 wk after the end of supplementation (5). Clinical anti-inflammatory effects of n-3 FAs have been documented in several chronic inflammatory conditions including rheumatoid arthritis and inflammatory bowel diseases (6). In cancer, n-3 FAs may have positive effects on cachexia and survival (3, 4, 7, 8), but evidence from randomized clinical trial was equivocal and results of additional trials are awaited (9, 10). Likewise, supplementation of n-3 FAs in other highly inflammatory circumstances, such as in surgery, sepsis, and ARDS, appears to have beneficial effects (11). Cancer, surgery, and critical care are areas with a high potential for the application of n-3 FAs in hospitals. Our objective was to systematically review effects of oral or enteral and parenteral n-3 FA supplementation on clinical outcomes in patients with cancer who underwent surgery or critical care. Second, knowledge on the incorporation into phospholipids of plasma, blood cells, and mucosal tissue and the subsequent washout after oral or enteral and parenteral n-3 FA supplementation in these patient populations is extremely limited. Therefore, we also reviewed this area.

Methods

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement was followed (12).

Eligibility criteria

Types of studies

To address the primary research objective to study the supplementation of enteral and parenteral n-3 FA supplementation, we included only RCTs. RCTs were either double-blinded, single blinded, or nonblinded, performed in a hospital or community setting, and studied the supplementation of oral or enteral and parenteral n-3 FA supplementation.
For the secondary research objective on the incorporation and washout of n-3 FAs, we included controlled and noncontrolled studies.

*Types of participants*
Types of participants included adult human subjects with any type of cancer who received chemotherapy and/or radiotherapy or palliative care, subjects who underwent elective surgery (abdominal, head and neck, or gastrointestinal), and subjects who received critical care (defined as admission to the medical ICU after a diagnosis of systemic inflammatory response syndrome, sepsis, ARDS, acute lung injury, or surgery).

*Types of intervention*
For the primary research objective, we included studies that compared supplementation of n-3 FAs to a control or placebo intervention. The means of n-3 FAs were fish-oil capsules, ONSs, and enteral or parenteral nutrition. We excluded studies with dietary interventions of multiple immune-enhancing compounds (eg, arginine, glutamine, nucleotides, and n-3 FAs) or studies with concurrent use of appetite stimulants. For the secondary research objective, we included controlled and noncontrolled studies that investigated supplementation of n-3 FAs by fish-oil capsules, ONSs, and enteral or parenteral nutrition.

*Types of outcome measures*
For the primary research objective, we defined the following outcome measures: nutritional status (body weight, LBM, mid-upper arm circumference, and appetite), morbidity, mortality, length of hospital stay, length of ICU stay, and quality of life. Quality-of-life variables included symptoms (eg, fatigue), physical function, and performance status, which were measured by validated self-administered questionnaires or classification methods for clinicians.
For the second research objective, we included studies that reported on the incorporation and washout of n-3 FAs in phospholipids of plasma, blood cells, and mucosal tissue.
Table 1. MeSH and key words used to search for publications in PubMed

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<tr>
<th>Subject</th>
<th>MeSH (medical subject headings) and keywords</th>
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<tr>
<td>Morbidity</td>
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</tr>
<tr>
<td>Mortality</td>
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</tr>
<tr>
<td>Length of stay</td>
<td>length of stay OR LOS OR “length of stay”[MeSH Terms]</td>
</tr>
</tbody>
</table>

29
Search methods for identification of studies
We searched the electronic databases PubMed (www.pubmed.com) and EMBASE (www.embase.com). In PubMed, we used MeSH and key words to select relevant studies (Table 1).
In PubMed, we applied limit criteria to search for publications in humans published in English. Subsequently, we searched EMBASE for additional publications that were not retrieved by PubMed. In EMBASE, we used Emtree expansion searches, key words, the map to preferred terminology option, explosion searches and the study-type filter (i.e., RCT). We searched for studies published from the start date of the electronic databases (1948 for PubMed and 1986 for EMBASE) until 1 April 2011.
Search terms for n-3 FAs and enteral and parenteral nutrition were combined with search terms for cancer, surgery, critical care, and the respective primary and secondary outcome variables (Table 1).
In addition, reference lists of included publications were inspected for references that were not retrieved by the database search.

Data collection and analysis
Selection of studies
We excluded studies if the title and abstract were not relevant; to achieve consensus, researchers discussed all areas of disagreement.

Data extraction and management
Both reviewers read the selected articles. One reviewer extracted the following data from each study with the use of data extraction forms: study design, characteristics of trial participants (number, condition, and treatment), and the type of n-3 FA supplementation or control intervention (duration, form and daily dose, and results on primary and secondary outcome measures). After extraction, a second reviewer checked the extracted data to minimize the possibility of errors. We only used data from original publications. For studies published more than once, we used the publication that reported at least one relevant outcome measure in the largest study population.

Assessment of risk bias in included studies
Two researchers independently, who were not blinded to authors or journals, assessed risk of bias in studies that met the inclusion criteria. We used the Jadad instrument (0–5-point rating scale, with 5 as the maximum score for methodologic quality) (13) and the
Table 2: Quality assessment and grading of clinical trials

<table>
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<th>Jadad instrument</th>
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<td>No: +0</td>
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<tr>
<td>Was the study described as double blind?</td>
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<tr>
<td>Was there a description of withdrawals and dropouts?</td>
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<td>No: +0</td>
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<tr>
<td>Was the method to generate the sequence of randomization described and was it appropriate? and/or</td>
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<td>No: -1</td>
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<td>Was the method of double blinding described and was it appropriate?</td>
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<td>Cochrane concealment assessment</td>
<td>A: Adequate</td>
</tr>
<tr>
<td></td>
<td>B: Uncertain</td>
</tr>
<tr>
<td></td>
<td>C: Clearly inadequate</td>
</tr>
<tr>
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<td>D: Not used</td>
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Quality Grading

| 0 or 1 A-D                     | Poor  |
| 2 A-D                          | Average |
| 3 A-D                          | Good  |
| 4 or 5 A-D                     | Excellent |

Cochrane concealment assessment criteria for risk of bias, which included the following criteria: adequacy of randomization, sequence generation, and blinding of participants, personnel, and outcome assessors; addressing of incomplete outcome data; and allocation concealment. Each study was graded to be of poor (0 or 1 A–D), average (2 A–D), good (3 A–D), or excellent (4 or 5 A–D) quality (Table 2). For the literature study on primary outcome measures, studies with 0 points were excluded (13). Disagreements between researchers were discussed and solved by consensus.

Results

Clinical outcome

Twenty-eight of 201 potential studies on effects of n-3 FA supplementation on clinical outcomes met our predefined inclusion criteria as follows: 8 RCTs in cancer patients, 13 RCTs in patients who underwent surgery, and 7 RCTs in patients who received critical care.
Chapter 2

(Figure 1). Trials used fish-oil or placebo capsules (3, 14–16), ONSs (17–20), enteral nutrition (21–25), ONSs followed by enteral nutrition (26), or parenteral lipid emulsions as part of the total parenteral nutrition (27–40) (Tables 3–5).

Cancer (nonsurgical oncology)

Oral or enteral supplementation of n-3 FAs

We included the following 8 studies in cancer patients: 2 studies were performed during chemotherapy (15, 18), one study was performed during chemoradiotherapy (20), one study was performed after hospital discharge for oral or laryngeal cancer surgery (19), and 4 studies were performed in palliative-care patients (3, 14, 16, 17) (Table 3). In one study in patients who received chemotherapy and bone marrow transplantations, the intervention consisted of fish-oil capsules (15), and in 3 studies (18–20), the intervention consisted of ONSs that contained n-3 FAs (~2 g EPA) compared with an isocaloric control supplement. In palliative-care patients, the intervention consisted of fish-oil capsules in 3 studies (3, 14, 16) and ONSs in one study (17).

Risk of bias in included studies

The quality of included studies was shown to range from poor to excellent; studies that were conducted during chemotherapy were both nonblinded and of poor (18) or average (15) quality. The one study conducted during chemoradiotherapy was of excellent quality (20). In palliative care, the quality was excellent in 2 large-scale studies (14, 17); 3 small, nonblinded studies were of average (16) and poor (3, 19) quality.

Clinical outcome

Seven studies reported on body-weight changes. One study showed a beneficial effect (20), whereas 4 studies did not show a beneficial effect (3, 16, 17, 19). A trend for a beneficial effect was observed in one study (14). Finally, one study reported weight maintenance in the intervention group, whereas body weights in the control group decreased. However, this study failed to report changes compared with those with the placebo (18). Five studies measured the effects of n-3 FA supplemented by ONSs on LBM. One small study observed a significant maintenance of LBM (20), and 2 studies showed a nonsignificant maintenance compared with that of a control intervention (14,17); 2 studies showed no effects on LBM after supplementation of n-3 FAs during 2 (16) and 12 (19) wk.
Quality of life was an endpoint in 4 studies. In lung cancer patients, improvements of quality of life variables in the n-3 FA group were shown in time (18). In a large-scale, double-blinded RCT, patients who received n-3 FAs showed a tendency for a better maintenance of physical functioning than did control patients (14). In a post hoc analysis of the Fearon et al (14) study, weight gain was associated with an improved quality of life in the n-3 FA group (17). One trial of a 2-wk supplementation of n-3 FAs via fish-oil capsules did not find improvements in wellbeing, but there was a trend for a reduction of tiredness in the n-3 FA group (16).

With regard to Karnofsky Performance Status, no differences between n-3 FAs and control patients were observed in one large excellent-quality trial (14) and one small average-quality trial of 2 wk (16). One small poor-quality trial showed an increase of Karnofsky Performance Status in a subgroup of malnourished patients who received n-3 FAs (3). Morbidity was studied in 2 trials; one study observed less graft versus host disease in patients who received n-3 FAs around bone marrow transplants (15), and a study in patients with head and neck cancer did not observe differences for complications between n-3 FAs and the control group (19).

Mortality was reported on in 5 studies: 2 small RCTs (of poor and average quality) showed beneficial effects on survival in bone marrow–transplant patients (15) and in patients with various types of cancer (3), whereas 2 high-quality RCTs (14, 17) and one small study (19) did not show such beneficial effects.

In summary, we showed some evidence for beneficial effects of oral supplementation of n-3 FAs for 5–8 wk on body weight (but not on LBM) and quality of life in cancer patients during chemo(radio)therapy and in palliative care. Effects on Karnofsky Performance Status and survival were inconsistent.

**Parenteral supplementation of n-3 FAs**

We showed no RCTs that investigated the effects of parenteral supplementation of n-3 FAs in nonsurgical oncology.

**Surgery: surgical oncology**

**Oral or enteral supplementation of n-3 FAs**

We identified 3 studies that applied enteral supplementation of n-3 FAs in surgical oncology in patients with gastrointestinal (24, 25) and esophageal cancer (26) (Table 4). Studies applied ONSs (26) and/or enteral nutrition (24–26).
Risk of bias in included studies
In a quality appraisal, we showed 2 trials to be of average (24, 25) methodologic quality because of a lack of details on randomization (24) and blinding (24) procedures or details on withdrawals and dropouts (24, 25). Only the study in esophageal cancer patients was shown to be of excellent methodologic quality (26).

Clinical outcome
One study reported on perioperative body weight and LBM changes in esophageal cancer patients and showed a postoperative maintenance of body weight and LBM, whereas this effect decreased in the control group (26).
One study reported on nitrogen balance and showed no differences between n-3 FAs and control groups after 7 d of postoperative enteral nutrition (25).
Three studies measured morbidity in terms of postoperative complications; the number of complications between n-3 FAs and control enteral feedings was not significantly different (24–26). Two studies reported a tendency for fewer infectious complications per patient in the n-3 FA group who received enteral nutrition during 7 d after gastrointestinal surgery (24, 25).
Only one study reported on the length of stay and mortality. In patients with upper gastrointestinal malignancies, no significant differences were observed between patients who received postoperative n-3 FAs that contained enteral nutrition and controls (24).
In summary, there was no evidence for beneficial effects of postoperative enteral supplementation of n-3 FAs on nutritional status, length of stay, infectious complications, and mortality in surgical oncology.

Parenteral supplementation of n-3 FAs
In 5 studies that involved patients with pancreas or colorectal cancer, n-3 FAs were parenterally supplemented during 5–7 d postoperatively (28–30, 38, 39) (Table 4).

Risk of bias in included studies
Three studies were rated of excellent quality (28, 29, 39). In the good-quality study, blinding was not described and probably not carried out (30). In the average-quality study, concealment was unsure because of the use of a randomization table with odd and even numbers (38).
Clinical outcome
One study reported on body-weight changes after gastrointestinal or pancreatic cancer surgery and showed no significant difference between groups that received n-3 FAs or control parenteral nutrition (28). In 3 studies, no differences in complications (28, 29) or infections (30) were observed between n-3 FAs and control groups after gastrointestinal cancer surgery. One small study showed a lower incidence of infections in the n-3 FA group than in the control group (38). One excellent-quality study observed a tendency for a lower incidence of infections and a significant lower incidence of systemic inflammatory response syndrome in the group that received parenteral n-3 FAs (39).
All studies reported on the length of hospital stay. Patients who received parenteral n-3 FAs had a significant shorter length of hospital stay (39) or a tendency for a shorter length of stay (29). A study in patients with gastrointestinal or pancreatic cancer observed a tendency for a shorter ICU stay in a post hoc analysis in which only patients with an increased risk of sepsis were selected (28). One study did not observe differences for a hospital or ICU stay after major intestinal surgery (30). A small study in major gastrointestinal surgery did not show any differences in the length of hospital stay between n-3 FAs and control parenteral nutrition (38).
In 3 studies, effects on mortality were investigated; none of the studies showed any differences on mortality between parenteral n-3 FAs or controls (28, 29, 38).
In summary, short-term (5–7 d) perioperative parenteral supplementation of n-3 FAs might have shortened the length of an ICU or hospital stay but did not improve other clinical outcome variables in surgical oncology.

Surgery: general surgery
Oral or enteral supplementation of n-3 FAs
We did not identify any RCTs that investigated effects of enteral supplementation of n-3 FAs in general, noncancer surgery.

Parenteral supplementation of n-3 FAs
Five studies described clinical effects of parenteral supplementation of n-3 FAs after general surgery. Four studies compared clinical effects of 5 d of parenteral n-3 FA supplementation after major abdominal, thoracic, or gastrointestinal surgery in mixed groups of patients with benign and malignant diseases (27, 31–33); one study investigated effects of perioperative parenteral n-3 FAs in coronary artery bypass surgery (36).
Risk of bias in included studies

The quality of study design was either excellent (31, 33), good (32), or poor (27, 36) (Table 4). In studies rated of poor quality, randomization and blinding procedures were either unclear or inappropriate. Two studies included small groups (27, 33), and 3 studies included large groups (31, 32, 36). Although the duration of supplementation was similar in most studies, the dosage of n-3 FAs was not reported in a consistent way, and there may have been large differences.

Clinical outcome

None of the studies measured nutritional status variables. Infectious complications were reported in 3 studies; 2 studies, a small and a large trial, did not find differences for the incidence of infections between n-3 FAs and control groups (32, 33). One large study in coronary artery bypass grafting surgery showed less atrial fibrillation after perioperative n-3 FA supplementation (36).

Hospital mortality was measured in 2 studies, of which neither study observed significant differences between n-3 FA and control groups (31, 32). Out of 4 studies, 2 studies showed a significant shorter length of hospital stay (27, 32), and one study showed a tendency for a shorter length of hospital stay after 5 d of parenteral n-3 FA supplementation in major abdominal or gastrointestinal surgery (31). The study in coronary artery bypass grafting showed a shorter ICU stay in the n-3 FA group (36).

In summary, these clinical trials indicated that 5-d postoperative parenteral n-3 FAs did not appear to reduce the postoperative infection rate or to have any effect on mortality. The evidence was not consistent regarding the length of the hospital or ICU stay in major abdominal or thoracic surgery.

Critical care

Oral or enteral supplementation of n-3 FAs

Reviewed publications in critical care included 3 studies on enteral supplementation of n-3 FAs, one study in patients with sepsis (21), and 2 studies in patients with Acute Lung Injury (22) or ARDS (23) (Table 5). All studies applied enteral nutrition.

Risk of bias in included studies

Large groups of patients were included, and the methodologic quality was judged average (21) or good (22, 23). One trial did not describe blinding and randomization procedures
(21), one study was nonblinded (22), and one study did not describe a blinding procedure (23).

Clinical outcome
One study in acute lung injury described body-weight development over 7 d of intervention; no differences between n-3 FAs and control groups were observed (22). Two studies assessed new organ failures, and showed a lower incidence in n-3 FA groups with sepsis (21) or ARDS (23).

Significant improvements in oxygenation were shown in patients who received enteral nutrition that contained n-3 FAs in patients with acute lung injury (22), ARDS (23), and severe sepsis (21) after 4–7 d (23), 14 d (22), and 4 wk (21) of supplementation, respectively. One study in patients with acute lung injury showed a better respiratory function and lower requirements for mechanical ventilation in the group that received enteral nutrition that contained n-3 FAs (22).

Ventilator-free days increased in patients with sepsis, acute lung injury, and ARDS who received enteral nutrition that contained n-3 FAs (21–23). Two of these studies reported on ICU stay, which was decreased in n-3 FA groups (21, 23). The length of hospital stay was not different between n-3 FA and control groups after 4–7 d of supplementation (22).

Mortality decreased in the n-3 FA group in the study in sepsis patients (21), but in one study in ARDS patients there was no between-group difference for mortality (22). In summary, enteral nutrition that contained n-3 FAs in critical care, especially in patients with sepsis, acute lung injury, or ARDS, resulted in a reduction of the length of ICU stay, mechanical ventilation, and, in sepsis patients, a reduced mortality rate.

Parenteral supplementation of n-3 FAs
Four studies on parenteral supplementation of n-3 FAs (during 12 h to 7 d) in patients who received critical care were reviewed in patients with sepsis (37), ARDS (34), ICU patients (35), and patients admitted to the ICU after abdominal aorta aneurysm surgery (40) (Table 5).

Risk of bias in included studies
One study was rated of excellent quality (35), and 3 studies were rated of average quality (34, 37, 40). Of the 3 studies of average quality, 2 studies did not describe blinding and randomization procedures (34, 40), and one study was single blinded (37). Two studies did
not describe details on withdrawals and dropouts (34, 37).

Clinical outcome

None of the studies reported on nutritional status variables. One excellent study in patients admitted to the medical ICU did not observe any differences between groups after 7 d of parenteral nutrition (n-3 FAs or control) for the infection rate or organ failures (35). In one study in ICU patients who underwent abdominal aorta aneurysm surgery, body temperatures in the n-3 FA group tended to be lower than in the control group (40). Other variables included gas variables and the duration of ventilation. One of the 2 studies showed improvements in gasexchange variables in sepsis patients (37); in a small 12-h intervention study in ARDS patients, there were no differences in gas-exchange variables between n-3 FA and control groups (34). Two studies reported on the duration of mechanical ventilation and did not find any differences between n-3 FA and control groups (35, 37).

The length of hospital stay was not significantly different between n-3 FA and control groups in ICU patients (35). In 2 studies, one study in patients admitted to the ICU after abdominal aorta aneurysm surgery (40) and one study in sepsis patients (37), the length of hospital stay tended to be shorter. Hospital mortality was measured in 3 studies, none of which observed significant differences between n-3 FA and control groups (34, 35, 37).

In summary, there was insufficient evidence for effects of parenteral supplementation of n-3 FAs on the clinical outcome in critical care.
Table 3: Clinical effects of supplementation of n-3 FA in cancer (non-surgical oncology)\(^1\)

<table>
<thead>
<tr>
<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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<tbody>
<tr>
<td>Guarcello, 2007 (18)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 46 lung cancer patients who underwent chemotherapy Baseline: &gt;10% weight loss in 6 mo</td>
<td>n = 26: n-3 FA ONSs (2.2 g EPA, 1.0 g DHA; Prosure; Abbott Laboratories) n = 20: isonitrogenous control ONSs</td>
<td>Patients in the n-3 FA group showed significant increases in body weight, energy and protein intakes, quality of life, appetite, and prealbumin. Body weight at 0, 30, and 60 d n-3 FAs: 57.7, 58.6, and 58.6 kg, respectively (P &lt; 0.05 compared with 0 d); control: 59.1, 57.0, and 59.1 kg, respectively. Differences between groups and blinding methods were not reported.</td>
<td>1B</td>
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<td>Takatsuka, 2001 (15)</td>
<td>Nonblinded, randomized, and controlled</td>
<td>n = 16 patients who underwent chemotherapy and allogeneic BMT from unrelated donors Baseline: no details on nutritional status</td>
<td>21 d before BMT to 180 d after BMT n = 7: 3 capsules (1.8 g EPA/d) n = 9: no capsules</td>
<td>Significantly higher survival rate in EPA group (p &lt; 0.01); EPA reduced complications (graft-versus-host disease; n = 2 grade III in the EPA group; n = 3 grade III or IV) (p-value not reported)</td>
<td>2D</td>
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<td>Gogos, 1998 (3)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 60 cancer patients (breast, gastrointestinal, lung, liver, and pancreas) Baseline: 50% malnourished (defined by weight loss &gt;10% in 6 mo, serum albumin &lt;30 g/L, serum transferrin &lt;2.0 g/L, and KPS &lt;60)</td>
<td>40 d n = 30: 18 fish oil capsules (3.1 g EPA, 2.0 g DHA) n = 30 control (sugar) tablets</td>
<td>n-3 FAs compared with control group: increased survival, no differences for body weight, serum albumin, or serum transferrin; malnourished n-3 FA subgroup compared to other subgroups: increase in KPS</td>
<td>1B</td>
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<td>Fearon, 2006 (14)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 198 patients with gastrointestinal cancer. n = 231 lung cancer patients Baseline: ≥ 5% loss of pre-illness stable weight</td>
<td>8 wk n = 175: 2 g EPA (95% diester capsules) n = 172: 4 g EPA (95% diester capsules) n = 171: control capsules</td>
<td>Compared with placebo: 2 g EPA; positive trend for body weight after 8 wk (weight change +1.2 kg, p = 0.66). Mean weight change in 4-g EPA group was +0.3 kg. Physical function improved by 7% in 2-g EPA group (p = 0.04) and</td>
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<tr>
<td>Fearon, 2006 (14) (continued)</td>
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<td>decreased by ~5% in the 4-g EPA group. Weakness tended to decrease in the 2-g EPA group at 4 and 8 wk, whereas there was little change in the 4-g EPA group; no significant differences between groups for albumin, KPS, survival, LBM, appetite, nausea, vomiting, diarrhea</td>
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<td>Bruera, 2003 (16) Nonblinded, randomized, and placebo controlled</td>
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<td>n = 60 cancer patients Baseline: anorexia and weight loss &gt;5%</td>
<td>14 d n = 30: 18 fish oil capsules (3.2 g EPA, 2.2 g DHA) n = 30: 18 control (olive oil) capsules</td>
<td>No difference between groups for body weight, appetite, tiredness, nausea, well-being, energy intake, LBM, arm circumference and triceps skinfold thickness. No correlation between fish-oil doses and changes in effect variables between days 1 and 14</td>
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<tr>
<td>Fearon, 2003 (17)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 200 pancreatic cancer patients</td>
<td>Baseline ≥ 5% weight loss in 6 mo</td>
<td>Body weight and LBM stabilized in both groups; no significant differences between groups in performance scores or any of the quality-of-life measures. Increased concentrations of plasma phospholipids EPA in the control group; noncompliance in both groups. Post hoc analysis showed a significant correlation between supplement intake and body weight and LBM in the n-3 FA group; significant correlation between plasma phospholipids EPA concentrations and LBM and body weight in n-3 FA group. Weight gain was associated with improved quality of life in n-3 FA group.</td>
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<tr>
<td>De Luis, 2008 (19)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 65 patients with oral and laryngeal cancer</td>
<td>12 wk postoperative (starting at hospital discharge)</td>
<td>No differences in plasma proteins, anthropometric variables (weight, LBM, fat mass, triceps skinfold)</td>
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<tr>
<td>De Luis, 2008 (19) (continued)</td>
<td>controlled</td>
<td>Baseline: recent weight loss</td>
<td>(3.7) of n-3:n-6 FA (2.0 g EPA, 0.9 g DHA) n = 34: control ONSs with a low ratio (0.99) of n-3:n-6 FA (1.8 g EPA, 1.2 g DHA)</td>
<td>thickness, arm circumference, postoperative infectious complications, or wound complications.</td>
<td>1C</td>
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<td>van der Meij, 2010 (20)</td>
<td>Double‐blinded, randomized, and placebo controlled</td>
<td>n = 40 patients with lung cancer undergoing chemoradiotherapy Baseline: 20% malnourished</td>
<td>5 wk n = 20: n-3 FA ONSs (2.02 g EPA, 0.92 g DHA; Prosure) n = 20: isocaloric control ONSs (Ensure; Abbott Laboratories)</td>
<td>Differences compared with controls: after 4 wk, the n-3 FA group showed higher energy and protein intakes [2456 kJ (p = 0.03) and 25.0 g (p = 0.01), respectively]. The n-3 FA group had better weight maintenance after 1, 2, and 4 wk [1.1 kg (p = 0.07), 1.3 kg (p = 0.02), and 1.7 kg (p = 0.04)], and less decrease in LBM [1.5 kg (p = 0.05), 1.9 kg (p = 0.02)] after 3 and 5 wk, respectively. REE in n-3 FAs decreased by 16.7% of predicted (p = 0.01) compared with that in controls after 3 wk.</td>
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1BMT, bone marrow transplantation; FA, fatty acid; KPS, Karnofsky Performance Status; LBM, lean body mass; ONSs, oral nutritional supplements; REE, resting energy expenditure.
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<td><strong>Enteral - Surgical oncology</strong></td>
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| Kenler, 1996 (24)             | Double-blinded, randomized, and placebo controlled | n = 50 patients with upper-gastrointestinal cancer | 7 d postoperative  
    n = 17: n-3 FA enteral nutrition  
    (4.0 g EPA; 1.9 g DHA)  
    n = 18: isocaloric, isonitrogenous control enteral nutrition  
    (Osmolite HN; Abbott Laboratories) | No differences between groups for number of infections, LoS, mortality, and nitrogen balance; fewer gastrointestinal complications in n-3 FA group (p = 0.053); 50% reduction in the total number of infections in n-3 FA group (p = 0.037); lower number of infected patients with more than one infection in n-3 FA group (p = 0.09). | 2B |
| Swails, 1997 (25)             | Double-blinded, randomized, and placebo controlled | n = 20 patients with upper-gastrointestinal cancer | 7 d postoperative  
    n = 8: n-3 FA enteral nutrition  
    (2.8 g EPA, 1.4 g DHA)  
    n = 10: isocaloric, isonitrogenous control enteral nutrition  
    (Osmolite HN) | No differences between groups for number of infections (n-3 FA group: 6, control: 5, p > 0.05) or nitrogen balance; n-3 FA group: trend for fewer infections per infected patient (n-3 FA group: n = 1, control: n = 2) (exact p-values not reported). | 2B |
Table 4 (continued)

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<th>First author, Year (reference)</th>
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<tr>
<td>Ryan, 2009 (26)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 53 cancer patients undergoing oesophagectomy</td>
<td>5 d pre-operative (ONSs) to 21 d postoperative (enteral nutrition)</td>
<td>n-3 FA group: lower number of patients with 5% weight loss at 1 mo postoperative (n-3 FAs: n = 2, control: n = 10; p = 0.03); maintenance of LBM (perioperative LBM difference n-3 FAs: +0.3 kg, p = 0.8; control: -1.9 kg, p = 0.03); no differences in incidence of major complications (n-3 FAs: n = 19, control: n = 24) or SIRS (n-3 FAs: 4%; control: 22%; p = 0.34).</td>
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<td>Baseline prevalence of malnutrition: ± 63%</td>
<td>n = 28: n-3 FA enteral nutrition (2.3 g EPA, 1.0 g DHA; Prosure; Abbott Laboratories) n = 25: isocaloric, iso-nitrogenous control enteral nutrition (Ensure Plus; Abbott Laboratories)</td>
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**Parenteral - Surgical oncology**

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<th>First author, Year (reference)</th>
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<tbody>
<tr>
<td>Heller, 2004 (28)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 44 gastrointestinal or pancreatic cancer who underwent surgery</td>
<td>5 d postoperative</td>
<td>No weight loss in fish oil group (mean ± SD: fish oil, 0.0 ± 2.9 kg, soybean oil: -1.1 ± 2.2 kg; NS); patients with increased risk of sepsis: tendency to shorter ICU stay. Complications and mortality: NS</td>
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<td>Described as in the methods of Liang, 2008 (29): n-3:n-6 FA ratio of 1:4 (eg, body weight of 70 kg, 2.8 g EPA, and 3.2 g DHA)</td>
<td>n = 24: soybean oil and fish oil (0.2 g/kg body weight)</td>
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<td>n = 20: soybean oil</td>
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<tr>
<td>Liang, 2008 (29)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 42 colorectal cancer patients who underwent surgery</td>
<td>7 d postoperative n = 21: n-3 lipid emulsion (0.2 g/kg body weight) and soybean oil emulsion (1.0 g/kg body weight) n-3:n-6 FA ratio of 1:3 (eg, body weight of 70 kg, 2.8 g EPA, and 3.2 g DHA) n = 21: soybean oil emulsion (1.2 g/kg body weight)</td>
<td>n-3 group: tendency to shorter postoperative hospital stay (mean ± SD: 17.45 ± 4.80 d compared with 19.62 ± 5.59 d; p = 0.19); Complications and mortality: NS</td>
<td>5A</td>
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<tr>
<td>Jiang, 2010 (39)</td>
<td>Randomized, double-blinded, and placebo controlled</td>
<td>n = 206 gastrointestinal or colonic cancer surgery</td>
<td>7 d postoperative n = 100: soybean oil plus n-3 FA emulsion (1.0 g/kg and 0.2 g/kg body weight, respectively) n = 103: soybean oil emulsion (1.2 g/kg body weight)</td>
<td>Fewer infectious complications (4 compared with 12 on day 8; p = 0.066) and SIRS (4 compared with 13; p = 0.039). Hospital stay was significantly shorter (mean ± SD: 15 ± 5 vs. 17 ± 8 d; p = 0.041) in the n-3 FA group. Total postoperative medical costs were comparable in the 2 groups (mean ± SD: 1269 ± 254 and 1302 ± 324 US $ in n-3 FA and control groups, respectively; p = 0.424).</td>
<td>4B</td>
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<td>First author, Year (reference)</td>
<td>Design</td>
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<td>n-3 (duration, form, and daily dose)</td>
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<td>Wachtler, 1997 (30)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 40 patients with cancer who underwent major intestinal surgery</td>
<td>5 d postoperatively n = 19: 20% n-3 FA lipid emulsion (Lipoplus; B Braun) n = 21: 20% isocaloric MCT/LCT lipid emulsion Daily dose: not specified</td>
<td>Intervention group compared to control group: no clinically significant changes in postoperative infections, Acute Physiology and Chronic Health Evaluation II scores, LoS in the hospital or ICU.</td>
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<td>Badia-Tahull, 2010 (38)</td>
<td>Randomized, double-blind, and placebo controlled</td>
<td>n = 27 patients with high-risk elective major GI surgery, that required TPN</td>
<td>5 d n = 13: olive oil emulsion, partially replaced with FO (16.6%, w/w) n = 14: olive oil emulsion</td>
<td>Significantly lower incidence of infections in the n-3 FA group (23.1% compared with 78.6%, p = 0.007). No differences in mortality, sepsis, LoS in hospital, prealbumin and safety parameters.</td>
<td>2B</td>
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<tr>
<td>Grimm, 2006 (27)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 33 patients with major abdominal surgery</td>
<td>5 d postoperative n = 19: 20% n-3 FA lipid emulsion (SMOFlipid; Fresenius Kabi) (4.7 g EPA/L, 5.3 g DHA/L) n = 14: control soybean oil emulsion (Lipovenoes 20%, Fresenius Kabi). Daily dose: 1.5 g fat/kg body weight</td>
<td>n-3 FAs compared to soybean oil on day 6: shorter LoS (mean ± SD: 13.4 ± 2.0 compared with 20.4 ± 10.0 d; p &lt; 0.05).</td>
<td>1B</td>
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<tr>
<td>First author, Year (reference)</td>
<td>Design</td>
<td>Condition</td>
<td>n-3 (duration, form, and daily dose)</td>
<td>Results</td>
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<td>Mertes, 2006 (31)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 199 elective abdominal surgeries</td>
<td>5 d postoperative&lt;br&gt;n = 99: 20% n-3 FA lipid emulsion (SMOFlipid; Fresenius Kabi)&lt;br&gt;n = 100: 20% control lipid emulsion (Lipovenoes; Fresenius Kabi)&lt;br&gt;Daily dose: 1.5 g fat/kg body weight: days 3-5: 1.4 g fat/kg body weight</td>
<td>No difference in mortality, tolerance, or? safety. Tendency for shorter LoS in per protocol patients in the n-3 FA group (n-3 FA: 15.7 ± 6.3 d, control: 17.8 ± 13.2 d, p-values not reported).</td>
<td>4A</td>
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<tr>
<td>Wichmann, 2007 (32)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 256 elective abdominal surgeries</td>
<td>5 d postoperative&lt;br&gt;n = 127: 10% fish oil, 50% MCTs, 40% LCTs (Lipoplus)&lt;br&gt;n = 129: 100% LCT lipid emulsion (Intralipid; Fresenius Kabi)&lt;br&gt;Daily dose: days 1 and 2: 0.7 g fat/kg body weight&lt;br&gt;days 3-5: 1.4 g fat/kg body weight</td>
<td>Significantly shorter LoS in hospital (n-3 FAs: 17.2 d; control: 21.9 d; p = 0.0061) in fish oil group; no differences for routine laboratory variables. No significant differences for complications, LoS in ICU, or mortality.</td>
<td>3A</td>
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Table 4 (continued)

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<tr>
<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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<tr>
<td>Senkal, 2007 (33)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 40 patients who underwent colorectal surgery</td>
<td>5 d postoperative n = 19: 20% n-3 FA lipid emulsion (Lipoplus) n = 21: 20% control MCT and LCT lipid emulsion (Lipofundin; B Braun) Daily dose: days 1 and 2: 50 g fat days 3 to 5: 100 g fat</td>
<td>Postoperative infections occurred in 4 patients of the n-3 group compared to 7 patients in the control group (NS).</td>
<td>5A</td>
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<tr>
<td>Heidt, 2009 (36)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 102 patients with elective CABG surgery</td>
<td>Perioperative, from hospital admission to discharge from ICU n = 52: 10% n-3 FA lipid emulsion (Omegaven; Fresenius Kabi) n = 50: soybean oil emulsion (Lipovenoes) Daily dose: 100 mg fat/kg body weight: 1 mL/kg body weight</td>
<td>Perioperative, from hospital admission to discharge from ICU n = 52: 10% n-3 FA lipid emulsion (Omegaven; Fresenius Kabi) n = 50: soybean oil emulsion (Lipovenoes) Daily dose: 100 mg fat/kg body weight: 1 mL/kg body weight</td>
<td>1B</td>
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1 AA, arachidonic acid; CABG, coronary artery bypass grafting; FA, fatty acid; FO, fish oil; GLA, γ-linolenic acid; ICU, intensive care unit; KPS, Karnofsky Performance Status; LBM, lean body mass; LCT, long-chain triglyceride; LoS, length of stay; MCT, medium-chain triglyceride; ONS, oral nutritional supplement; REE, resting energy expenditure; SIRS, systemic inflammatory response syndrome; TPN, total parenteral nutrition.
### Table 5: Clinical effects of supplementation of n-3 FA in critical care

<table>
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<tr>
<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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</table>
| Pontes-Arruda, 2006 (21)      | Randomized, double-blinded, and placebo controlled | n = 165 patients with severe sepsis or septic shock who required mechanical ventilation | 28 d  
 n = 55: n-3 FA enteral nutrition (4.9 g EPA, 2.2 g DHA, 4.6 g GLA, and anti-oxidant vitamins)  
 n = 48: isonitrogenous, isocaloric control enteral nutrition | Intervention compared with control: significant reduction in mortality rate (19.4%; 95% CI: 0.3%, 36.7%; \( p = 0.037 \)); significant improvements in oxygenation status, more ventilator-free days and ICU-free days, and lesser development of new organ dysfunctions. | 2B |
| Singer, 2006 (22)             | Randomized, nonblinded, and placebo controlled study | n = 95 patients with acute lung injury | 14 d  
 n = 46: n-3 FA enteral nutrition (5.4 g EPA, 2.5 g DHA, 5.1 g GLA, anti-oxidant vitamins; Oxepa; Ross Laboratories)  
 n = 49: isocaloric, isonitrogenous control enteral nutrition | Intervention compared with control: no significant differences in body weight at days 1 and 7; significant shorter length of ventilation (\( P < 0.04 \)); no difference in survival. Significant improvement in oxygenation; mean ± SD PaO\(_2\):FiO\(_2\) ratio, 317.3 ± 99.5 compared with 214.3 ± 56.4 and 296.5 ± 165.3 compared with 236.3 ± 79.8, respectively; \( p < 0.05 \) | 3D |
Table 5 (continued)

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<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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</table>
| Gadek, 1999 (23)               | Randomized, double-blind, and placebo controlled | n = 146 ARDS caused by sepsis/pneumonia, trauma or aspiration injury | ≥ 4–7 d  
n = 51: n23 FA enteral nutrition (6.9 g EPA, 2.9 g DHA, and 5.8 GLA)  
n = 47: isonitrogenous, isocaloric control enteral nutrition | Intervention compared with control: significant improvements in oxygenation; fewer days of ventilatory support (11 compared with 16.3 d; p = 0.011); decreased length of ICU stay (12.8 compared with 17.5 d, p = 0.016); less patients with new organ failure (8 compared with 28%, p = 0.015). | 38 |

**Parenteral**

| Sabater, 2008 (34) | Double-blind, randomized, and placebo controlled | n = 16 patients with ARDS, intolerance to enteral nutrition | 12 h  
n = 8: Lipoplus 20% (B Braun) (50% MCT, 40% LCT, and 10% n-3 FA)  
n = 8: Intralipid (Fresenius Kabi) (100% LCTs)  
Dose: 1.44 g fat/kg body weight | Significantly lower pulmonary capillary pressure in the n-3 group. No differences between groups for hemodynamics, gas exchange parameters, adverse events, or survival. | 28 |
Table 5 (continued)

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<tr>
<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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<tr>
<td>Friesecke, 2008 (35)</td>
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<td>n = 166 patients admitted to medical ICU</td>
<td>7 d n = 83: 10% n-3 FA lipid emulsion (Omegaven; Fresenius Kabi) TPN n-3:n-6 ratio of 1:2 n = 82: 20% MCT and LCT lipid emulsion (Lipofundin; B Braun) TPN n-3:n-6 ratio of 1:7 Daily dose: minimum of 0.5 g fat/kg body weight</td>
<td>No differences between groups for nosocomial infections, duration of mechanical ventilation, LoS in ICU, 28-d mortality, and organ failures.</td>
<td>4A</td>
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<td>Berger, 2008 (40)</td>
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<td>n = 24 patients admitted to the surgical ICU after abdominal aorta aneurysm surgery</td>
<td>4 d n = 12: n-3 FA lipid emulsion (Lipoplus; 20 g fat/L, of which there are 10.45 g EPA + DHA/L) Mean body weight: 72.5 kg; mean daily dose: 0.15 g fat/kg body weight: 0.15 x 72.5 = 10.88 g fat, 568 mg EPA + DHA n = 12: MCT and LCT lipid emulsion (Lipofundin)</td>
<td>Temperature increased in both groups, with a trend to lower values in the n-3 FA group (p = 0.09). Nonsignificant shorter ICU stay (p = 0.22) and hospital stay (p = 0.19) in n-3 FA group. REE increased in both groups (NS).</td>
<td>2B</td>
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Table 5 (continued)

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<tr>
<th>First author, Year (reference)</th>
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<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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<tr>
<td>Barbosa, 2010 (37)</td>
<td>Randomized, single blinded, and placebo controlled</td>
<td>n = 25 patients with sepsis 6 d n = 13: MCTs, soybean oil, and fish oil (50:40:10) n = 10: MCTs and soybean oil (50:50)</td>
<td>Ratio of P02:FiO2 was significantly higher in the fish oil group (p = 0.047). The n-3 FA group tended to have a shorter LoS (mean ± SD: 22 ± 7 compared with 55 ± 16 d, p = 0.079). No differences in days on ventilation, LoS in ICU and mortality between groups.</td>
<td>2A</td>
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1AA, arachidonic acid; ARDS, acute respiratory distress syndrome; FA, fatty acid; GLA, γ-linolenic acid; ICU, intensive care unit; LCT, long-chain triglyceride; LoS, length of stay; MCT, medium-chain triglyceride; REE, resting energy expenditure; TPN, total parenteral nutrition.
Incorporation and washout of n-3 FAs after supplementation

The second aim of this review was to investigate the dosedependent incorporation of n-3 FAs into phospholipids of plasma, blood cells, and mucosal tissue and the subsequent washout after n-3 FA supplementation (Figure 2). In total, we retrieved 23 RCTs and 13 open-label studies that reported incorporation or washout in cancer, surgery, and critical care after supplementation of n-3 FAs via fish-oil capsules (7, 41–59), ONSs (4, 17, 20, 26, 60), and/or enteral nutrition (23, 24, 26), or n-3 FA lipid emulsions for parenteral administration (27, 32, 33, 37, 40, 61–64) (see Supplemental Tables 1–3 under “Supplemental data” in the online issue). The following paragraphs summarize the most important findings.

Cancer

The findings of 23 clinical studies that investigated the incorporation of n-3 FAs in different tissues after supplementation by fish-oil capsules or ONSs are summarized in Supplemental Table 1. The studies were performed in patients with cancer or in subjects with a high risk or history of cancer. We included 10 RCTs (17, 20, 41–44, 55, 57–59) and 13 open-label prospective studies (4, 7, 45–49, 51–55, 60). The doses of supplementation ranged from 0.47 to 9 g EPA/d and from 0.24 to 3.6 g DHA/d. The duration of supplementation ranged from 14 d to 2 y.

Oral or enteral supplementation of n-3 FAs

In general, oral or enteral supplementation of 1.5–3 g EPA (either combined with DHA or not) increased the average concentration of EPA in plasma phospholipids from ≤ 2.0% to 2.5–6.5% of total FAs, which was a 2.5–10-fold increase in the plasma EPA concentration (4, 7, 17, 20, 23, 24, 26, 44, 45, 47, 48, 50–55, 57, 60), and decreased the concentration of the n-6 FA AA (24, 57). The concentration of DHA in phospholipids increased in most studies after supplementation of EPA and DHA (4, 20, 44, 45, 47, 48, 50, 51, 54, 55, 60). Two studies reported unchanged plasma phospholipids concentrations of DHA after supplementation of EPA and DHA (24, 57).

Studies showed variable increases of plasma n-3 FAs after supplementation of 0.5–6.9 g EPA and 0.2–3.6 g DHA during variable periods as follows: short-term [4 d (23), 7 d (23, 24), and 2 wk (55, 65), respectively] and long-term [2–6 mo (42, 44, 45, 48, 50, 53, 57)] supplementation of n-3 FAs. In a few dose escalation studies, a plateau of the maximal incorporation into plasma phospholipids was reached at doses > 2 g EPA; 2.1 g EPA (46)
and 2.8–4.2 g EPA (41) did not further increase plasma EPA concentrations after 15 d and 6 mo, respectively.

With regard to membrane phospholipids of blood cells, one study showed the incorporation of EPA, DPA, and DHA in RBCs after supplementation of 1.2 g EPA + 0.9 g DHA during 2 mo (46) but not in WBCs. A second study showed the incorporation of EPA and DHA and a decrease of AA in RBCs (41). One study did not find an increase of the EPA concentration of neutrophils after 2 wk of supplementation of 3.2 g EPA + 2.2 g DHA (55). In addition, oral supplementation of EPA (1.4–4 g) during ≥ 1 mo, either combined with DHA or not, resulted in increased concentrations of EPA in colorectal mucosa (42, 58, 59). One of these studies observed a dose-dependent increase in EPA and DHA in rectal mucosa and a decrease in mucosal AA in groups that received 1.4, 2.7, or 4.1 g EPA combined with 1.1, 2.4 or 3.6 g DHA, respectively (59). None of the studies described a washout of n-3 FAs from plasma, blood cells, or mucosal tissue.

In summary, there is a large body of evidence that showed increases of EPA and DHA concentrations in plasma phospholipids and colorectal mucosa after supplementation of EPA and DHA, which demonstrate incorporation to occur after short and long-term oral or enteral supplementation in patients with cancer. Supplementation of 3 g EPA/d seemed to suffice to reach maximal incorporation. Too few studies have been performed to conclude on incorporation in other blood compartments (eg, RBCs and WBCs). Moreover, the duration of washout of n-3 FAs from plasma and cell membranes in patients with cancer is unknown to our knowledge.

**Parenteral supplementation of n-3 FAs**

We retrieved no studies that investigated incorporation or washout after parenteral supplementation in cancer patients.

**Surgery**

Eight RCTs reported on the incorporation and washout in patients who underwent surgery after enteral supplementation (24, 26, 56) or parenteral supplementation of n-3 FAs (27, 32, 33, 61, 62) (see Supplemental Table 2).

**Oral or enteral supplementation of n-3 FAs**

Perioperative enteral supplementation of n-3 FAs was applied in 3 studies; the methodologic quality was rated to be excellent (26), good (56), and average (24). One study applied fish-oil capsules (56), and the other studies used ONSs and/or enteral
nutrition that contained n-3 FAs (24, 26). Two studies showed a significant increase in the concentration of plasma or serum EPA (24, 26). Seven days of postoperative supplementation of 4.0 g EPA and 1.9 g DHA in gastrointestinal cancer surgery resulted in increased plasma phospholipid EPA concentrations (0.6–11.3%); DHA concentrations did not significantly change (24). An excellent-quality study in esophageal cancer surgery applied 7-d preoperative ONSs and 3-wk postoperative enteral nutrition (2.3 g EPA and 0.95 g DHA) and showed the incorporation of EPA in serum (1.2–3.2%) (26). These studies also observed an increase of EPA concentrations in RBCs (24) and peripheral blood mononuclear cells (26). One study observed an increase of EPA, but not of DHA, in colonic mucosa after 7–21 d of preoperative supplementation of 1.4 g EPA and 1.0 g DHA with fish-oil capsules (56).

In summary, perioperative enteral supplementation of n-3 FAs only increased EPA, but not DHA, concentrations of plasma phospholipids and colonic mucosa. The washout after perioperative enteral supplementation of n-3 FAs is unknown.

**Parenteral supplementation of n-3 FAs**

A short-term (5–7 d) parenteral supplementation of n-3 FAs in surgery was applied in 5 studies, 2 studies of which were of poor and average quality in cancer surgery (61, 62) and 3 studies of which were of poor, good, and excellent quality in general surgery (27, 32, 33). Most studies only described the type of lipid emulsion and the daily dose of fat administered during the study and not the exact dose of EPA and DHA.

One study in gastrointestinal cancer surgery showed increases of plasma EPA, not DHA, after 5 d postoperative parenteral supplementation of n-3 FAs (61). Blood samples after 1 d of infusion did not show increases of plasma EPA and DHA (27). In general surgery, plasma EPA and DHA increased after 5 d of supplementation (27, 33), whereas at the same time, plasma AA decreased in one of the studies (27); the other study did not find a decrease of AA (33). In the latter study, FA concentrations of RBCs were also measured; EPA increased, but there were no significant changes in DHA and AA concentrations in RBCs (33). A study in esophageal surgery also measured the incorporation in platelet FAs; after 7 d of supplementation, a peak in EPA was reached at day 8. For DHA, no significant changes in platelets were observed (62).

Two studies reported on the washout after cessation of supplementation. In patients who underwent an esophagectomy, the washout of EPA and DHA from platelet phospholipids was > 7 d (62). In patients with colorectal surgery, the washout of EPA and DHA from plasma free FAs was > 5 d (33).

In summary, the short-term (5–7 d) postoperative parenteral supplementation of n-3 FAs
increased EPA in plasma and cell membranes (platelets and RBCs) and also, in some studies, plasma DHA. The washout from plasma and platelets after postoperative parenteral supplementation of n-3 FAs appeared to be ≥ 5–7 d.

**Critical care**

Five RCTs on incorporation of n-3 FAs in critical care were retrieved, of which 4 RCTs on parenteral supplementation of n-3 FAs (37, 40, 63, 64) (see Supplemental Table 3).

**Oral or enteral supplementation of n-3 FAs**

One good-quality study investigated plasma incorporation after 4–7 d of enteral nutrition (7 g EPA and 3 g DHA) in patients with ARDS; EPA was incorporated after 4 d and further increased after 7 d (23).

In summary, enteral supplementation of n-3 FAs in critical care resulted in the incorporation of EPA in plasma phospholipids and sometimes of DHA. After 4–7 d of supplementation, incorporation was observed. The washout after enteral supplementation of n-3 FAs in critical care is unknown.

**Parenteral supplementation of n-3 FAs**

A short-term (4–10 d) parenteral supplementation of n-3 FAs was applied in patients with sepsis (37, 63, 64) and patients after aorta aneurysm surgery (40). The methodologic quality was average (37, 63, 64) and poor (40). Doses of n-3 FA supplementation were ~7 g EPA + 6 g DHA (63, 64), 6 g EPA + DHA (40) and 2.3 g EPA + DHA (37).

All studies investigated plasma incorporation; 4 studies showed an increase in the concentrations of plasma EPA (37, 40, 63, 64), and 3 studies showed an increase in plasma DHA (37, 63, 64).

Two studies, one study in patients with sepsis (63) and one study after abdominal aorta aneurysm surgery (40), showed an increase of EPA and DHA in plasma-free FAs and phospholipids, respectively, after 1 d of infusion, which reached a peak concentration of EPA after 3 d. For AA, no significant changes in plasma free FAs were observed in the study in sepsis patients (63). Another study in sepsis patients observed increases of EPA and DHA in mononuclear leukocyte membranes; EPA already increased after 1 d. Concentrations of AA did not significantly change (64).

After the cessation of infusion, the washout of EPA and DHA from plasma and mononuclear leukocyte membranes occurred within 5 d (63).

In summary, parenteral supplementation of n-3 FAs in critical care resulted in the
incorporation of EPA in plasma phospholipids, and sometimes of DHA, after 1–3 d of supplementation. The washout in patients who received critical care occurred in ≤5 d.

Discussion
The use of n-3 FA supplementations in clinical practice is still a subject of debate. What is the preferred supplementation method (oral or enteral compared with parenteral), which patients should be supplemented, when, and for how long?
The current systematic literature review included 28 RCTs that evaluated the clinical effects of n-3 FAs in patients with cancer who underwent surgery or received critical care. The following conclusions and recommendations were elaborated from the results of this literature review.

Nonsurgical oncology
Earlier reviews have stated that evidence for the recommendation of n-3 FAs for their beneficial effects in cancer patients with weight loss and cachexia was too weak (9, 66). The current review, which included 4 new studies that were not discussed in the earlier reviews, showed that 6–8 wk of supplementation of n-3 FAs by capsules or ONSs may have had beneficial effects on body weight and the quality of life in cancer patients who received chemo(radio)therapy or palliative care. Because of the absence of RCTs on parenteral supplementation of n-3 FAs in nonsurgical oncology, no recommendation on this subject can be made. Studies in patients who regularly receive parenteral nutrition, in particular, in patients with hematologic malignancies, would be useful.

Surgery
There is no evidence for any beneficial effects for enteral supplementation of n-3 FAs on nutritional status, postoperative complications, or mortality for surgery patients. Five to 7 d of perioperative parenteral supplementation of n-3 FAs might have reduced the length of postoperative hospital or ICU stays but did not improve other clinical variables. The effect on the length of hospital stay was confirmed by a recent meta-analysis, which also showed fewer infectious complications in patients who received perioperative parenteral n-3 FA supplementation (67). Therefore, supplementation of n-3 FAs may be considered in patients who receive perioperative parenteral nutrition.
**Critical care**

There are convincing results of the beneficial effects of enteral supplementation of n-3 FAs on the morbidity and mortality in critical care patients, especially in patients with sepsis, ARDS, or acute lung injury. Benefits were observed after 4–7 d and 2 and 4 wk. These results were in line with those in earlier meta-analyses (68). Therefore, we recommend enteral nutrition that contains n-3 FAs for ≥ 4 d for patients with sepsis, ARDS, or acute lung injury.

Studies on the parenteral supplementation of n-3 FAs in critical care did not consistently show any beneficial effects on the clinical outcome. Before applying parenteral n-3 FAs in critical care, more research that shows clinical benefits would be required.

**Incorporation and washout**

A second aim of this review was to provide an overview of the incorporation into phospholipids of plasma, blood cells, and mucosal tissue and the subsequent washout after n-3 FA supplementation.

The studies included 23 RCTs and 13 open-labeled trials in cancer, surgery, and critical care and showed that the supplementation of n-3 FAs by oral or enteral or parenteral nutrition resulted in the incorporation of EPA, and sometimes of DHA, in plasma, serum, blood cells, and mucosal tissues. The incorporation after enteral supplementation of n-3 FAs occurred after 4–7 d. In a few studies that measured the incorporation on the first days after parenteral supplementation of n-3 FAs, concentrations increased within 1–3 d. Studies administered variable doses of EPA and DHA and measured the FA composition of various tissues. Because of the heterogeneity of study designs, it was hard to draw conclusions for a dose-effect relation of n-3 FA incorporation in plasma, blood cells, and mucosal tissue. In general, any dose between 0.5 and 4 g of EPA was expected to result in incorporation in a dose- and time-dependent manner.

The washout from plasma and blood cells after the cessation of supplementation has been studied only after the short-term parenteral administration of n-3 FAs. It should be noted that the washout might involve complex pathways. After long-term supplementation, n-3 FAs accumulate in adipose tissue and, after reaching a new steady state; there may be a prolonged washout period because of the release of n-3 FAs during subsequent postabsorptive periods (69). This pathway was not unraveled by the current review.

To our knowledge, the duration of the washout of n-3 FAs from plasma and cell membranes in patients with cancer is unknown, but in surgical oncology, the washout from plasma and platelets after postoperative parenteral supplementation appeared to be
≥ 5–7 d. The washout of n-3 FAs from plasma and cell membranes of platelets and leukocytes after parenteral supplementation of n-3 FAs appeared to be somewhat longer after surgery (> 5 d) than in patients with sepsis (< 5 d), which we could not explain after having reviewed the underlying literature. Rapid washout might be related to the disease severity.

**Strengths and limitations of the review**

The strength of our review was the systematic inclusion of RCTs for the evaluation of clinical evidence by following the PRISMA statement, added by studies on the incorporation and washout of FAs.

This review had a number of potential limitations. At first, we focused on one type of nutritional formula; we selected studies that investigated only the supplementation of n-3 FAs, thereby excluding studies on immune-modulating formulas. Immunemodulating formulas contain a mix of immune-modulating nutrients, such as glutamine, arginine, ribonucleic acids, and n-3 FAs. Meta-analyses have shown positive effects of immunemodulating formulas on the postoperative infection rate and length of hospital stay in patients who undergo gastrointestinal surgery (70). A combination of immunemodulating nutrients is probably more effective than the exclusive supplementation of n-3 FAs. In patients with critical illnesses, n-3 FAs alone proved to have beneficial effects above arginine and glutamine (71).

This review restricted itself to n-3 FA supplementation in patients with cancer (who received no treatment, any cancer treatment, or palliative care) around surgery or in patients who underwent critical care at the ICU (eg, because of sepsis, ARDS, or postsurgery). No conclusions can be drawn from this literature study on the effects of n-3 FAs in patients with, eg, inflammatory bowel diseases or HIV. We were particularly interested in differences between oral or enteral and parenteral supplementation of n-3 FAs. Unfortunately, no studies that compared these areas were shown.

For the successful application of n-3 FA supplementation, it is important to evaluate the safety and feasibility of parenteral and enteral supplementation. Only a few well-designed studies reported possible adverse effects. The supplementation of n-3 FAs (with doses of ≥ 2 g EPA /d or ≤ 0.2 g n-3 FAs /kg body weight) appeared to be safe in the patient populations of our interest (3, 14, 16–18, 20, 26). Oral or enteral supplementation of n-3 FAs may cause diarrhea or a fishy taste.
Implications for practice
This systematic review provided a summary and update of the evidence for effects of supplementation of n-3 FAs in patients with cancer around surgery and in critical care. Although studies were heterogeneous with regard to the n-3 FA dose, supplementation method, endpoints, and quality, we believe there is enough evidence to advise the oral or enteral supplementation of n-3 FA in cancer patients with a high risk of weight loss and in critical care patients (provided that the digestive tract is functioning and platelet and coagulation function are adequate). Parenteral supplementation might be considered around surgery.
Supplementation of the optimal dose should be continued as long as the initial indication for n-3 FA supplementation exists, taking the incorporation period (which is a few days longer for enteral than parenteral supplementation) and the relative short washout period into account. During the washout period, clinical beneficial effects of n-3 FAs probably extinguish.

Acknowledgements
We thank Sanne Hesselink for her efforts in carrying out parts of the literature study.

FA, fatty acid; RCTs, randomized controlled trials.

Search result:
PubMed: 2043
EMBASE: 878

ENTERAL: 1146
PubMed: 794
EMBASE: 352

Potential studies: 93 (based on title and abstract)
PubMed: 78
EMBASE: 15

Excluded: 63
Reasons for exclusion:
non-RCTs: 17
no enteral supplementation of n-3 FA: 26
no clinical outcome parameters: 11
multiple immune enhancing compounds and/or use of appetite stimulants: 17
no cancer/surgery/critical care: 5
in vivo/in vitro: 2
no adult: 0

RCTs included: 14
Cancer: 8
Surgery: 3
Critical care: 3

PARENTERAL: 1775
PubMed: 1249
EMBASE: 526

Potential studies: 98 (based on title and abstract)
PubMed: 71
EMBASE: 27

Excluded: 85
Reasons for exclusion:
non-RCTs: 22
no parenteral supplementation of n-3 FA: 31
no clinical outcome parameters: 6
multiple immune enhancing compounds and/or use of appetite stimulants: 12
no cancer/surgery/critical care: 4
in vivo/in vitro: 8
no adult: 2

RCTs included: 14
Cancer: 0
Surgery: 10
Critical care: 4
Figure 2: Flowchart literature search on incorporation and washout. EMBASE, www.embase.com; PubMed, www.pubmed.com.
FA, fatty acid; RCTs, randomized controlled trials.
## Supplemental Table 1: Incorporation of n–3 fatty acids (FAs) in blood after enteral supplementation of n–3 FAs in cancer

<table>
<thead>
<tr>
<th>First author, Year (reference)</th>
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<tr>
<td>Barber, 1999 (4)</td>
<td>Open label</td>
<td>n = 20 patients with unresectable adenocarcinoma of the pancreas (with ongoing weight loss)</td>
<td>3 wk ONSs (2.2 g EPA and 0.96 g DHA/d)</td>
<td>EPA and DHA in plasma phospholipids increased significantly. Plasma phospholipids (%): median (IQR) EPA Baseline: 0.5 (0.2–1.6); week 3: 5.2 (2.6–6.7) ( p = 0.0003 ) compared with baseline DHA Baseline: 2.9 (1.6–3.9); week 3: 4.8 (3.3–5.3) ( p = 0.0086 ) compared with baseline AA Baseline: 6.4 (4.1–7.5); week 3: 5.1 (4.3–6.4) ( p = 0.33 ) compared with baseline</td>
<td>1D</td>
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<tr>
<td>Read, 2006 (60)</td>
<td>Phase II study</td>
<td>n = 23 patients with advanced colorectal cancer</td>
<td>9 wk ONSs (2.2 g EPA and 0.92 g DHA/d) 3 wk, n = 20 patients completed 3 wk, n = 15 patients completed 9 wk</td>
<td>EPA and DHA in plasma phospholipids increased and AA decreased significantly over the first 3 wk and remained high by the end of week 9. Plasma phospholipids (%): median (range) EPA Baseline: 0.53 (0–3.62); week 3: 5.3 (0.3–7.4); week 9: 4.8 (1.6–7.1) ( p = 0.002 ) DHA Baseline: 2.6 (1.0–7.9); week 3: 7.0 (1.5–8.6); week 9: 6.8 (1.6–9.2) ( p = 0.005 )</td>
<td>1C</td>
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<td>Read, 2006 (60) (continued)</td>
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<td>Fearon, 2003 (17)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 200 pancreatic cancer patients</td>
<td>8 wk 1. n = 95; n-3 FA ONSs (2.2 g EPA and 1.0 g DHA) 2. n = 105: isonitroge-nous control ONSs</td>
<td>EPA in plasma phospholipids significantly increased in the intervention group; 26% of the intervention group did not have an elevation of plasma phospholipid EPA at weeks 4 and/or 8. Plasma phospholipids (%) (mean ± SEM) EPA 1. 5.57 ± 0.70 (range: &gt;0.5 to &gt;7.5) 2. 1.70 ± 0.49</td>
<td>5A</td>
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<tr>
<td>van der Meij, 2010 (20)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 40 patients with nonsmall cell lung cancer who underwent chemo(radio)-therapy</td>
<td>5 wk 1. n = 20: n-3 FA ONSs (2.0 g EPA + 0.9 g DHA) 2. n = 20: isocaloric placebo ONSs</td>
<td>EPA and DHA in plasma phospholipids increased and AA did not change. Difference between groups EPA: 1.5% (p = 0.06) DHA: 1.1% (p = 0.04) AA: 0.3% (p = 0.65)</td>
<td>5A</td>
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| Courtney, 2007 (58)           | Nonblinded, randomized, and controlled | n = 30 patients with colorectal cancer | 3 mo  
   1. n = 14: 2 g EPA (free FA form, 4 capsules)  
   2. n = 14: no supplement | EPA of colonic mucosa significantly increased (p = 0.005 difference from baseline) in the EPA group but not in the control group. AA decreased more in the EPA group than in the control group (p = 0.021 difference between groups). Mucosal FAs (% of total FAs): mean ± SD; pre; post; change  
   EPA  
   1. 1.15 ± 0.62; 2.65 ± 1.60; 1.51 ± 1.17  
   2. 0.80 ± 0.46; 0.79 ± 0.53; -0.009 ± 0.15  
   DHA  
   1. 2.38 ± 1.06; 2.46 ± 1.37; 0.08 ± 0.94  
   2. 1.73 ± 0.83; 1.92 ± 0.82; 0.18 ± 0.36  
   AA  
   1. 9.44 ± 3.02; 7.99 ± 2.46; -1.45 ± 2.67  
   2. 8.35 ± 2.83; 9.10 ± 1.86; 0.75 ± 1.43 | 3A |
| Huang, 1996 (57)              | Double-blinded, randomized, and placebo controlled | n = 27 patients with resected colon/rectum cancer or adenomatous polyps | 6 mo  
   9 capsules  
   1. n = 17: 4.0 g EPA and 2.4 g DHA  
   2. n = 10: placebo (corn oil) | n-3 FA group: plasma phospholipids EPA and DHA increased (p < 0.01 and P < 0.5) and AA decreased (p < 0.01); mean plasma phospholipid n-6:n-3 ratio | 2B |
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<tr>
<td>Anti, 1994 (59)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 60 patients after clearing polypectomies for sporadic adenomatous colorectal polyps</td>
<td>30 d Fish-oil or placebo capsules (n = 15 per group) 1. 1.4 g EPA and 1.1 g DHA 2. 2.7 g EPA and 2.4 g DHA 3. 4.1 g EPA, and 3.6 g DHA 4. Placebo capsules</td>
<td>Dose-dependent increase in EPA and DHA in rectal mucosa and decrease in mucosal AA in all fish-oil groups (p &lt; 0.05, comparisons between groups). Rectal mucosa (% of total FAs): mean ± SEM; baseline; 30 d, p-value compared with 1, 2 and 3 EPA 1. 0.78 ± 0.15; 1.64 ± 0.20 2. 0.85 ± 0.10; 1.93 ± 0.28 3. 1.16 ± 0.08; 2.52 ± 0.24 4. 0.97 ± 0.13; 1.09 ± 0.11 (p &lt; 0.01) DHA 1. 1.29 ± 0.16; 2.19 ± 0.42 2. 1.41 ± 0.16; 2.61 ± 0.24 3. 2.01 ± 0.51; 4.02 ± 0.63 4. 1.54 ± 0.23; 1.45 ± 0.16 (p = 0.03) AA 1. 6.28 ± 0.51; 5.03 ± 0.37 2. 5.69 ± 0.59; 3.64 ± 0.33 3. 6.54 ± 0.46; 4.23 ± 0.25 4. 5.80 ± 0.50; 6.10 ± 0.46 (p &lt; 0.01)</td>
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<tr>
<td>Pratt, 2002 (55)</td>
<td>Single-blinded, randomized, and placebo controlled</td>
<td>n = 23 advanced cancer patients</td>
<td>14 d</td>
<td>Cancer patients exhibited low concentrations of plasma phospholipids EPA and DHA and elevated concentrations of AA and n-6 FAs in neutrophil phospholipids. Fish-oil capsules raised EPA and DHA concentrations in plasma phospholipids but not in neutrophils. A significant reduction of AA in a neutrophil fraction (phosphatidyl-inositol). Plasma phospholipids (% of total FAs) EPA 1. Baseline: 0.7 ± 0.1; 14 d: 4.4 ± 0.7 (p &lt; 0.05) DHA 1. Baseline: 4.1 ± 0.6; 14 d: 5.8 ± 0.8 (p &lt; 0.05) AA 1. Baseline: 10.2 ± 0.7; 14 d: 9.2 ± 0.8 2. No significant changes compared with baseline values of plasma and neutrophil phospholipids fatty acids (data not shown)</td>
<td>4A</td>
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<tr>
<td>West, 2010 (42)</td>
<td>Double-blind, randomized, and placebo controlled</td>
<td>n = 55 patients who underwent endoscopic surveillance of a retained rectum postcolectomy</td>
<td>6 mo 1. n = 28: EPA capsules (2 x 500 mg/d; mean daily dose: 1.78 ± 0.38 g EPA) 2. n = 27: placebo capsules (capric and caprylic acid MCTs)</td>
<td>Rectal mucosa (%); mean ± SD EPA 1. Baseline: 0.97 ± 0.89; 6 mo: 2.50 ± 1.98; change: 1.56 (95% CI: 0.97, 2.15) 2. Baseline 0.73 ± 0.72; 6 mo: 1.30 ± 1.09; change: 0.54 (95% CI: -0.06, 1.13) p = 0.018 compared with placebo DHA 1. Baseline: 1.92 ± 1.30; 6 mo: 1.71 ± 0.94; change: -0.07 (95% CI: -0.42, 0.28) (p = 0.875 compared with placebo) 2. Baseline: 1.39 ± 0.61; 6 mo: 1.42 ± 1.05; change: -0.11 (95% CI: -0.46–0.24) AA 1. Baseline: 8.47 ± 1.68; 6 mo: 8.82 ± 1.91; change: -0.01 (95% CI: -0.72, 0.69) (p = 0.228 compared with placebo) 2. Baseline: 9.63 ± 2.18; 6 mo: 9.88 ± 1.68; change: 0.61 (95% CI: 0.10, 1.31)</td>
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<td>Anti, 1992 (43)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 24 patients with sporadic adenomatous colorectal polyps</td>
<td>12 wk 1. n = 12: fish-oil capsules (4.1 g EPA + 3.6 DHA) 2. n = 12: placebo (0.8 g olive oil per capsule)</td>
<td>Fish-oil group: significant increase in EPA content of the rectal mucosa over time (p = 0.0016), accompanied by decreases of mucosal LA (p = 0.0001) and AA (p = 0.042). DHA did not show significant changes in fish oil of placebo group. Rectal mucosa (%); mean ± SEM EPA 1. Baseline: 1.3 ± 0.3; week 2: 2.6 ± 0.6; week 12: 3.3 ± 0.6 2. Baseline: 1.5 ± 0.2; week 2: 1.3 ± 0.3; week 12: 1.8 ± 0.1 DHA 1. Baseline: 1.5 ± 0.3; week 2: 1.9 ± 0.3; week 12: 1.8 ± 0.2 2. Baseline: 1.8 ± 0.1; week 2: 1.9 ± 0.2; week 12: 1.6 ± 0.3 AA 1. Baseline: 5.7 ± 0.6; week 2: 5.0 ± 0.8; week 12: 4.4 ± 0.6 2. Baseline: 6.1 ± 0.5; week 2: 6.3 ± 0.8; week 12: 6.2 ± 0.3</td>
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| Yee, 2010 (44)               | Randomize and open label | n = 48 women with increased breast cancer risk | 6 mo  
6. n = 12: 0.47 g EPA + 0.38 g DHA (1 capsule)  
7. n = 12: 1.40 g EPA + 1.13 g DHA (3 capsules)  
8. n = 12: 2.79 g EPA + 2.25 g DHA (6 capsules)  
9. n = 12: 4.19 g EPA + 3.38 g DHA (9 capsules)  
Breast adipose tissue FA concentrations not shown | All doses led to increased serum and breast adipose tissue EPA and DHA concentrations and a decrease in AA. The response to 1 capsule/d was less than the maximum possible response with 3, 6, or 9 capsules/d. Serum (% total fatty acids); mean ± SD  
EPA  
Baseline: 0.63 ± 0.38; 6 mo: 1.52 ± 0.46  
Baseline: 0.57 ± 0.26; 6 mo: 2.75 ± 1.07  
Baseline: 0.96 ± 1.03; 6 mo: 5.65 ± 1.94  
Baseline: 0.68 ± 0.40; 6 mo: 5.98 ± 2.03  
DHA  
Baseline: 1.59 ± 0.62; 6 mo: 2.90 ± 0.72  
Baseline: 1.99 ± 0.84; 6 mo: 4.23 ± 0.86  
Baseline: 2.20 ± 1.38; 6 mo: 5.57 ± 1.43  
Baseline: 2.07 ± 0.77; 6 mo: 6.26 ± 1.64  
AA  
Baseline: 7.72 ± 1.72; 6 mo: 7.11 ± 1.64  
Baseline: 8.27 ± 2.25; 6 mo: 7.04 ± 2.26  
Baseline: 7.89 ± 1.49; 6 mo: 7.25 ± 2.10  
Baseline: 9.07 ± 2.93; 6 mo: 6.88 ± 1.10 | 3A |
**Supplemental Table 1 (continued)**

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<tr>
<td>Bagga, 1997 (45)</td>
<td>Open label</td>
<td>n = 25 patients with high-risk localized breast cancer</td>
<td>3 mo Low-fat diet; 10 fish-oil capsules (1.8 g EPA and 1.2 g DHA/d) and 800 IU vitamin E</td>
<td>Significant reduction of total n–6 FAs in the plasma; total n–3 FAs increased 3-fold; increase in total n–3 FAs in breast adipose tissue and gluteal adipose tissue (data not shown in this table). Plasma FAs (mmol/L): mean ± SEM EPA Baseline: 33.8 ± 6.6; 3 mo: 686.1 ± 82.8 (p = 0.0001) DHA Baseline: 330.8 ± 58.3; 3 mo: 529.2 ± 61.8 (p = 0.001) AA Baseline: 777.8 ± 61.1; 3 mo: 581.5 ± 38.1 (p = 0.0001)</td>
<td>1D</td>
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<tr>
<td>Witte, 2010 (46)</td>
<td>Pilot</td>
<td>n = 11 patients with early stage chronic lymphocytic leukemia</td>
<td>1 mo, fish-oil capsules 1. n = 11: no capsules 2. n = 11: 1.2 g EPA and 0.9 g DHA (3 capsules) 3. n = 9: 2. g EPA and 1.8 g DHA (6 capsules)</td>
<td>n–3 FA concentrations (sum of ALA, EPA, DPA, and DHA) of RBCs and WBCs significantly increased after supplementation: RBCs incorporated a higher fraction of n–3 fatty acids than did WBCs. RBCs - Incorporation of EPA, DPA, and DHA: linear and</td>
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<td>Witte, 2010 (46) (continued)</td>
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<td>n = 36 patients with stage IIB or IV non–small cell lung cancer</td>
<td>4. n = 3: 3.6 g EPA and 2.7 g DHA (9 capsules)</td>
<td>dose responsive increases (p &lt; 0.02) WBCs Incorporation of EPA, DPA, and DHA: not linear and at a slower rate than for RBCs. EPA and DHA were not dose responsive (NS).</td>
<td>1D</td>
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<td>Murphy, 2011 (47)</td>
<td>Open label with contemporaneous control group</td>
<td>n = 46</td>
<td>± 10 wk n = 15: n-3 FAs: 2.2 g EPA + 240–500 mg DHA (4 × 1-g capsules or 7.5 mL liquid fish oil) 2. n = 31: none</td>
<td>Plasma EPA and DHA increased significantly after supplementation of n-3 FAs, not in control group. Plasma phospholipids (%): mean ± SD EPA 1. Baseline: 1.0 ± 0.5; 10 wk: 3.6 ± 1.3 (p &lt; 0.05) 2. Baseline: 0.9 ± 0.6; 10 wk: 1.2 ± 0.6 DHA 1. Baseline: 2.3 ± 0.7; 10 wk: 2.7 ± 1.0 (p &lt; 0.05) 2. Baseline: 2.3 ± 0.8; 10 wk: 2.2 ± 0.6</td>
<td>1D</td>
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<tr>
<td>Aronson, 2001 (48)</td>
<td>Open label</td>
<td>n = 9 patients with untreated prostate cancer</td>
<td>3 mo Reduction of dietary fat intake; 10 fish-oil capsules (1.8 g EPA and 1.2 g DHA/d)</td>
<td>Significant increase in EPA and DHA in plasma and gluteal adipose stores Plasma (mmol/L): mean ± SEM EPA Baseline: 180.3 ± 79.4; 3 mo: 1183.4 ± 180.7</td>
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<td>Aronson, 2001 (48) (continued)</td>
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<td>(p = 0.001) DHA Baseline: 287.8 ± 75.1; 3 mo: 892.8 ± 108.7 (p = 0.001) AA Baseline: 163.9 ± 45.4; 3 mo: 1171.2 ± 91.6 (NS)</td>
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<tr>
<td>Taylor, 2009 (49)</td>
<td>Open label</td>
<td>n = 31 tumor patients</td>
<td>6 wk n = 31: n-3 FAs: 1.5 g/d as soft gel capsules (0.53 g EPA and 0.85 g DHA)</td>
<td>Significant increase in EPA and DHA in plasma phospholipids (p = 0.002 and p = 0.0007) and mononuclear lymphocytes (p = 0.044); only a slight increase in RBCs (NS). Significant decrease of AA in RBCs (p = 0.037) but not in plasma phospholipids and mononuclear lymphocytes. EPA increased between 27% and 70% in all 3 blood compartments; the greatest relative increase was shown in plasma phospholipids (p = 0.002). Median relative DHA change varied from -8% to 35% (p = 0.0007); AA decreased slightly by -2% to -17% (NS).</td>
<td>1D</td>
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Baseline: 287.8 ± 75.1; 3 mo: 892.8 ± 108.7 (p = 0.001) AA Baseline: 163.9 ± 45.4; 3 mo: 1171.2 ± 91.6 (NS)
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<td>Wigmore, 1996 (50)</td>
<td>Open label</td>
<td>n = 38 patients with unresectable adenocarcinoma of the pancreas</td>
<td>3 mo n = 18: 1-g fish oil-capsules (180 mg EPA and 120 mg DHA/d) increased at weekly intervals by 2 g to a maximum dose of 16 g/d (2.9 g EPA and 2.2 g DHA)</td>
<td>EPA group: EPA and DHA in plasma phospholipids increased, AA decreased significantly. Plasma phospholipids (%): median (IQR) EPA Baseline: undetectable; 1 mo: 5.3 (3.5–6.2) (p &lt; 0.05) DHA Baseline: 3.5 (2.7–5.5); 1 mo: 6.6 (4.7–7.9) (p &lt; 0.05) AA Baseline: 13.2 (11.6–19.8); 1 mo: 7.1 (6.6–8.2) (P &lt;0.05)</td>
<td>1D</td>
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<td>Persson, 2005 (51)</td>
<td>Randomized, nonplacebo controlled, and nonblinded</td>
<td>n = 24 patients not amenable to anticancer treatment (with documented weight loss)</td>
<td>4 wk 30 mL fish-oil (4.9 g EPA and 3.2 g DHA) 18 mg melatonin</td>
<td>EPA and DHA in plasma phospholipids increased significantly after fish-oil supplementation. Serum (%); mean ± SD EPA Baseline: 1.3 ± 0.5; 4 wk: 6.0 ± 3.5 (p &lt; 0.001) DHA Baseline: 4.4 ± 1.3; 4 wk: 6.3 ± 1.9 (p &lt; 0.001) AA Baseline: 7.8 ± 1.9; 4 wk: 7.1 ± 1.3 (NS)</td>
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<tr>
<td>Barber, 2001 (52)</td>
<td>Open label and dose-escalation study</td>
<td>n = 5 patients with unresectable pancreatic adenocarcinoma</td>
<td>EPA diester 2 wk: 25 mL (4.5 g EPA) 2 wk: 50 mL (9 g EPA) 2 wk: 100 mL (18 g EPA) 2 wk: 200 mL (36 g EPA) Dose increase if tolerated After 8 wk, emulsion was continued at the choice of the patient; assessments every 4 wk</td>
<td>EPA in plasma phospholipids increased significantly; a less marked increase in EPA in RBCs. Plasma phospholipids (%): median (range) EPA Baseline: 1.5; 4 wk: 10.3 (8.4–17.6); 8 wk: 18.5 (13.6 – 22.6) Plasma phospholipid EPA of 3 subjects after 4 mo (long-term daily dose) Subject 1. 10.7 (13.5 g EPA) Subject 2. 15.3 (18 g EPA) Subject 3. 20.3 (27 g EPA) RBCs (%): median (range) EPA Baseline: 0.8; 8 wk: 7.3 (2.0–13.7) 4 mo Subject 1. 10.5 (13.5 g EPA) Subject 2. 7.2 (18 g EPA) Subject 3. 14.8 (27 g EPA)</td>
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<tr>
<td>Burns, 1999 (53)</td>
<td>Open label and dose-escalation study</td>
<td>FA analyses: n = 1 chronic lymphocytic leukemia (study population: n = 22 patients with neoplastic disease)</td>
<td>2 mo 31 d: fish-oil capsules (1 g per capsule; 0.38 mg EPA/g and 0.25 mg DHA/g); 0.2 g · kg^{-1} · d^{-1}</td>
<td>EPA and DHA in leukemic cells and serum increased. Leukemic cells EPA: 3-fold higher DHA Baseline: 5.6 ± 0.1; after supplementation: 4.0 ± 0.2 (NS) Serum (molar%); mean ± SD EPA: 4-fold increase (no p-value reported)</td>
<td>1D</td>
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<tr>
<td>Wigmore, 2000 (7)</td>
<td>Open label</td>
<td>n = 26 patients with unresectable adenoma of the pancreas</td>
<td>4 wk Fish-oil capsules 1 wk: 1 g EPA 2 wk: 2 g EPA 3 wk: 4 g EPA Thereafter: 6 g EPA</td>
<td>EPA in plasma phospholipids increased significantly; AA decreased significantly. Plasma phospholipids (%); mean (IQR) EPA Week 0: 0 (0–0); 4 wk: 10.0 (9.0–14.0) (p = 0.03 compared with week 0) AA Week 0: 8.6 (5.8–9.2); 4 wk: 6.1 (4.8–6.8) (p &lt; 0.05 compared with week 0)</td>
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| Higashihara, 2010 (41)         | Randomized, nonblinded, and controlled | n = 62 prostate cancer patients who underwent prostatectomy | 2 y  
1. n = 32: 2.4 g EPA ethyl ester  
2. n = 30: Control (none) | EPA and DHA in the total phospholipid fraction in RBCs significantly increased, and AA and DHA decreased, in the EPA group. EPA, AA, and DHA did not change in the control group.  
RBCs (area%); mean ± SD; baseline; 4 mo; 24 mo; at recurrence  
EPA  
1. 2.7 ± 1.0; 6.1 ± 1.4; 5.8 ± 1.2; 6.1 ± 0.9 (p < 0.001 after 4 and 24 mo)  
2. 2.6 ± 1.2; 2.6 ± 1.0; 2.3 ± 1.0; 3.0 ± 1.7  
DHA  
1. 8.0 ± 1.1; 6.6 ± 1.3; 6.3 ± 1.0; 7.3 ± 2.0 (p < 0.001 after 4 and 24 mo)  
2. 8.0 ± 1.1; 8.1 ± 1.2; 7.7 ± 1.1; 9.0 ± 0.9  
AA  
1. 9.7 ± 1.2; 7.6 ± 1.2; 7.1 ± 1.0; 7.7 ± 0.9 (p < 0.001 after 4 and 24 mo)  
2. 9.8 ± 1.3; 9.6 ± 1.6; 9.3 ± 1.3; 9.2 ± 0.7 | 2D      |
### n-3 PUFAs in cancer, surgery and critical care

#### Supplemental Table 1 (continued)

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<tr>
<th>First author, Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
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</thead>
<tbody>
<tr>
<td>Purasiri, 1994 Phase II</td>
<td>n = 14 patients with advanced colorectal cancer</td>
<td>15 d before surgical treatment, n-3 capsules in increasing doses: 15 d: 1.056 g EPA, 0.160 g DHA, and 1.168 g GLA; 15 d: 1.584 g EPA, 0.240 g DHA, and 1.752 g GLA; 150 d: 2.112 g EPA, 0.320 g DHA, and 2.336 g GLA</td>
<td>Significant increase in serum concentrations of GLA, EPA and DHA in serum triacylglycerol, cholesterol and phospholipids. Serum phospholipids (%): mean ± SEM; day 0; day 15; day 30; month 6</td>
<td>0D</td>
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</table>

#### Notes:

- AA, arachidonic acid; DPA, docosapentaenoic acid; GLA, γ-linolenic acid; MCT, medium-chain triglyceride; ONS, oral nutritional supplement; WBC: white blood cell.

- AA: significantly different from baseline.

- Day 0, 15, 30, and month 6: serum phospholipids (%): mean ± SEM; day 0; day 15; day 30; month 6.

- EPA: 1.59 ± 0.24; 3.05 ± 0.76; 5.45 ± 0.68; 6.54 ± 0.92

- DHA: 5.27 ± 0.56; 5.36 ± 0.30; 5.33 ± 0.23; 6.63 ± 0.34

- GLA: 11.24 ± 0.35; 10.57 ± 0.60; 10.98 ± 0.97; 9.68 ± 0.68
Supplemental Table 2: Incorporation of n–3 fatty acids (FAs) in blood after supplementation of n–3 FAs in patients undergoing

<table>
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<th>First author, Year (reference)</th>
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</thead>
<tbody>
<tr>
<td>Kenler, 1996 (24)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 50 patients with upper gastrointestinal cancer</td>
<td>7 d postoperative 1. n = 17: n–3 FA enteral nutrition (4.0 g EPA and 1.9 g DHA) 2. n = 18: isocaloric, isonitrogenous control enteral nutrition</td>
<td>Increase of EPA and EPA:AA ratio and decrease of n–6:n–3 ratio (p &lt; 0.01) in plasma triglycerides, phospholipids, and RBC membrane phospholipids in n–3 FA patients; no significant differences between groups for DHA. Plasma phospholipids (% of total FA): mean ± SEM EPA 1. Day 1: 0.60 ± 0.15; day 7: 11.33 ± 1.37 (p &lt;0.01) 2. Day 1: 0.76 ± 0.14; day 7: 0.57 ± 0.08 DHA 1. Day 1: 3.10 ± 0.23; day 7: 3.62 ± 0.22 2. Day 1: 3.56 ± 0.34; day 7: 5.73 ± 2.80 Plasma triglycerides (% of total FA): mean ± SEM EPA 1. Day 1: 0.12 ± 0.05; day 7: 5.50 ± 1.17 (p &lt; 0.01) 2. Day 1: 0.18 ± 0.05; day 7: 0.18 ± 0.09 DHA 1. Day 1: 0.37 ± 0.11; day 7: 2.03 ± 0.41 2. Day 1: 0.90 ± 0.36; day 7: 0.34 ± 0.07</td>
<td>2B</td>
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**Enteral Surgical oncology**
Supplemental Table 2 (continued)

<table>
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<th>First author, Year (reference)</th>
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<tbody>
<tr>
<td>Kenler, 1996 (24)</td>
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<td>RBC membrane (% of total FA): mean ± SEM EPA</td>
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<td>1. Day 1: 0.31 ± 0.12; day 7: 2.59 ± 0.39 (p &lt; 0.01)</td>
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<td>2. Day 1: 0.42 ± 0.11; day 7: 0.40 ± 0.11</td>
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<td>DHA</td>
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<td>1. Day 1: 2.52 ± 0.39; day 7: 3.29 ± 0.43</td>
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<td>2. Day 1: 3.44 ± 0.45; day 7: 3.41 ± 0.43</td>
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<tr>
<td>Gee, 1999 (56)</td>
<td>Single-blinded, randomized, and controlled</td>
<td>n = 50 colon cancer patients who required surgery</td>
<td>7–21 d before surgery</td>
<td>Significant increase in EPA, EPA + DHA: linoleic acid ratio (p &lt; 0.001) and n−3:n−6 ratio (p &lt; 0.005) in colonic mucosa in the fish-oil group, DHA slightly increased (NS)</td>
<td>3C</td>
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<td>1. n = 25: fish-oil capsules (1.4 g EPA and 1.0 g DHA)</td>
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<td>2. n = 24: 2-g safflower oil capsules</td>
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<td>Colonic mucosa (% of weight); mean ± SEM EPA</td>
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<td>1. »1.0 (p &lt; 0.001)</td>
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<td>2. »0.5</td>
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<td>Mesenteric adipose tissue (% of weight) EPA</td>
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<td>1. 0.064 ± 0.007 (NS)</td>
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<td>2. 0.074 ± 0.017</td>
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</table>
| Ryan, 2009 (26) | Double-blinded, randomized, and placebo controlled | n = 53 patients with esophageal cancer who underwent and esophagectomy | 5 d preoperative (oral nutritional supplements) to 21 d postoperative (enteral nutrition) 1. n = 28: n-3 FA ONSs or enteral nutrition (2.3 g EPA and 0.95 g DHA) 2. n = 25: control ONSs or enteral nutrition | EPA concentrations increased in serum and PBMC membranes.  
Serum (%); mean EPA  
1. Baseline: 1.2; day 7: 3.2 (p = 0.04); day 14: 2.8 (p = 0.05)  
PBMCs (%)  
EPA  
1. Baseline: 0.3; day 7: 1.7 (p = 0.005)  
2. Baseline 0.7; day 7: 0.7 (p = 0.174) | 5A |

**General surgery: 0 studies**
Supplemental Table 2 (continued)

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<td><strong>Surgical oncology</strong></td>
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<tr>
<td>Linseisen, 2000 (61)</td>
<td>Double-blinded, randomized, and controlled</td>
<td>n = 33 patients who underwent major abdominal surgery for upper and lower gastrointestinal cancer, adenomas, or stenosis of the small intestine</td>
<td>5 d postoperative 1.4 g fat/kg body weight 1. n = 17: 20% n-3 FA fat emulsion, 3.1% EPA, and 2.3% DHA (Lipoplus® B. Braun) Mean body weight: 75 kg Mean dose 1.4 g/kg × 75 kg × 3.1% = 3.3 g EPA 1.4 g/kg × 75 kg × 2.3% = 2.4 g DHA 2. n = 16: 20% soybean oil emulsion (Intra-lipid® Fresenius Kabi)</td>
<td>After 5 d, plasma phospholipid EPA was significantly higher in the n-3 lipid group (p ≤ 0.05, difference between groups); DHA was not significantly different. Plasma phospholipids (molar %); mean ± SEM EPA 1. Day 1: 0.65 ± 0.006; day 6: 2.07 ± 0.33 2. Day 1: 0.66 ± 0.10; day 6: 0.77 ± 0.20 DHA 1. Day 1: 5.05 ± 0.48; day 6: 5.68 ± 0.98 2. Day 1: 5.67 ± 0.66; day 6: 5.72 ± 0.67</td>
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Supplemental Table 2 (continued)

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<tbody>
<tr>
<td>Roulet, 1997 (62)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 19 surgical patients with esophageal cancer who underwent an elective total esophagectomy</td>
<td>7 d postoperative 1. n = 10: fish-oil emulsion (Omegaven® Fresenius Kabi); 0.028 g/kg body weight EPA and 0.028 g/kg body weight DHA Mean body weight: 65 kg Mean dose 0.028 g/kg × 65 kg = 1.8 g EPA 0.028 g/kg × 65 kg = 1.8 g DHA 2. n = 9: 20% soybean-fat emulsion (Lipovenoes® Fresenius Kabi)</td>
<td>Large increase in platelet phosphatidylcholine and phosphatidylethanolamine EPA. DHA did not change significantly. EPA:AA ratios doubled in patients in the fish-oil group in platelet phospholipids. ALA: no sign of change during lipid infusion. Peak at days 8 and 15; values still significantly increased. Platelet phosphatidylcholine (% of weight) EPA Peak at day 8 (&gt;1.5 compared with &gt;0.3) Day 15: &gt;0.7 compared with &gt;0.5. Values were still different from day before operation (p &lt; 0.01) DHA Peak at day 8 (2.0 compared with 1.3) (NS) Day 15: &gt;1.8 compared with &gt;1.3 (NS) (5 platelet phospholipids measured, similar results for FAs)</td>
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### Supplemental Table 2 (continued)

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<td><strong>General surgery</strong></td>
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<tr>
<td>Senkal, 2007 (33)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 40 patients who underwent colorectal surgery</td>
<td>5 d postoperative 1. n = 19: 20% lipid emulsion with n-3 FAs (Lipoplus® B. Braun); Days 1 and 2: 2 g fish oil Days 3–5: 4 g fish oil 2. n = 21: 20% control MCT and LCT lipid emulsion (Lipofundin® B. Braun)</td>
<td>EPA and DHA of plasma phospholipids significantly increased at days 6 and 10 in the n-3 group but not in the control group; n-3 group: n-3:n-6 ratio significantly higher at days 6 and 10 (data not shown). EPA and EPA:AA ratio of RBC membranes significantly increased on days 6 and 10; DHA did not show differences between groups. Plasma phospholipids (%) EPA 1. Day 1: »1; day 6: »7; day 10: »3.5 (p &lt; 0.05) 2. Day 1: »1; day 6: »1; day 10: »1 DHA 1. Day 1: »8; day 6: »11.5; day 10: »11 (p &lt; 0.05) 2. Day 1: »8; day 6: »8; day 10: »8 AA 1. Day 1: »14; day 6: »10.5; day 10: »11 (NS) 2. Day 1: »13.5; day 6: »11.5; day 10: »12.5</td>
<td>5A</td>
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Supplemental Table 2 (continued)

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<th>First author, Year (reference)</th>
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<td>Senkal, 2007 (33) (continued)</td>
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<td>RBC membranes (%)</td>
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<td>EPA</td>
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<td>1. Day 1: »0.5; day 6: »2; day 10: »2 (p &lt; 0.05)</td>
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<td>2. Day 1: »0.5; day 6: »0.7; day 10: »0.9</td>
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<td>DHA</td>
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<td>1. day 1: »3; day 6: »6; day 10: »7.5 (NS)</td>
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<td>2. day 1: »3; day 6: »5.5; day 10: »6</td>
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<td>AA</td>
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<td>1. Day 1: »14; day 6: »12; day 10: »11.5 (NS)</td>
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<td>2. Day 1: »14; day 6: »11; day 10: »12.5</td>
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<tr>
<td>Wichmann, 2007 (32)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 256 patients with elective abdominal surgery</td>
<td>5 d postoperative Days 1 and 6: 0.7 g fat/kg body weight Days 3–5: 1.4 g fat/kg body weight</td>
<td>Plasma phospholipid EPA significantly increased, and the EPA:AA ratio decreased after 5 d. AA decreased significantly in both groups. Plasma phospholipids (molar %) EPA</td>
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<td>1. n = 127: 10% fish oil, 50% MCTs, and 40% LCTs (Lipoplus® B. Braun)</td>
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<td>2. n = 129: control lipid emulsion (Intralipid® Fresenius Kabi)</td>
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<td>Grimm, 2006 (27)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 33 patients with major abdominal surgery</td>
<td>5 d postoperative 1.5 g fat/kg body weight 1. n = 19: 20% lipid emulsion (SMOFlipid® Fresenius Kabi); 4.7 g EPA/L and 5.3 g DHA/L Mean body weight: 63 kg Mean dose (63 kg × 1.5 g/kg) ÷ 200 g/L = 0.4725 L SMOF 4.7 g/L × 0.4725 L = 2.2 g EPA 5.3 g/L × 0.4725 L = 2.5 g DHA 2. n = 14: control soybean oil emulsion (Lipovenoes® Fresenius Kabi)</td>
<td>EPA, DHA, and ratio of n-3:n-6 FAs were profoundly elevated in the n-3 group. Plasma phospholipids (molar %); mean ± SD EPA 1. Baseline: 0.64 ± 0.33; day 6: 3.32 ± 0.97 (p &lt; 0.05) 2. Baseline: 0.63 ± 0.41; day 6: 0.44 ± 0.18 DHA 1. Baseline: 5.05 ± 1.46; day 6: 6.88 ± 1.81 (p &lt; 0.05) 2. Baseline: 4.95 ± 0.94; day 6: 3.75 ± 0.80 AA 1. Baseline: 6.51 ± 1.00; day 6: 5.02 ± 0.58 (p &lt; 0.05) 2. Baseline: 6.56 ± 2.08; day 6: 4.31 ± 1.09 Phospholipid FAs in leukocytes and platelets similar to those seen in plasma phospholipids (results not shown)</td>
<td>1B</td>
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</tbody>
</table>

1AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; GLA, γ-linolenic acid; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; ONS, oral nutritional supplement; PBMC, peripheral blood mononuclear cell; RBC, red blood cell; SMOF, soybean oil, medium-chain triglycerides, olive oil and fish oil; WBC, white blood cell.

2p-values of differences compared with baseline.
### Supplemental Table 3: Incorporation of n–3 fatty acids (FAs) in blood after supplementation of n–3 FAs in critical care

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<tr>
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<tbody>
<tr>
<td>Gadek, 1999 (23)</td>
<td>Randomized, double-blinded, and placebo controlled</td>
<td>n = 146 ARDS caused by sepsis or pneumonia, trauma, or aspiration injury</td>
<td>≥4–7 d</td>
<td>EPA and EPA:AA ratio in plasma phospholipids increased from baseline to day 4 and further increased to day 7. Significant differences compared with baseline and compared with control group. Plasma phospholipids (%) EPA 1. Baseline: ≥0.3; day 4: ≥7; day 7: ≥8.4 (p &lt; 0.001) 2. Baseline: ≥0.6; day 4: ≥0.2; day 7: ≥0.2</td>
<td>3B</td>
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<tr>
<td>Mayer, 2003 (63)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 21 patients with sepsis who required parenteral nutrition</td>
<td>5 d</td>
<td>Significant increase of EPA in mononuclear leukocyte membranes, and rapid decrease after cessation of infusion regimen. Concentrations of n–3 FA remained largely unchanged in the control group (p-value of comparison between groups). Mononuclear leukocyte membrane (mmol/L) EPA 1. Day 1: ≥1; day 4: ≥35 (p &lt; 0.01). Washout: within 5 d after cessation.</td>
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Supplemental Table 3 (continued)

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<tbody>
<tr>
<td>Mayer, 2003 (63) (continued)</td>
<td>Nonblinded, randomized, and placebo controlled</td>
<td>n = 10 patients with sepsis who required parenteral nutrition</td>
<td>10 d 400-mL fat emulsion</td>
<td>2. Day 1: »3; day 4: »0 DHA 1. Day 1: »26; day 4: »66 (p &lt; 0.05). Washout: ≤ 5 d after cessation. 2. Day 1: »19; day 4: »25 AA 1. Day 1: »38; day 4: »37; day 11: »31 (NS) 2. Day 1: »37; day 4: »31; day 11: »57</td>
<td>1B</td>
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<tr>
<td>Mayer, 2003 (64)</td>
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<td>1. n = 5: 10% n-3 lipid emulsion (Omegaven® Fresenius Kabi); 7.5 g EPA and 6.6 g DHA 2. n = 5: 10% n-6 lipid emulsion (Lipoven® Fresenius Kabi)</td>
<td>Marked increase in plasma EPA and DHA in the fish-oil group. Plateau after 7 and 10 d. EPA and DHA sum surpassed the AA concentration ×2-fold. AA was markedly higher in both infusion groups than in healthy control subjects at baseline. Plasma free fatty acids (mmol/L) 1. EPA: Day 1: 5.6 ± 1.5; day 10: 121.9 ± 31.0 (p &lt; 0.05) 2. DHA: Day 1: 2.6 ± 1.2; day 10: 3.5 ± 3.5</td>
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### Supplemental Table 3 (continued)

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<td>AA</td>
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<td>1. Day 1: 111.8 ± 37.7; day 10: 173.2 ± 62.9 (p &lt; 0.05)</td>
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<td>2. Day 1: 42.7 ± 9.7; day 10: 66.4 ± 19.8</td>
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<tr>
<td>Berger, 2008 (40)</td>
<td>Double-blinded, randomized, and placebo controlled</td>
<td>n = 24 patients admitted to the surgical ICU after abdominal aorta aneurysm surgery</td>
<td>4 d 1: n = 12: fish-oil that contained lipid emulsion (Lipoplus® B. Braun; 20 g fat/L, of which 10.45 g/L EPA + DHA) Mean body weight: 72.5 kg Mean daily dose: 0.15 g fat/kg body weight: 0.15 g/kg × 72.5 kg = 10.9 g fat, 5.69 g EPA + DHA 2. n = 12: MCT and LCT lipid emulsion (Lipofundin® B. Braun)</td>
<td>Plasma phospholipid concentrations of EPA and DHA increased from days 2 to 4 in the FO group compared with at baseline, although not in the control group. AA did not differ in time or between groups.</td>
<td>2B</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Plasma phospholipids (mg/L) EPA 1. Baseline: 4.6 ± 1.9; day 4: 30.5 ± 8.2 (p &lt; 0.001) 2. Baseline: 4.5 ± 1.9; day 4: 4.8 ± 1.7</td>
<td></td>
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<tr>
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<td></td>
<td>DHA 1. Baseline: 26.9 ± 11.6; day 4: 50.0 ± 12.6 (p &lt; 0.001) 2. Baseline: 25.4 ± 9.9; day 4: 31.4 ± 4.0</td>
<td></td>
</tr>
</tbody>
</table>
### Supplemental Table 3 (continued)

<table>
<thead>
<tr>
<th>First author, Year (reference)</th>
<th>Design</th>
<th>Condition</th>
<th>n-3 (duration, form, and daily dose)</th>
<th>Results</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbosa, 2010 (37)</td>
<td>Randomized, single-blinded, and placebo controlled</td>
<td>n = 25 patients with sepsis</td>
<td>6 d n = 13: MCTs, soybean oil, fish oil 50:40:10: Lipoplus® B. Braun n = 10: MCTs and soybean oil (50:50): Nutriflex LipidSpecia® B. Braun</td>
<td>EPA in plasma phosphatidylcholine increased in the n-3 FA group; concentrations were higher at day 6 than at admission ($p &lt; 0.001$), day 1 ($p &lt; 0.001$), and day 2 ($p = 0.003$). EPA was higher in the fish-oil group than in the n-3 FA group at day 6 ($p &lt; 0.001$). DHA and AA did not differ between the 2 groups. Plasma phosphatidylcholine (mg/mL) EPA 1. Baseline: »10; day 1: »10; day 2: »12; day 6: »32 2. Baseline: »10; day 1: »10; day 2: »7; day 6: »8 DHA 1. Baseline: »40; day 1: »40; day 2: »38; day 6: »42 2. Baseline: »47; day 1: »46; day 2: »45; day 6: 38 AA 1. Baseline: »100; day 1: »100; day 2: »90; day 6: »100 2. Baseline: »130; day 1: »140; day 2: »125; day 6: »100</td>
<td>2A</td>
</tr>
</tbody>
</table>

1 AA, arachidonic acid; ARDS, acute respiratory distress syndrome; FO, fish oil; GLA, γ-linolenic acid; ICU, intensive care unit; LCT, long-chain triglyceride, MCT, medium-chain triglyceride.

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n-3 PUFAs in cancer, surgery, and critical care
Appendix: Example of search strategy in PubMed; effects of enteral supplementation of n−3 fatty acids on the clinical outcome in cancer, surgery, or critical care

<table>
<thead>
<tr>
<th>Search</th>
<th>Subject</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>n−3 Fatty acids</td>
<td>115,051</td>
</tr>
<tr>
<td>#2</td>
<td>Cancer</td>
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</tr>
<tr>
<td>#3</td>
<td>Surgery</td>
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<tr>
<td>#4</td>
<td>Critical care</td>
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<tr>
<td>#5</td>
<td>#2 OR #3 OR #4</td>
<td>2,943,025</td>
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<tr>
<td>#6</td>
<td>Clinical outcome variables</td>
<td>2,595,002</td>
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<tr>
<td>#7</td>
<td>Enteral nutrition</td>
<td>323,729</td>
</tr>
<tr>
<td>#8</td>
<td>#1 AND #5 AND #7</td>
<td>794</td>
</tr>
</tbody>
</table>

*Medical subject headings and key words are described in Table 1.*
References


Precachexia and cachexia
at diagnosis of stage III non-small cell lung carcinoma:
an exploratory study comparing two consensus-based frameworks

BS van der Meij
CP Schoonbeek
EF Smit
M Muscaritoli
PAM van Leeuwen
JAE Langius

British Journal of Nutrition 2012 Nov 16: 1-9
Abstract

Despite the development of consensus-based frameworks to define cancer cachexia, the validity and usefulness of these frameworks are relatively unknown. Our aim was to study the presence of precachexia and cachexia in patients with stage III non-small-cell lung carcinoma (NSCLC), by using a cancer-specific framework and a general framework for cachexia and to explore the prognostic value of precachexia and cachexia. In 40 patients at diagnosis of stage III NSCLC, weight loss, FFM, handgrip strength, anorexia and serum biochemistry, assessed before the first chemotherapy were used to define ‘cancer cachexia’ or ‘cachexia’. The cancer-specific framework also classified for precachexia and refractory cachexia. Additionally, quality of life was assessed by the EORTC-QLQC30. Groups were compared by independent t-tests, ANOVA, Kaplan Meier and Cox survival analyses. Based on the cancer-specific framework, precachexia was present in 9 patients (23%) and cancer cachexia was present in 7 patients (18%). Cancer cachexia was associated with a reduced quality of life ($p = 0.03$) and shorter survival (HR = 2.9; $p = 0.04$). When using the general framework, cachexia was present in 11 patients (28%), and associated with a reduced quality of life ($p = 0.08$) and shorter survival (HR = 4.4; $p = 0.001$). In conclusion, precachexia and cachexia are prevalent in this small population of patients at diagnosis of stage III NSCLC. For both frameworks, cachexia appears to be associated with a reduced quality of life and shorter survival. Further studies are warranted to more extensively explore the validity and prognostic value of these new frameworks in cancer patients.
**Introduction**

Cachexia is a complex metabolic syndrome characterised by ongoing loss of body weight and skeletal muscle mass, which cannot be fully reversed by conventional nutritional support (1). The pathophysiology of cachexia encompasses a negative protein and energy balance, driven by a variable combination of reduced food intake and abnormal metabolism. Cachexia is frequently observed in patients with cancer, and is associated with progressive functional impairment, intolerance to anticancer treatment and shorter survival (1-3).

The severity of cachexia in patients with cancer varies from non-symptomatic inflammatory derangements and minimal weight and muscle loss in the early stage, to severe muscle wasting and low performance status in patients not responding to anticancer treatment (4).

In order to define and stage cachexia, a number of frameworks in patients with chronic diseases (1;5) and cancer (4;6-8) have been described. Recently, an international expert group proposed a conceptual framework for cancer cachexia, with a classification for 3 stages of clinical relevance: precachexia, cachexia and refractory cachexia (4). Overall, existing instruments use slightly different nutritional and inflammatory parameters and cut-off points to define precachexia and cachexia.

Despite the growing understanding of the pathophysiology and staging of cachexia, assessment of cachexia in clinical practice is limited. These studies clearly showed the occurrence of weight loss and features of cachexia in patients with cancer.

In patients with lung cancer, high prevalences of involuntary weight loss have been reported (9-11). Lung cancer is frequently associated with cachexia. Weight loss in patients with lung cancer was associated with systemic inflammation, loss of muscle mass, an increased acute-phase response, decreased levels of the anabolic hormone insulin-like growth factor-I (12) and hypermetabolism (12;13). Weight loss was also associated with reduced quality of life (14), response to chemotherapy (15) and survival (9;16) in patients with lung cancer.

The staging of cachexia in patients with lung cancer has not been described, but could help clinicians to decide on early interventions or cachexia treatment. Up to now, the validity and usefulness of cachexia instruments in patients with cancer is unknown, and the recognition and nutritional management of cancer cachexia remains unsatisfactory (1).

Comprehensive data of cancer populations could give more insight in the pathophysiology of (pre)cachexia, and could be used to apply cachexia frameworks, and investigate the
outcomes and differences between frameworks. Therefore, we aimed to retrospectively study the presence of (pre)cachexia at diagnosis of stage III non-small cell lung cancer (NSCLC), using recently described consensus-based frameworks (1;4;5), and to explore the prognostic value of precachexia and cachexia. Secondly we explored quality of life, nutritional and inflammatory parameters associated with (pre)cachexia. We hypothesize that (pre)cachexia is present in this locally advanced patient population, and that cachexia is associated with a decreased quality of life and shorter survival.

Materials and methods

Patients
Between March 2005 and October 2007, 40 patients with histological or cytological proven stage III NSCLC, 18–80 years of age and a life expectancy of at least 3 months were included at the start of concurrent chemoradiotherapy. Patients were excluded if they had undergone surgery, chemotherapy, or radiotherapy during the previous month; if they had oedema, ascites, or severe comorbidities; or if they used high-dose corticosteroids or fish oil supplements. Data used for this retrospective analysis were collected at the inclusion for a prospective double blind RCT that has been carried out in our centre in 2005 to 2008. Out of fifty-five enrolled patients, four patients did not meet inclusion criteria, nine patients refused to participate, and 2 had disease progression (Supplemental Figure 1). We used the baseline and survival data of 40 patients, irrespective of the intervention in the trial. After carrying out baseline measurements, patients were randomly assigned to receive 2 cans per day of either a protein- and energy-dense oral nutritional supplement containing n-3 polyunsaturated fatty acids or an isocaloric control oral nutritional supplement during 5 weeks of chemoradiotherapy (17).
Throughout chemoradiotherapy, the dietician monitored dietary intake and provided dietary counselling. Tube feeding was indicated in case of (expected) oral intake < 75% of energy requirements for more than 3 days, combined with the inability to increase energy intake by oral food or sip feeds.
This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human patients were approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam, The Netherlands. Written informed consent was obtained from all patients.
Precachexia and cachexia at diagnosis of stage III NSCLC

Baseline measurements
At baseline, before the start of chemoradiotherapy, weight loss, BMI, fat-free mass (FFM), energy expenditure, anorexia, inflammation, muscle strength, quality of life and physical activity were assessed.

Weight loss and BMI
Pre-illness weight, unintentional weight loss in the last month and last 6 months and height were recorded. Body weight, without shoes and wearing light clothing, was measured on a compact digital flat scale (SECA 888) to the nearest 0.2 kg. Body Mass Index (BMI) was calculated by dividing body weight (kg) by the square of the height (m).

Fat-free mass
Bioelectrical impedance spectroscopy (BIS, Hydra 4200, Xitron Technologies) was performed to assess FFM. Whole-body resistance was measured with four surface electrodes placed on the right wrist and ankle, as previously described (18). Briefly, the principle was based on the application of a variable electrical current between 50 µA and 700 µA produced by a generator and applied to the skin using adhesive electrodes (3M red Dot Ag/AgCl) with the subject lying supine (19). FFM was calculated from resistance and reactance at the frequency of capacitance by using the Kyle Geneva equation (20). The phase angle of bioelectrical impedance at 50 kHz was calculated using the following equation: phase angle = (resistance/reactance) x (180/π). The cut-off point for patients with lung cancer, described by Gupta and colleagues, was used to classify patients with a low (≤ 5.3) and high (> 5.3) phase angle (21).

Energy expenditure
Resting energy expenditure (REE) was measured by a ventilated hood system (Deltatrac, Datex); CO₂ production and O₂ consumption were measured at complete rest during a period of 30 minutes. REE was calculated using a modified Weir equation (22;23). To estimate total energy expenditure (TEE), 30% was added to REE, assuming a physical activity level of 1.3 for sedentary patients with cancer (24).

Anorexia
Patients recorded their appetite on a visual analogue scales (VAS), 10 cm in length (25). Patients’ energy intake, assessed by a 24-h dietary recall, was expressed as percentage of TEE. Anorexia and/or reduced food intake were identified by the presence of either:
appetite < 5 cm (VAS), energy intake < 84 kJ / kg body weight per day (20 kcal / kg) (11), or energy intake < 70% of TEE (1).

**Inflammation**

Non-fasting blood samples were taken simultaneous with usual blood samples for chemotherapy. Plasma concentrations of C-reactive protein (CRP) were measured with an automated latex-enhanced immunoturbidimetric assay on a Modular P analyser [reference: 0 - 8 mg/L] (26). Serum IL-6 was measured by commercially available ELISA (Pelikine compact human ELISA kits, Sanquin) [reference: 0 - 4 pg/ml]. Whole blood haemoglobin (Hb) was determined by spectrofotometry on a Cell-Dyn Sapphire analyzer [reference: ≥ 7.3 mmol/L or ≥ 11.7 g/dL] (27). Serum albumin concentrations were chemically determined on a Modular P analyzer (ACN 760, 11815148 216, Roche Diagnostics, Almere, The Netherlands) [reference: ≥ 32 g/dL] (28).

**Muscle strength**

Muscle strength was measured by handgrip strength in the non-dominant hand using a hydraulic hand dynamometer (Baseline, Fabrication Enterprises, USA). The patient performed 2 maximal isometric contractions while sitting, with the shoulder adducted and neutrally rotated, elbow flexed at 90 degrees, the forearm and wrist in neutral position. The average of 2 measurements was recorded, and compared with age- and sex dependent reference values for handgrip strength (29).

**Additional parameters**

We assessed additional parameters, which could be related to cancer cachexia, such as quality of life, physical activity level and survival. The investigator recorded the Karnofsky Performance Score, a valid and widely used instrument to quantify the functional status of cancer patients. The Karnofsky Performance Score ranges from 0 to 100, with a higher score indicating a better ability to carry out normal daily activities and work (30;31). Patients filled out the EORTC-QLQC30 questionnaire, a multidimensional validated cancer specific measure that includes global health status, physical status, functional and symptom scales (i.e. fatigue) (17;18;12). Physical activity was assessed by the PAM (Physical Activity Monitor) accelerometer. Patients were instructed to wear the PAM for 7 consecutive days on the hip (model AM101, 28 g, 59x43x10mm, PAM B.V., the Netherlands) (32). The PAM produces a single
index score, which is a proxy measure of total daily physical activity. Every 3 points of the physical activity score reflects about 10 minutes walking. The PAM also produces minutes of low and moderate intensity activities; low intensity physical activity corresponds with small in-house movements; moderate intensity corresponds with walking (33).

Definition of precachexia and cachexia
We used two consensus-based frameworks to define cachexia; a cancer-specific and a not disease-specific general framework. With the cancer-specific framework we defined precachexia, cancer cachexia and refractory cancer cachexia, as proposed by respectively the ESPEN Special Interest Group (SiG) ‘cachexia-anorexia in chronic wasting diseases’ (1) and an international panel of experts in clinical cancer cachexia research (4). Second, we used the general framework for cachexia in chronic illness, as described by Evans and colleagues) (5).

Cancer-specific framework for cachexia
Cancer precachexia (1):
1. Unintentional weight loss of 0 to ≤5% during the previous 6 months
2. Anorexia (the presence of either*: appetite < 5 cm (VAS), energy intake < 84 kJ / kg body weight per day (20 kcal/kg) (11), or energy intake < 70% of TEE (1))
3. Systemic inflammation (CRP ≥ 8 mg/L, the upper limit of normality*)

Cancer cachexia (4):
1. Weight loss > 5% during the previous 6 months, or BMI < 20 kg/m² and weight loss > 2%, or sarcopenia (FFM index < 5th percentile of age and sex specific reference values (34) and weight loss > 2%)
2. Reduced food intake (the presence of either*: appetite < 5 cm (VAS), energy intake < 84 kJ / kg body weight per day (20 kcal / kg) (11), or energy intake < 70% of TEE (1))
3. Systemic inflammation (CRP ≥ 8 mg/L, the upper limit of normality*)

Refractory cancer cachexia (4):
1. Variable degree of ‘cancer cachexia’
2. Cancer disease both procatabolic and not responsive to anticancer treatment
3. Low performance score (Karnofsky Performance Score < 50*, indicating a patient is unable to care for self)
< 3 months expected survival

**General framework**
The not disease-specific general framework for cachexia (5) includes the combination of weight loss of ≥ 5% in 6 months or BMI < 20 kg/m², combined with at least 3 of the following 5 criteria:

1. Decreased muscle strength
   Handgrip strength below the lowest tertile extracted from age- and sex specific reference values (29).
2. Fatigue (score of 3 or 4 according to the EORTC QLQ-C30 symptom scale (12))
3. Anorexia (the presence of either: appetite < 5 cm (VAS)*, energy intake < 84 kJ / kg body weight per day (20 kcal / kg) (11), or energy intake < 70% of TEE (1))
4. FFM index below the 10th percentile by age- and sex specific reference values (13)
5. One or more abnormal serum biochemistry parameters: CRP > 5 mg/L, Hb < 12 g/dL or 11.7 g/dL, serum albumin < 32 g/L, or IL-6 > 4 pg/mL (6).

* Parameter(s) and cut-off point(s) not described in frameworks, and therefore retrieved from the available literature, and if necessary from experts

**Statistical analysis**
Statistical analysis was performed using SPSS for Windows (version 17.0, SPSS inc. United Kingdom). Groups with no cachexia, precachexia and cachexia were compared for serum biochemistry, REE, quality of life and physical, role, emotional, cognitive and social functioning. Independent samples t-tests were performed to compare groups with no cachexia and cachexia. For variables, which were not normally distributed, non-parametric tests were performed to compare group differences. Frequencies within groups for nominal characteristics were compared by Pearson chi-square tests. Differences between 3 groups (no cachexia, precachexia and cachexia) were tested by one-way ANOVA. Correlations between variables were investigated by Pearson correlation tests.

Group survival, from the date of the start of concurrent chemoradiotherapy (from March 15th 2005 until October 30th 2007) until death or follow-up visit (November 17th 2011), was generated by the method of Kaplan and Meier and compared by means of the log-rank test. Secondly, the multivariate Cox’s regression proportional hazards model was used to analyze hazard ratios (HR) for survival. Cachexia was the independent variable, and the model was adjusted for confounding factor(s) (based on a > 10% change of OR, after
Precachexia and cachexia at diagnosis of stage III NSCLC

adding a single factor: sex, age and/or tumour stage: IIla vs. IIlb). Median survival was displayed ± SE; p-values < 0.05 were considered to be statistically significant.

Results

Patients
Forty patients with histological or cytological proven stage IIla (n = 16) or stage IIlb (n = 24) NSCLC were studied, 19 females and 21 males, with a median age of 57 y (range 39-80). The average amount of weight loss during the previous 6 months was 1.9 ± 6.5% of pre-illness weight. The overall median survival was 25.0 ± 8.7 months. Baseline patient characteristics are displayed in Table 1.

Cancer-specific framework
Using the two consensus-based frameworks of the ESPEN SIG and Fearon and colleagues, we classified precachexia in 9 patients (23%), and cachexia in 7 patients (18%). The remainder 24 patients were classified as no-cachexia patients (Table 2). None of the patients met the criteria of refractory cancer cachexia: measurements were carried out at diagnosis, just before starting anticancer treatment, Karnofsky performance score was relatively high (70 to 100), and expected survival was at least 3 months in all patients. Quality of life was significantly different between no-cachexia, precachexia and cachexia groups (p = 0.03), but other function scales (such as physical function) did not significantly differ between groups. Survival was not significantly different between no-cachexia, precachexia and cachexia groups in univariate analysis (24 ± 11.6 vs. 32 ± 1.5 vs. 9 ± 9.2 months, respectively; p = 0.21) (Figure 1). Multivariate analysis with no cachexia as

Table 2: Number of patients with stage III NSCLC classified as having no cachexia, precachexia and cachexia

<table>
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<th></th>
<th>Cancer</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>No cachexia</td>
<td>Precachexia</td>
<td>Cachexia</td>
<td></td>
</tr>
<tr>
<td>General</td>
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<td></td>
<td></td>
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<td>No cachexia</td>
<td>20</td>
<td>9</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Cachexia</td>
<td>4</td>
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<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>9</td>
<td>7</td>
<td>40</td>
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</table>
Table 1: Baseline characteristics for patients with stage III NSCLC, specified for groups with no cachexia, precachexia and cachexia, as defined by different consensus-based frameworks

<table>
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<th>General</th>
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<tr>
<td></td>
<td>Overall (n = 40)</td>
<td>No cachexia (n = 24)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.8 ± 10.1</td>
<td>57.7 ± 11.1</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>19 (47.5%)</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>Tumour stage n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>16 (40%)</td>
<td>11 (46%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>24 (60%)</td>
<td>13 (54%)</td>
</tr>
<tr>
<td>Weight change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>previous 6 months (%)</td>
<td>-1.3 ± 4.5</td>
<td>+ 0.3 ± 5.5</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>23.9 ± 3.5</td>
<td>24.5 ± 3.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>ANOVA (comparing no-cachexia, precachexia and cachexia groups)

<sup>b</sup>Pearson chi-square test (comparing no-cachexia and (pre-)cachexia groups)

<sup>c</sup>Independent samples t-test for equality of means (comparing no-cachexia and cachexia groups)
Precachexia and cachexia at diagnosis of stage III NSCLC

Figure 1: Kaplan Meier survival functions for no cachexia, precachexia and cachexia in patients with stage III NSCLC (n = 40), defined by the ESPEN SIG and cancer-specific framework of Fearon and colleagues.

reference category, corrected for sex and tumour stage, showed a significantly shorter survival in patients with cancer cachexia (HR 2.93; 95% CI 1.03;8.34; p = 0.04), not in patients with precachexia (HR 0.78; 95% CI 0.30;2.03; p = 0.62).

General framework
Using the general framework to define cachexia, we identified 11 (28%) out of 40 patients with cachexia and 29 patients (72%) as having no cachexia (Table 2). The four patients who were classified as cachectic using the general definition, but not when using the cancer-specific framework, did not experience anorexia, but scored positive on at least 3 other features of the general definition (Table 3). Cachexia tended to be associated with a trend for a lower quality of life (p = 0.08).

Between the general no-cachexia and the cachexia groups, median survival was significantly different (respectively 32.0 ± 4.5 vs. 10.0 ± 3.7 months; p < 0.01) (Figure 2). In multivariate analysis, corrected for confounding by sex and tumour stage, cachexia remained significantly associated with a shorter survival (HR 4.2; 95% CI 1.7-10.0; p = 0.001).
**Cachexia features**

Approximately 50% of non-cachectic patients scored positively on cachexia features, such as fatigue, anorexia, reduced handgrip strength and upper arm circumference, or increased CRP. In general, low percentages of patients scored positively on a reduced FFM index, albumin or Hb (Table 3).

For all instruments, groups with cachexia showed higher levels of CRP and IL-6, and a lower Hb and serum albumin than patients with no cachexia \( (p < 0.01) \) (Table 4). CRP was positively correlated to IL-6 \( (r = 0.55, p < 0.01) \) and negatively correlated to Hb \( (r = -0.47, p < 0.01) \) and serum albumin \( (r = -0.71, p < 0.01) \). The remaining inflammatory parameters were also significantly correlated to one another. Using different cut-off points for CRP (>5 or 10 mg/L instead of >8 mg/L) did not change the presence of pre-cachexia and cachexia in individual patients (data not shown).

Of all patients, 12 (30%) had a weight loss of at least 5% in the previous 12 months or less, 3 (8%) had a fat-free mass index below the 5th percentile of reference values, 27 (68%) had decreased handgrip strength (below the lowest tertile of reference values), 19 (48%) experienced fatigue and 23 (58%) experienced anorexia or reduced food intake. When comparing individual levels of inflammatory parameters with their reference values, CRP and serum IL-6 were elevated in respectively 28 (70%) and 20 (50%) patients, and Hb and serum albumin were decreased in 8 (20%) and 7 (18%) patients (Table 3).

Figure 2: Kaplan Meier survival functions for no cachexia and cachexia in patients with stage III NSCLC \( (n = 40) \), defined by the no disease specific, general framework of Evans and colleagues.
Table 3: Number of patients with stage III NSCLC scoring on cachexia features according to the applied criteria\(^a, b, c\)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Overall (n = 40)</th>
<th>No cachexia (n = 24)</th>
<th>Precachexia(^a) (n = 9)</th>
<th>Cachexia(^b) (n = 7)</th>
<th>No cachexia (n = 29)</th>
<th>Cachexia (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight loss / BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to ≤ 5% (6 months)(^a)</td>
<td>19 (48)</td>
<td>19 (79)</td>
<td>9 (100)</td>
<td>0</td>
<td>18 (62)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>&gt; 5% (6 months)(^b)</td>
<td>12 (30)</td>
<td>5 (21)</td>
<td>0</td>
<td>7 (100)</td>
<td>2 (7)</td>
<td>10 (91)</td>
</tr>
<tr>
<td>&gt; 2% &amp; BMI &lt; 20 kg/m(^2)(^b)</td>
<td>3 (8)</td>
<td>0</td>
<td>0</td>
<td>3 (43)</td>
<td>0</td>
<td>3 (27)</td>
</tr>
<tr>
<td>≥ 5% (12 months or less)(^c)</td>
<td>12 (30)</td>
<td>5 (21)</td>
<td>0</td>
<td>7 (100)</td>
<td>2 (7)</td>
<td>10 (91)</td>
</tr>
<tr>
<td>BMI &lt; 20 kg/m(^2)</td>
<td>4 (10)</td>
<td>1 (4)</td>
<td>0</td>
<td>3 (43)</td>
<td>0</td>
<td>4 (36)</td>
</tr>
<tr>
<td><strong>Fat-free mass index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFM index &lt; 5(^{th}) percentile (BIS)(^b)</td>
<td>3 (8)</td>
<td>1 (4)</td>
<td>1 (11)</td>
<td>1 (14)</td>
<td>1 (3)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>FFM index &lt; 10(^{th}) percentile for age and gender (BIS)(^c)</td>
<td>6 (15)</td>
<td>3 (13)</td>
<td>2 (22)</td>
<td>1 (14)</td>
<td>4 (14)</td>
<td>2 (18)</td>
</tr>
<tr>
<td><strong>Upper arm circumference</strong> &lt; 10(^{th}) percentile(^c)</td>
<td>23 (58)</td>
<td>15 (63)</td>
<td>2 (22)</td>
<td>6 (86)</td>
<td>15 (52)</td>
<td>8 (73)</td>
</tr>
<tr>
<td><strong>Muscle strength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handgrip strength &lt; lowest tertile(^c)</td>
<td>27 (68)</td>
<td>13 (54)</td>
<td>7 (78)</td>
<td>7 (100)</td>
<td>16 (55)</td>
<td>11 (100)</td>
</tr>
<tr>
<td><strong>Fatigue (EORTC-QLQc30 question 18, score 3 or 4)</strong></td>
<td>19 (48)</td>
<td>10 (42)</td>
<td>6 (67)</td>
<td>3 (43)</td>
<td>14 (48)</td>
<td>5 (45)</td>
</tr>
<tr>
<td><strong>Anorexia / reduced food intake</strong>(^a, b, c)</td>
<td>23 (58)</td>
<td>7 (29)</td>
<td>9 (100)</td>
<td>7 (100)</td>
<td>16 (55)</td>
<td>7 (64)</td>
</tr>
</tbody>
</table>
Table 3 (continued)

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Overall (n = 40)</th>
<th>No cachexia (n = 24)</th>
<th>Pre-cachexia (n = 9)</th>
<th>Cachexia (n = 7)</th>
<th>General (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer</td>
<td>General</td>
<td>Cancer</td>
<td>General</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CRP &gt; 5.0 mg/Lc</td>
<td>33 (83%)</td>
<td>18 (75%)</td>
<td>8 (89%) (100%)</td>
<td>22 (76%) (100%)</td>
</tr>
<tr>
<td></td>
<td>CRP ≥ 8.0 mg/La,b</td>
<td>28 (70%)</td>
<td>13 (54%)</td>
<td>8 (89%) (100%)</td>
<td>17 (59%) (100%)</td>
</tr>
<tr>
<td></td>
<td>IL-6 &gt; 4.0 pg/mLc</td>
<td>20 (50%)</td>
<td>7 (29%)</td>
<td>8 (89%) (100%)</td>
<td>14 (48%) (55%)</td>
</tr>
<tr>
<td></td>
<td>Albumin &lt; 32 g/Lc</td>
<td>8 (20%)</td>
<td>1 (4%)</td>
<td>2 (22%)</td>
<td>4 (57%)</td>
</tr>
</tbody>
</table>

**FFM**, fat free mass; **BIS**, bioelectrical impedance spectroscopy; **EORTC-QLQc30**, 30-item Quality of Life Questionnaire of the European Organisation for Research and Treatment of Cancer; **Hb**, haemoglobin; **CRP**, C-reactive protein; **IL-6**, interleukin-6.

*a*ANOVA (comparing no-cachexia, precachexia and cachexia groups)

*b*Independent samples t-test for equality of means (comparing no-cachexia and cachexia groups)

*c*1 kJ = 0.239 kcal

**Additional parameters**

REE per kg FFM, physical activity and phase angle were not significantly different between groups. However, physical activity appeared to be lower in cachexia patients (Table 4).

**Discussion**

The purpose of this explorative study was to study the presence of precachexia and cachexia in patients with stage III NSCLC, by using consensus-based conceptual frameworks, which have not yet been applied or validated in populations of patients with cancer. Second, we explored the association of (pre)cachexia with survival and quality of life. Although we are gaining knowledge on the pathophysiology and treatment of cancer cachexia, little is known about the typical profile and staging of cachexia. We chose to apply the only two available consensus-based frameworks to define cachexia. These frameworks were both comprehensive, but differed in the kind of parameters to define cachexia. The cut-off point of essential parameters, e.g. weight loss, is still a subject of
Table 4: Differences in biochemistry, phase angle, REE, physical activity and quality of life between cachexia groups with stage III NSCLC

<table>
<thead>
<tr>
<th></th>
<th>Cancer</th>
<th>General</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cancer</th>
<th>General</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.3 ± 1.3</td>
<td>13.6 ± 1.5</td>
<td>11.3 ± 2.5</td>
<td>0.01</td>
<td>13.5 ± 1.3</td>
<td>11.7 ± 2.1</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>38.3 ± 3.8</td>
<td>37.1 ± 4.0</td>
<td>32.0 ± 6.4</td>
<td>0.01</td>
<td>38.4 ± 3.1</td>
<td>32.8 ± 6.2</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>30.4 ± 53.6</td>
<td>40.1 ± 33.8</td>
<td>92.0 ± 57.0</td>
<td>0.03</td>
<td>22.3 ± 25.2</td>
<td>96.5 ± 71.9</td>
</tr>
<tr>
<td>IL-6 serum (pg/ml)</td>
<td>3.2 ± 2.5</td>
<td>7.3 ± 3.1</td>
<td>14.9 ± 12.2</td>
<td>&lt;0.001</td>
<td>4.6 ± 3.3</td>
<td>11.3 ± 11.6</td>
</tr>
<tr>
<td>Phase angle (°)</td>
<td>6.9 ± 2.2</td>
<td>6.2 ± 0.6</td>
<td>6.3 ± 0.7</td>
<td>0.55</td>
<td>6.8 ± 2.0</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>REE (kJ)</td>
<td>6406 ± 895</td>
<td>6623 ± 950</td>
<td>5845 ± 527</td>
<td>0.19</td>
<td>6510 ± 937</td>
<td>5954 ± 519</td>
</tr>
<tr>
<td>REE (kJ/kg FFM)</td>
<td>129 ± 24</td>
<td>137 ± 22</td>
<td>140 ± 15</td>
<td>0.50</td>
<td>130 ± 24</td>
<td>139 ± 16</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM day score</td>
<td>6.7 ± 5.3</td>
<td>5.5 ± 2.8</td>
<td>4.3 ± 2.1</td>
<td>0.57</td>
<td>6.7 ± 5.0</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>Low intensity (min/d)</td>
<td>46.5 ± 23.5</td>
<td>50.8 ± 37.2</td>
<td>26.4 ± 11.0</td>
<td>0.24</td>
<td>47.5 ± 26.4</td>
<td>32.5 ± 20.0</td>
</tr>
<tr>
<td>Moderate intensity (min/d)</td>
<td>32.0 ± 21.0</td>
<td>28.2 ± 17.4</td>
<td>25.7 ± 20.6</td>
<td>0.81</td>
<td>32.2 ± 20.5</td>
<td>24.0 ± 17.5</td>
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### Table 4 (continued)

<table>
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<tr>
<th></th>
<th>Cancer</th>
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<th></th>
<th>General</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>No cachexia (n = 24)</td>
<td>Pre-cachexia (n = 9)</td>
<td>Cachexia (n = 7)</td>
<td>p&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No cachexia (n = 29)</td>
<td>Cachexia (n = 11)</td>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EORTC-QLQc30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality of life</td>
<td>62.3 ± 20.7</td>
<td>56.5 ± 21.6</td>
<td>34.7 ± 22.6</td>
<td>0.03</td>
<td>60.4 ± 21.6</td>
<td>45.8 ± 24.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Physical functioning</td>
<td>75.7 ± 19.8</td>
<td>68.9 ± 21.3</td>
<td>63.6 ± 23.9</td>
<td>0.37</td>
<td>75.0 ± 19.3</td>
<td>64.1 ± 23.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Role functioning</td>
<td>59.4 ± 34.8</td>
<td>40.7 ± 29.0</td>
<td>41.7 ± 41.8</td>
<td>0.29</td>
<td>53.6 ± 32.2</td>
<td>48.3 ± 43.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Emotional functioning</td>
<td>62.0 ± 25.0</td>
<td>73.1 ± 23.9</td>
<td>60.7 ± 20.8</td>
<td>0.46</td>
<td>63.7 ± 25.6</td>
<td>65.9 ± 20.2</td>
<td>0.80</td>
</tr>
<tr>
<td>Cognitive functioning</td>
<td>72.5 ± 26.9</td>
<td>81.5 ± 15.5</td>
<td>76.2 ± 23.3</td>
<td>0.64</td>
<td>73.2 ± 25.0</td>
<td>80.3 ± 20.8</td>
<td>0.41</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>65.9 ± 31.6</td>
<td>64.8 ± 29.4</td>
<td>44.4 ± 40.4</td>
<td>0.35</td>
<td>64.9 ± 29.9</td>
<td>55.0 ± 40.1</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; CRP, C-reactive protein; IL-6, interleukin-6; REE, resting energy expenditure; PAM, Physical Activity Monitor; EORTC-QLQc30, 30-item Quality of Life Questionnaire of the European Organisation for Research and Treatment of Cancer; BIS, bioelectrical impedance spectroscopy.

<sup>a</sup>ANOVA (comparing no-cachexia, precachexia and cachexia groups).

<sup>b</sup>Independent samples t-test for equality of means (comparing no-cachexia and cachexia groups)

<sup>c</sup>1 kJ = 0.239 kcal
debate. Therefore we were interested in the outcomes of these two instruments when applied in a small, heterogeneous population of patients with locally advanced cancer. These frameworks defined cachexia and described the clinical features associated with cachexia. More recently published proposals aim to grade the severity of cachexia lead to the definition of pre-cachexia (1;4). Using these proposals, it is possible to identify cancer patients with precachexia; early-stage cachexia, characterised by moderate systemic inflammation and metabolic alterations, and minimal weight loss. Patients with precachexia are not always recognized by clinicians or nutritional screening instruments, while nutritional support is expected to prevent progressive loss of body weight and FFM. On the contrary, treatment options for cachexia are limited.

In the current population of patients at diagnosis of stage III NSCLC, precachexia was prevalent in 23%, but only the framework proposed by the ESPEN special interest group (1) defines precachexia, the general framework of Evans and colleagues only defines cachexia (5). Cachexia was also prevalent in our population, but the cancer-specific framework and the general framework for cachexia found a different number of patients with cachexia (respectively 18% and 28% by the cancer-specific and not disease-specific general framework).

A number of studies showed the association of survival and weight loss in general cancer populations (10;14;35) and in patients with gastrointestinal (36) and lung cancer (9). One of the first papers on this topic found the combination of weight loss, food intake and systemic inflammation to be related to poor outcome in pancreatic cancer patients (35). Because the definition of cachexia includes the presence of severe weight loss, the association with survival in the current study is consistent with these findings. The difficulty is that in the literature, weight loss and cachexia are used disorderly, and that it is not possible to isolate starvation from cancer cachexia. Another component of the cachexia definition is inflammation. Systemic inflammation, amongst others reflected by elevated CRP and hypoalbuminemia, is also negatively associated with survival (37;38).

After carrying out baseline measurements at diagnosis, patients received different anticancer treatments and participated in a placebo-controlled RCT, comparing oral nutritional supplements containing n-3 polyunsaturated fatty acids (PUFAs) with an isocaloric placebo. Yet, the percentages of patients with (pre)cachexia and survival did not significantly differ among groups with different cancer treatments (data not shown). Preclinical studies suggest an increased intake of omega-3 PUFAs decreases the risk of cancer development and progression. A few clinical studies support the potential benefit of omegas-3 PUFAs on chemotherapy efficacy (39) or cancer cell proliferation (40). In our population, patients who received oral nutritional supplements containing n-3 PUFAs did
not show a significantly different presence of cachexia or survival than control patients (data not shown).

On average, the current population of patients with stage III NSCLC showed a moderate amount of weight loss (on average 1.9% of pre-illness weight) during the previous 6 months and consequently, a low prevalence of malnutrition (20%). Other studies in patients with lung cancer (all types and stages) reported high percentages of malnutrition, i.e. 15.6% (7), 30% (41), 36% (10) and 50 to 61% (11). A study by Bozetti and colleagues showed an average weight loss of 9.5% in outpatients with lung cancer (6). Consequently, the percentage of patients with cachexia in the current patient population was relatively low (18% by the cancer-specific framework, and 28% by the general definition). This could be explained by the selection of stage III NSCLC. As this is one of the first studies to assess precachexia in stage III lung cancer, it is hard to compare these findings with other data. Op den Kamp and colleagues found a comparable amount of weight loss (average 3.1%) in a group of 16 newly diagnosed patients with stage I to III NSCLC. Compared to healthy controls, these patients also showed systemic inflammation, but no apparent loss of FFM. However, this exploratory study did not describe precachexia features (such as inflammation and anorexia) in individual patients (42).

Weight loss was associated with an elevated REE (12;41), systemic inflammatory response (12;41) and a reduced dietary intake (41) in patients with SCLC and NSCLC. Metabolic and inflammatory derangements seemed to be mainly related to the tumour; after resection (43) or chemotherapeutic treatment (44), REE in patients with lung cancer decreased. We did not find differences for REE per kg FFM between cachexia groups, probably due to high CRP and inflammation in the majority of patients. When uncorrected for FFM, REE in patients with cachexia (defined by the general framework for cachexia) was significantly lower, but this could be explained by the lower body weight in patients with cachexia. Because we used bioelectrical impedance spectroscopy to assess FFM (and not the gold standard DXA), this may have resulted in overestimation or underestimation of FFM.

We also showed that approximately 50% of non-cachectic patients scored positively on cachexia features, such as moderate weight loss, systemic inflammation, fatigue, anorexia, reduced handgrip strength and upper arm circumference. The frameworks we used defined patients as precachectic or cachectic when they experience a combination of cachexia features, inflammation, and weight loss, which is consistent with the existing knowledge on the pathophysiology of cachexia. Other cachexia frameworks, e.g. the proposal of the SCRNIO working group (6) and the cachexia score (CASCO) (8), were not consensus-based and therefore not selected to address the current research question.
A secondary aim of this study was to explore quality of life and physical activity, and their association with cachexia. Overall, our small sample size resulted in a low statistical power, which made it hard to demonstrate significant associations. Precachexia and cachexia were associated with a reduced overall quality of life, but not with other quality of life parameters, such as physical function. In the literature, an association between nutritional status, inflammation and wellbeing in lung cancer has been described, but these studies did not assess cachexia in the way we did (45;46). Physical activity is an important indicator of quality of life and performance status in cancer patients (47), and found to be reduced in patients with SCLC (48) and pancreatic cancer (24). Current patients with stage III NSCLC also showed a lower physical activity than healthy subjects (approximately 6 vs. 20) (33), and patients with pre-cachexia and cachexia showed a non-significant lower physical activity than no-cachexia patients.

When using the selected frameworks, we encountered some issues. First, patients with weight loss as well as complaints and/or inflammation, were incorrectly justified as having no cachexia by the general framework, which requires 3 positive scores on complaints and inflammation. Patients with ≥ 5% weight loss, in combination with 2 positive scores on complaints and inflammation, were not classified as cachectic. Also, the ESPEN SIG did not classify these patients as precachectic, as their weight loss was more than 5%. Second, cut-off points for anorexia and CRP, and FFM index were lacking for the precachexia and cancer-specific frameworks. For precachexia, weight loss ≤ 5% was described, but it was unclear if this accounted for patients with a weight loss of 0%. We solved these issues by consulting the authors. In line with the current knowledge, we found a positive correlation between pro-inflammatory indexes (CRP and serum IL-6), and these were negatively correlated to Hb and serum albumin. Interestingly, when other cut-off points for inflammatory parameters were applied, we observed the same presence of (pre)cachexia.

Validation of cachexia instruments in large groups of patients with cancer is still required, but a gold standard is lacking. The association of cachexia with survival is informative, but validation of instruments against one or more indicators of cachexia (e.g. standardized assessment of muscle mass) is preferable. Further studies in larger populations are warranted to validate these new instruments and to more extensively explore the prognostic value in patients with cancer. Ideally, worldwide cancer centres record a number of biomarkers and cachexia parameters, follow up treatment adherence and survival, and merge these data in order to validate definitions and their prognostic value. A promising parameter might be proteolysis inducing factor (PIF), which has been found in the urine of cachectic patients with cancer (49).

In conclusion, new consensus-based frameworks show that precachexia and cachexia are
prevalent in patients with stage III NSCLC. Cachexia appears to be associated with a shorter overall survival and a reduced quality of life.

**Acknowledgements**

We would like to thank N. Kok (Department of Pulmonary Diseases, VU University Medical Center, Amsterdam, Netherlands) and V. van Adrichem (Department of Nutrition and Dietetics, VU University Medical Center, Amsterdam, Netherlands) for their assistance with patient inclusion.
Supplemental Figure 1: CONSORT diagram

Assessed for eligibility (n=55)

Excluded (n=13)
Not meeting inclusion criteria (n=4)
Refused to participate (n=9)

Enrollment

Randomization

Allocation

Allocated to intervention (n=21):
Received allocated intervention (n=20)
Did not receive allocated intervention:
Disease progression (n=1)

Allocated to placebo (n=21):
Received allocated placebo (n=20)
Did not receive allocated placebo:
Disease progression (n=1)

INTERVENTION GROUP (n=20)

CONTROL GROUP (n=20)

Baseline

INTERVENTION GROUP (n=20)

CONTROL GROUP (n=20)

Baseline
References

23. Moses AW, Slater C, Preston T et al. Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. Br J Cancer 2004;90:996-1002.
Precachexia and cachexia at diagnosis of stage III NSCLC


119
Oral nutritional supplements containing (n-3) polyunsaturated fatty acids affect the nutritional status of patients with stage III non-small cell lung cancer during multimodality treatment

BS van der Meij
JAE Langius
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MD Spreeuwenberg
BM von Blomberg
AC Heijboer
MA Paul
PAM van Leeuwen

Journal of Nutrition 2010;140(10):1774-80
Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), (n-3) fatty acids from fish oil, have immune-modulating effects and may improve nutritional status in cancer. The objective of this study was to investigate the effects of an oral nutritional supplement containing (n-3) fatty acids on nutritional status and inflammatory markers in patients with non-small cell lung cancer (NSCLC) undergoing multimodality treatment. In a double blind experiment, 40 patients with stage III NSCLC were randomly assigned to receive 2 cans/d of a protein- and energy-dense oral nutritional supplement containing (n-3) fatty acids (2.0 g EPA + 0.9 g DHA/d) or an isocaloric control supplement. EPA in plasma phospholipids, energy intake, resting energy expenditure (REE), body weight, fat free mass (FFM), mid-upper arm circumference (MUAC), and inflammatory markers were assessed. Effects of intervention were analyzed by generalized estimating equations and expressed as regression coefficients (B). The intervention group (I) had a better weight maintenance than the control (C) group after 2 and 4wk (B = 1.3 and 1.7 kg, respectively; p < 0.05), a better FFM maintenance after 3 and 5wk (B = 1.5 and 1.9 kg, respectively; p < 0.05), a reduced REE (B = 216.7% of predicted; p = 0.01) after 3 wk, and a trend for a greater MUAC (B = 9.1; p = 0.06) and lower interleukin-6 production (B = 227.9; p = 0.08) after 5 wk. After 4 wk, the I group had a higher energy and protein intake than the C group (B = 2456 kJ/24 h, p = 0.03 and B = 25.0g, p = 0.01, respectively). In conclusion, a protein- and energy-dense oral nutritional supplement containing (n-3) fatty acids beneficially affects nutritional status during multimodality treatment in patients with NSCLC.
Introduction

Lung cancer is the most common cause of cancer mortality worldwide. The 5-y survival of patients with lung cancer is ~15%, with earlier stage patients having a better chance of long-term survival (1,2). Non-small cell lung cancer (NSCLC) (10) is the main type of lung cancer, accounting for 80% of lung cancers (1,3). For patients with unresectable stage III NSCLC who have a good performance status and no severe comorbidities, concurrent multimodality treatment provides the best treatment outcome with respect to survival (1). Multimodality treatment consists of cisplatin-based induction chemotherapy with concurrent thoracic radiation (chemoradiotherapy) (4–6) followed by surgical resection in patients with overall mediastinal down staging after chemoradiotherapy. Chemoradiotherapy is associated with various acute and delayed toxicities, such as esophagitis, nausea, vomiting, and altered taste (1,4,7,8). These side effects lead to an impaired nutritional status, increased treatment-related morbidity and mortality, and a decreased quality of life (3,9).

Nutritional status in lung cancer patients is also affected by metabolic alterations induced by the tumor. Metabolic alterations lead to cachexia syndrome, which is characterized by anorexia, anemia, and weight loss (mostly loss of lean body mass) (10,11). The pathogenesis of cancer cachexia is multifactorial and involves the production of proinflammatory cytokines and acute phase reactants along with activation of proteolytic pathways (11,12).

Cancer cachexia is frequently observed in lung cancer (3). Several studies found weight loss, decreased lean body mass, and hypermetabolism were associated with higher levels of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-a (TNF-a) and lower levels of albumin in patients with lung cancer (13–15).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), (n-3) PUFA from fish oil, have immune-modulating effects (16–18). The (n-3) fatty acids, in particular EPA, reduce the production of proinflammatory cytokines by several mechanisms and may positively influence the cancer cachexia syndrome. An optimal dose of 2.0 g/d of EPA was selected, because this was previously demonstrated to reduce proinflammatory cytokine production in cachectic patients. However, clinical studies show contradictory effects of (n-3) fatty acids on cancer cachexia and nutritional status (7,19).

The aim of this study was to investigate the effects of an oral nutritional supplement containing (n-3) PUFA on nutritional status and inflammatory markers in patients with stage III NSCLC undergoing multimodality therapy.
Materials and Methods

Patients
From March 15 2005 until January 31 2008, 55 patients with histological or cytological proven stage IIIa-N2 or IIIb NSCLC were recruited. Patients 18–80 y of age were included if they were eligible for concurrent chemoradiotherapy and if their life expectancy was more than 3 mo.

Patients were excluded if they had undergone surgery, chemotherapy, or radiotherapy during the previous month; if they had edema, ascites, severe comorbidities (major gastrointestinal disease, chronic renal failure, uncontrolled diabetes mellitus, or HIV); or if they used medication that could modulate metabolism or body weight, in particular high-dose corticosteroids or fish oil supplements, during the previous month. Four patients did not meet inclusion and exclusion criteria and 9 patients refused to participate, leaving 42 patients to be enrolled and allocated to intervention (I) \(n = 21\) or control (C) \(n = 21\) groups (Supplemental Figure 1).

Treatment for stage III NSCLC consisted of chemotherapy with concurrent thoracic radiotherapy. Chemotherapy consisted of 2 courses of induction chemotherapy consisting of cisplatin-based doublet, 6 weekly courses of docetaxel and cisplatin, or 2 courses of induction chemotherapy and concurrent bevacizumab. Concurrent thoracic radiotherapy was given in fractions of 1.8–2 Gy (5 fractions/wk) up to a maximal individual dose of 45 Gy.

Study design
This study was a randomized, double-blind, placebo-controlled trial carried out at the VU University Medical Center Amsterdam (The Netherlands). The protocol was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam and written informed consent was obtained from all patients.

Patients were asked to consume 2 cans/d of either a protein- and energy-dense oral nutritional supplement containing \((n-3)\) PUFA providing 2.02 g/d EPA + 0.92 g/d DHA (480 mL ProSure) or an isocaloric control oral nutritional supplement without EPA and DHA (400 mL Ensure). The manufacturer (Abbott Nutrition, Abbott Laboratories) provided nutritional composition analyses of both oral nutritional supplements (Table 1). The oral nutritional supplements were commercially available and provided in blank cans, identical in texture, both vanilla flavored, ready to use and intended to act as a supplement to the patient’s usual diet. The prescribed daily dose of \((n-3)\) fatty acids is generally recognized as
Table 1: Nutritional composition of the oral nutritional supplements

<table>
<thead>
<tr>
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<th>Unit/can</th>
</tr>
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<tbody>
<tr>
<td>Volume, mL</td>
<td>240</td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>1234</td>
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<tr>
<td>Protein, g</td>
<td>16.0</td>
</tr>
<tr>
<td>Fat, g</td>
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</tr>
<tr>
<td>Monounsaturated fatty acids</td>
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<td>SFA</td>
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<tr>
<td>PUFA</td>
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<td>Linoleic acid</td>
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</tr>
<tr>
<td>DHA</td>
<td>0.46</td>
</tr>
<tr>
<td>(n-6):(n-3) fatty acids</td>
<td>0.3 : 1</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>44.0</td>
</tr>
</tbody>
</table>

safe (20) and no toxic effects of this dose have been described in cancer patients (21–23). Patients received oral nutritional supplements during 5 wk from the start of concurrent chemoradiotherapy and were monitored for clinical performance and nutritional and inflammatory markers.

Random assignment and stratification
Random assignment to the intervention group (I) was performed by the pharmacist via sequential randomization in blocks of 4 participants with stratification for 1 of the 3 chemotherapy schedules. Patients, investigators, and study personnel were unaware of the treatment group allocation.
In the pharmacy, study supplements were packaged identically and not distinguishable from each other except for randomization number.

Compliance with study supplements
To evaluate compliance with study supplements, patients were instructed to record supplement intake in a compliance diary.
Second, plasma phospholipid fatty acid concentrations at baseline and after 5 wk were assessed as an objective indicator of study supplement intake. For this purpose, EDTA
plasma was immediately separated from blood cells by low speed centrifugation at 1850 x g for 10 min (37°C) and stored at -80°C. Plasma phospholipid fatty acids were assessed while keeping the investigators unaware of individual fatty acids concentrations until the treatment allocation was revealed.

Lipids were extracted from plasma with a mixture of isopropanol:hexane (40:60) and separated by TLC into phospholipids, cholesterol, FFA, triglycerides, and cholesterol esters. Phospholipids were scraped off and transmethylated. FAME were extracted with hexane and the composition was analyzed by GC (Fisons 8000 series, Chrompack column CP Sil 88). The amount of fatty acids in plasma phospholipids was expressed as weight percentage of total measured fatty acids (24,25).

**Nutritional intake and energy balance**

To assess energy intake, a 24-h dietary recall was performed. Dietary energy and nutrient composition were calculated by a nutrition analysis software application with the use of the most recent Dutch Food Composition table (NEVO 2006) (26). Resting energy expenditure (REE) was measured by a ventilated hood system (Deltatrac, Datex). CO₂ production and O₂ consumption were measured at complete rest during a period of 30 min. REE was calculated using a modified Weir equation (27,28). The equipment was calibrated at the start of each experiment. To calculate total energy expenditure (TEE), 30% was added to REE, assuming a physical activity level of 1.3 for sedentary patients with cancer (29). Energy balance was expressed as energy intake as percentage of TEE. Expected REE was estimated using the predictive equation of the FAO/WHO/UNU including weight and height (30,31).

**Nutritional status**

At baseline, pre-illness weight, unintentional weight loss in the last month and last 6 mo, and height were recorded. Body weight, without shoes and wearing light clothing, was measured on a compact digital flat scale (SECA 888) to the nearest 0.2 kg. BMI was calculated as the ratio of body weight (kg)/height (m)². Patients with a BMI ≤ 18.5 and/or unintentional weight loss ≥ 5% in the previous month and/or ≥ 10% in the previous 6 mo were classified as malnourished (32,33). Mid-upper arm circumference (MUAC) was measured at the midpoint of the upper arm between the acromion process and the tip of the olecranon process by using a tape measure. The mean of 2 measurements was recorded.

To obtain fat free mass (FFM), bioelectrical impedance analysis (Hydra 4200, Xitron
 Technologies) was assessed. FFM was calculated from resistance and reactance at the frequency of capacitance by using the Kyle Geneva equation (34).

**Inflammatory markers**

Plasma concentrations of C-reactive protein (CRP) were measured with an automated latex-enhanced immunoturbidimetric assay on a Modular P analyzer (35). Serum albumin concentrations were chemically determined on a Modular P analyzer (Roche Diagnostics) (36). Whole blood leukocyte count was performed by impedance and optical flow cell measurement (Cell Dyn Sapphire, Abbott Diagnostics) (37). The ex vivo production of IL-6 in whole blood samples was measured upon stimulation at 37°C for 3 h using 0.01 and 10 mg/L of lipopolysaccharide (Difco Laboratories) (38). Commercially available ELISA were used to measure IL-6 (Pelikine compact human ELISA kits, Sanquin) and soluble tumor necrosis factor-p55 (sTNF-p55; Biosource Europe S) concentrations in serum and supernatants. The minimum detectable concentrations were 6 ng/L for IL-6 and 0.94 μg/L for sTNF-p55. Human leukocyte antigen-DR (HLA-DR) expression on CD14+ cells was evaluated by FACS analysis (FACS Calibur, Becton Dickinson) as previously described (38).

**Adverse events**

During the entire study period, adverse events were monitored by the treating physician.

**Statistics**

Statistical power was based on changes in weight from a study in patients with pancreatic cancer by Barber et al. (39). A sample size of 17 patients was calculated to detect a difference in FFM of 0.5 kg (± 0.5 kg) between groups with a significance level of 0.05 and a power of 0.8. Based on an anticipated 15% attrition rate, 40 patients were required to be enrolled to obtain a minimum of 34 patients for data analyses.

Differences between groups for patient characteristics at baseline for nominal and ordinal variables were analyzed by chi-square tests. For continuous baseline variables, differences between groups were analyzed by independent samples t tests and linear regression analyses with sex as covariate, as appropriate. Differences between malnourished and well-nourished patients at baseline were analyzed accordingly.

The primary analysis of the effect of (n-3) fatty acids containing oral nutritional supplements was performed on an intention-to-treat basis of all patients as randomized and allocated to the I or C group.
Second, per protocol analyses were performed to evaluate the effect of (n-3) fatty acids on primary effect parameters (body weight and FFM). For this purpose, compliant patients were selected according to their plasma phospholipid EPA after 5 wk: I patients with plasma phospholipid EPA ≥ 1.6% and C patients with plasma phospholipid EPA < 1.6%. In addition, Pearson correlation analysis tests were performed to investigate the relationship of plasma phospholipid EPA and inflammatory markers in I patients who had a plasma phospholipid EPA increase of at least 1.5% after 5 wk. We used generalized estimating equations (GEE), a longitudinal linear regression technique to account for the dependency of the observations in time, to analyze effects of intervention over time (40, 41). Adjustments were made by addition of baseline values and sex as covariates. Independent dummy variables for group (I or C group) and for separate time points (wk 1, 2, 3, 4, and 5) were entered into the GEE model. Absolute differences between the I and C group were expressed as B. We used an exchangeable correlation structure to analyze the data. SPSS 16.0 was used for data analyses. Values are displayed as mean ± SD, except where stated otherwise. All P-values were 2-sided at a significance level of α = 0.05 (p < 0.05).

**Results**

We included 40 eligible patients with stage III NSCLC, 21 men and 19 women, with a median age of 57.8 y (range 39–80 y). Sixteen patients had stage IIIa NSCLC and 24 patients had stage IIIb NSCLC. At baseline, the patients had lost 0.5 ± 2.5 kg in the previous month, 0.9 ± 3.7% of their pre-illness stable weight. Three patients in the I group and 5 patients in the C group were malnourished at baseline (BMI ≤ 18.5 and/or unintentional weight loss ≥ 5% in the previous month and/or ≥ 10% in the previous 6 mo).

The I and C groups did not differ in baseline characteristics except for sex. The I group consisted of more men (n = 16; 80%) than the C group (n = 5; 25%) (p < 0.01). After adjustments for sex, there was no difference in nutritional variables between groups at baseline (Table 2). In subsequent analyses, adjustments were made for baseline values and sex.
Table 2: General and baseline characteristics of 40 patients with stage III NSCLC

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>C</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>4 (20)</td>
<td>15 (75)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.4 ± 12.0</td>
<td>57.2 ± 8.1</td>
<td>0.70</td>
</tr>
<tr>
<td>Karnofsky Performance Score</td>
<td>84.0 ± 11.4</td>
<td>80.5 ± 10.0</td>
<td>0.31</td>
</tr>
<tr>
<td>Stage of disease, n (%)</td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>IIIa</td>
<td>9 (45)</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>11 (55)</td>
<td>13 (65)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy, n (%)</td>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>Cisplatin and docetaxel</td>
<td>11 (55)</td>
<td>8 (40)</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>8 (40)</td>
<td>11 (55)</td>
<td></td>
</tr>
<tr>
<td>Cisplatin and bevacizumab</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8 ± 4.1</td>
<td>23.0 ± 2.4</td>
<td>0.42*</td>
</tr>
<tr>
<td>Weight loss previous month, %</td>
<td>-0.3 ± 2.4</td>
<td>-1.5 ± 4.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Malnutrition, n (%)</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>0.70</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>77.1 ± 14.6</td>
<td>64.7 ± 7.4</td>
<td>0.12*</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>58.0 ± 8.7</td>
<td>48.0 ± 7.3</td>
<td>0.26*</td>
</tr>
<tr>
<td>FFM index, kg/m²</td>
<td>18.5 ± 2.0</td>
<td>16.6 ± 1.4</td>
<td>0.47*</td>
</tr>
<tr>
<td>MUAC, mm</td>
<td>289.4 ± 36.2</td>
<td>269.6 ± 23.1</td>
<td>0.43*</td>
</tr>
<tr>
<td>REE, % of expected</td>
<td>113.6 ± 15.1</td>
<td>110.5 ± 13.7</td>
<td>0.51</td>
</tr>
<tr>
<td>REE, kJ/kg body weight</td>
<td>102.5 ± 17.6</td>
<td>99.6 ± 15.5</td>
<td>0.57</td>
</tr>
<tr>
<td>REE, kJ/kg FFM</td>
<td>136.8 ± 4.7</td>
<td>141.0 ± 22.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum CRP, mg/L</td>
<td>39.1 ± 42.9</td>
<td>50.4 ± 66.8</td>
<td>0.54</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>35.9 ± 5.2</td>
<td>35.8 ± 6.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Leukocytes, x10⁹/L</td>
<td>8.7 ± 4.5</td>
<td>9.3 ± 7.4</td>
<td>0.76</td>
</tr>
<tr>
<td>Serum IL-6, mg/L</td>
<td>7.0 ± 6.5</td>
<td>4.9 ± 7.8</td>
<td>0.38</td>
</tr>
<tr>
<td>Serum sTNF-p55, ng/mL</td>
<td>3.0 ± 1.1</td>
<td>2.8 ± 1.0</td>
<td>0.65</td>
</tr>
<tr>
<td>HLA-DR expression on monocytes, kMESF</td>
<td>95.2 ± 43.1</td>
<td>88.1 ± 37.8</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*p-VALUE OF DIFFERENCE BETWEEN GROUPS (INDEPENDENT SAMPLES T-TEST).

1Results are mean ± SD or n (%).

2p-value of difference between groups after adjustment for sex (linear regression analysis with sex as covariate).
We assessed 40 patients (I: n = 20, C: n = 20) at baseline, 35 patients (I: n = 15, C: n = 20) after 3 wk and 33 patients (I: n = 14, C: n = 19) after 5 wk. Among the group of patients who dropped out before wk 3 (I: n = 5, C: n = 0), there were significantly more patients with malnutrition (dropouts: 60% vs. 14%; patients who reached wk 3; p = 0.02). Stage of disease and Karnofsky performance score were comparable between early dropouts and patients who reached wk 3. Reasons for dropout before wk 3 were withdrawal of consent (n = 3), disease progression (n = 1), or the occurrence of an adverse event (Supplemental Figure 1).

**Compliance with study supplements**

Consumption of study supplements during chemoradiotherapy was ~1 can/d (I: 1.1 ± 1.0 vs. C: 1.0 ± 0.9 can/d).

Plasma phospholipid EPA concentrations were assessed as objective markers of compliance with the intervention (Figure 1). In both groups (I: n = 1 vs. C: n = 3), there were patients with baseline plasma phospholipid EPA ≥ 1.6%, which is approximately the 90th percentile in free-living pancreatic cancer patients (39,42,43). After 5 wk, plasma phospholipid EPA concentrations in the I group were higher than in the C group (B = 1.5%; p = 0.06). Plasma phospholipid concentrations of DHA were also higher in the I group after 5 wk (B = 1.1%; p = 0.04), but there were no significant differences for arachidonic acid concentrations (B = 0.3%; p = 0.65).

Figure 1: Plasma phospholipid (PL) EPA concentrations of individual I (A) and C patients (B) with stage III NSCLC at baseline and wk 5. Difference between I (n = 14) and C (n = 18) groups after 5 wk (analyzed by GEE, with baseline value and sex as covariates): B = 1.5%, p = 0.06 (B > 0 indicates that I > C).
Table 3. Daily energy and macronutrient intake of 40 patients with stage III NSCLC at baseline and wk 5

<table>
<thead>
<tr>
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<th>C</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>wk 5</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Energy, kJ/24 h</td>
<td>6668 ± 2684</td>
<td>7646 ± 4132</td>
</tr>
<tr>
<td>Macronutrients, % of energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>16.5 ± 4.08</td>
<td>16.9 ± 3.81</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>51.9 ± 12.0</td>
<td>52.4 ± 7.51</td>
</tr>
<tr>
<td>Fat</td>
<td>28.6 ± 8.98</td>
<td>30.2 ± 8.50</td>
</tr>
<tr>
<td>PUFA</td>
<td>4.65 ± 1.82</td>
<td>6.11 ± 3.91</td>
</tr>
<tr>
<td>(n-3) PUFA, g</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.17</td>
</tr>
<tr>
<td>EPA</td>
<td>0.02 ± 0.08</td>
<td>0.87 ± 1.03</td>
</tr>
<tr>
<td>DHA</td>
<td>0.04 ± 0.10</td>
<td>0.43 ± 0.46</td>
</tr>
<tr>
<td>ALA</td>
<td>0.04 ± 0.17</td>
<td>1.58 ± 1.90</td>
</tr>
</tbody>
</table>

Results are mean ± SD.

1kJ = 0.239 kcal, 1 g protein = 17 kJ, 37 g fat = 37 kJ, 1 g carbohydrate = 17 kJ (44).

p < 0.05 (difference between groups, analyzed by GEE with baseline value and sex as covariates).

At baseline, daily intake of (n-3) fatty acids [α-linolenic acid (ALA), EPA, and DHA] was comparable in the 2 groups. After 5 wk, the I group had a higher intake of EPA (B = 0.6 g/d; p = 0.01) and ALA (B = 1.3 g/d; p = 0.003) but not of DHA (B = 0.2 g/d; p = 0.25) compared with the C group (Table 3) (44). The intake of (n-3) fatty acids from normal daily food did not significantly differ between groups after 5 wk (data not shown).

Nutritional intake and energy balance

At baseline, the REE of the study population was 112% of predicted REE and not significantly different between groups. The REE was 101 kJ/(24 h -1·kg body weight). Eleven patients (I: n = 6, 33%; C: n = 5, 26%; p = 0.64) had an elevated REE (defined as > 20% above expected).

After 3 and 5 wk, mean REE (percent of predicted) decreased to 109% (95 kJ/kg) and 108% (102 kJ/kg) in the I group, respectively, and 112% (103 kJ/kg) and 102% (99 kJ/kg) in the C group (p < 0.05). Compared with the C group, the REE in the I group decreased more after 3 wk (B = 216.7% of predicted, p = 0.01 and B = 24 kJ/kg body weight, p = 0.07) (Table 4).
Table 4: Energy metabolism and MUAC in patients with stage III NSCLC in the I and C group after 3 and 5 wk of chemoradiotherapy and supplementation

<table>
<thead>
<tr>
<th></th>
<th>wk 3</th>
<th></th>
<th>wk 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
<td>p</td>
</tr>
<tr>
<td>REE, % of predicted</td>
<td>-16.7</td>
<td>0.01</td>
<td>4.9</td>
<td>0.56</td>
</tr>
<tr>
<td>REE, kJ/kg body weight</td>
<td>-10.0</td>
<td>0.07</td>
<td>3.8</td>
<td>0.66</td>
</tr>
<tr>
<td>REE, kJ/kg FFM</td>
<td>-10.9</td>
<td>0.10</td>
<td>3.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Energy intake, kJ/24 h</td>
<td>1046</td>
<td>0.25</td>
<td>256</td>
<td>0.81</td>
</tr>
<tr>
<td>Energy balance, % of TEE</td>
<td>15.4</td>
<td>0.20</td>
<td>-3.8</td>
<td>0.78</td>
</tr>
<tr>
<td>MUAC, mm</td>
<td>6.6</td>
<td>0.37</td>
<td>9.1</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*B: difference between I and C groups (analyzed by GEE with baseline value and sex as covariates). B > 0 indicates that I > C.

**Nutritional status**

**Weight maintenance**

After 1, 2, and 4 wk, the I group had a better weight maintenance than the C group (B = 1.1 kg, p = 0.07; B = 1.3 kg, p = 0.02; and B = 1.7 kg, p = 0.04, respectively) (Figure 2). In the per protocol analysis, the effect on body weight after 1, 2, and 4 wk was stronger (B = 2.2 kg, p < 0.01; B = 2.2 kg, p < 0.01; and B = 2.2 kg, p = 0.04, respectively).

Figure 2: Weight change from baseline in I and C patients with stage III NSCLC. Values are means ± SD, n = 20 (baseline), 14 (wk 5, I), or 19 (wk 5, C). *Different from C, p < 0.05 (analyzed by GEE, with baseline value and sex as covariates).
Effects of n-3 PUFAs on nutritional status in stage III NSCLC

FFM
Over time, FFM in both groups decreased but less in the I group than in the C group after 3 and 5 wk (B = 1.5 kg, \( p = 0.05 \) and B = 1.9 kg, \( p = 0.02 \), respectively).

MUAC
The MUAC of the I group increased during chemoradiotherapy, whereas MUAC in the C group decreased. After 5 wk, the I group tended to have a greater MUAC than the C group (\( p = 0.06 \)) (Table 4).

Inflammatory markers
In both groups, baseline values of CRP and leukocytes were greater than the upper normal limit (35, 37) (Table 2) and decreased until wk 5. Malnutrition at baseline was associated with high leukocyte counts and serum CRP concentrations and low serum albumin concentrations. Malnourished (n = 8) and well-nourished (n = 32) patients differed in leukocyte counts (12.9 ± 8.4 vs. 8.1 ± 5.1 x 10^3, respectively; \( p = 0.04 \)), serum CRP (86.0 ± 67.17 vs. 33.8 ± 47.6 mmol/L; \( p = 0.02 \)), and albumin (31.8 ± 6.7 vs. 36.8 ± 4.8 g/L; \( p = 0.02 \)). At wk 5, the I group tended to have lower IL-6 production in response to whole blood stimulation with lipopolysaccharide than the C group (B = 227.9; \( p = 0.08 \)). For I patients who had a plasma phospholipid EPA increase of at least 1.5% (n = 6), serum IL-6 and CRP at wk 5 were negatively correlated with plasma phospholipid EPA levels (Pearson r: 20.8, \( p = 0.041 \) and 20.8, \( p = 0.048 \), respectively).

Serum CRP, IL-6, sTNF-p55, and albumin concentrations and HLA-DR expression on monocytes were not different between groups at any time point.

Adverse events
No serious adverse events related to the study supplements were observed. Five patients experienced an adverse event during the study period. In the I group, 1 patient experienced a cerebrovascular accident during chemoradiotherapy. Two patients in the I group and 2 patients in the C group experienced gastrointestinal complaints, which included nausea, vomiting, diarrhea, cramps, and belching, after consumption of the study supplement.
Discussion

In this double-blind, randomized, placebo-controlled study, we compared a protein- and energy-dense oral nutritional supplement containing (n-3) PUFA to an isocaloric control supplement for effects on nutritional status and inflammatory markers in stage III NSCLC patients undergoing multimodality treatment. To our knowledge, this is the first randomized controlled trial showing beneficial effects of a protein- and energy-dense oral nutritional supplement containing (n-3) fatty acids on nutritional status in patients with lung cancer during multimodality treatment.

Effects on nutritional status

The oral nutritional supplement containing (n-3) fatty acids resulted in a preservation of body weight and FFM during chemoradiotherapy, in particular after 4–5 wk of supplementation. When selecting patients with increased plasma phospholipid EPA concentrations, we found better preservation of body weight, confirming these effects could be ascribed to supplementation of (n-3) fatty acids. In addition to the benefits seen with body weight and FFM, MUAC of the I group tended to be ~1 cm higher after 5 wk, whereas MUAC in the C group decreased over time. Moreover, the I group showed a significantly higher energy and protein intake after 4 wk together with a clinically relevant reduced REE. These effects on body weight, FFM, energy expenditure, and energy intake might have resulted in improved physical functioning and quality of life during multimodality treatment (effects on physical functioning and quality of life in the same patient population; B. S. van der Meij, J. A. E. Langius, M. D. Spreeuwenberg, S. M. Slootmaker, M. A. Paul, E. F. Smit, P. A. M. van Leeuwen, unpublished data). Previous studies showed nutritional intervention improves nutritional intake in cancer patients and this improvement was positively associated with quality of life (45–48).

Previous studies, mostly performed in palliative care, showed comparable effects of oral nutritional supplements containing (n-3) fatty acids on body weight, FFM, and REE in pancreatic (7,22,39,49) and lung cancer (8) patients with cachexia. However, these studies were noncontrolled, nonblinded trials and, subsequently, placebo-controlled trials failed to show significant differences between I and C groups on body weight, FFM, and quality of life in cancer patients (7,8,19). Two randomized controlled studies observed changes in energy and protein intake (800–2000 kJ/d and 15 g protein/d, respectively) after 4–8 wk consumption of oral nutritional supplements containing (n-3) fatty acids. The authors reported a supplement intake of 2 cans/d by lung cancer patients (8) and 1.4 cans/d
pancreatic cancer patients (7). In the current study, compliance was considerably lower (~1 can/d in both I and C groups) and energy balance did not differ. However, the I group had a higher energy intake than the C group of an additional 2456 kJ/d and an additional higher protein intake at some time points (12–25 g/d). These results are rather comparable to the observations of Fearon et al. (7) and Guarcello et al. (8).

Compared with previous studies, the present study population showed a less advanced stage of disease and a low prevalence of malnutrition at baseline. Yet most patients showed signs of precachexia, such as increased levels of serum IL-6 and CRP, anorexia, and reduced muscle strength. By chance, patients who dropped out early from the study experienced more weight loss at baseline than patients who reached follow-up measurements. Moreover, there was a greater dropout in the I group compared with the C group. As a result of this, the required number of patients (as indicated by power calculations) was not achieved in the I group. This might have resulted in a reduced statistical power. Without this selective dropout, we possibly would have observed even stronger and more significant effects of the oral nutritional supplement containing (n-3) fatty acids. Overall, we observed consistent beneficial effects of (n-3) fatty acids on different nutritional variables in this small, precachectic study population.

Effects on inflammatory markers
Immune function may be modulated by (n-3) fatty acids and ~2 g of EPA/d has been shown to suppress inflammatory cytokines and CRP (23,50–52) levels in weight-losing patients with pancreatic and lung cancer (16–18,51,53,54) or surgery trauma (55–57). In the present study, levels of inflammatory markers decreased during chemoradiotherapy in both I and C groups. After 5 wk, only IL-6 production tended to be lower in the I group than in the C group. The effects of chemoradiotherapy, such as reduction of tumor volume and tumor-induced inflammation, possibly had greater effects on inflammatory markers than (n-3) fatty acids. On the other hand, patients’ supplement intake might have been too low to significantly affect inflammatory markers.

Compliance
Patient compliance is a limiting factor in nutrition intervention studies. In this study, patient compliance was monitored by a compliance diary. A compliance diary could be biased by a patient reporting a desirable amount of supplements to satisfy the
investigator or underreporting could take place when patients were too ill or forgot to complete the diary. Therefore, plasma phospholipid EPA was assessed as an objective marker of patient compliance and is known to represent the (n-3) fatty acid consumption of the previous week. Although plasma phospholipid EPA concentrations are generally used as a marker of (n-3) fatty acid consumption in healthy individuals as well as in cancer patients, cancer-induced inflammation and chemotherapy might alter the metabolism of phospholipid and in this way reduce the validity of these measurements (58).

Similarly to Fearon et al. (7), we found a number of patients in the C group with increased plasma phospholipid EPA concentrations. In general, suboptimal compliance with oral nutritional supplements is a common issue in cancer patients receiving nutritional support. In the current study, causes for suboptimal compliance of the study supplements, as mentioned by patients of both I and C groups, were anorexia, palatability and early satiety, and patients’ preference to consume normal oral food rather than oral nutritional supplements. Even though patients consumed a relatively low amount of oral nutritional supplements, we clearly found effects on nutritional status markers after a few weeks.

An issue of concern is the difference of nutrient composition of the oral nutritional supplements. The intervention and control supplements were isocaloric, although not isonitrogenous. The intervention supplement contained more protein, less fat, and slightly more carbohydrates than the control supplement. This might have influenced satiety and nutritional status in a different way and the observed effects of intervention may not be fully ascribed to (n-3) fatty acids.

However, the per protocol analyses showed greater differences for body weight and FFM between groups than the intention-to-treat analyses. This confirms the dose response effect of EPA on nutritional status markers and corresponds to results from other studies showing a positive dose response effect (7,21,42,59). The minimum dose of EPA to establish effects on nutritional status is probably lower than the proposed optimal dose of 2 g/d. In the current study, an EPA consumption of ~1 g/d resulted in a significantly better weight and FFM maintenance and a reduced REE.

In conclusion, this randomized, double-blind, placebo-controlled study indicates beneficial effects of a protein- and energy-dense oral nutritional supplement containing (n-3) fatty acids on nutritional status in stage III NSCLC patients.
Acknowledgments

We thank Natasja Kok (Pulmonology) for assistance with patient recruitment, Geraldine Droog and Vrouwke van Adrichem (Nutrition and Dietetics) for their help with data collection, Marlies Henning Platvoet for processing the nutritional intake data, Pierre Bet and Klara Bruyn (Pharmacy) for their help with randomization and packaging of the study supplements, and Petra Scholten, Martine Reijm, and Petra Bonnet (Pathology) for their efforts with blood sampling and laboratory analyses. This work has been supported by Abbott Nutrition.
Supplemental Figure 1: CONSORT diagram

Assessed for eligibility (n=55)

Excluded (n=13):
- Not meeting inclusion criteria (n=4)
- Refused to participate (n=9)

Enrollment

Randomization

Allocation

Allocated to intervention (n=21):
- Received allocated intervention (n=20)
- Did not receive allocated intervention:
  - Disease progression (n=1)

Allocated to placebo (n=21):
- Received allocated placebo (n=20)
- Did not receive allocated placebo:
  - Disease progression (n=1)

INTERVENTION GROUP (n=20)

n=15
- Lost to follow-up:
  - Patient withdrawal (n=3)
  - Adverse event (n=1)
  - Disease progression (n=1)

CONTROL GROUP (n=20)

n=20
- Follow-up & analysis 3 weeks

n=14
- Lost to follow-up:
  - Patient withdrawal (n=1)

n=19
- Lost to follow-up (n=1):
  - Adverse event (n=1)
References


Oral nutritional supplements containing n-3 polyunsaturated fatty acids affect quality of life and functional status in lung cancer patients during multimodality treatment: an RCT

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JAE Langius
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SM Slootmaker
MA Paul
EF Smit
PAM van Leeuwen

Abstract

Background / objectives
Our objective was to investigate effects of an oral nutritional supplement containing n-3 polyunsaturated fatty acids (FAs) on quality of life, performance status, handgrip strength and physical activity in patients with non-small cell lung cancer (NSCLC) undergoing multimodality treatment.

Subjects / methods
In a double blind experiment, 40 patients with stage III NSCLC were randomised to receive 2 cans/day of a protein- and energy-dense oral nutritional supplement containing n-3 polyunsaturated FAs (2.02 g eicosapentaenoic acid + 0.92 g docosahexaenoic acid/day) or an isocaloric control supplement, during multimodality treatment. Quality of life, Karnofsky Performance Status, handgrip strength and physical activity (by wearing an accelerometer) were assessed. Effects of intervention were analysed by generalised estimating equations. P-values < 0.05 were regarded as statistically significant.

Results
The intervention group reported significantly higher on the quality of life parameters, physical and cognitive function (B = 11.6 and B = 20.7, p < 0.01), global health status (B = 12.2, p = 0.04) and social function (B = 22.1, p = 0.04) than the control group after 5 weeks. The intervention group showed a higher Karnofsky Performance Status (B = 5.3, p < 0.04) than the control group after 3 weeks. Handgrip strength did not significantly differ between groups over time. The intervention group tended to have a higher physical activity than the control group after 3 and 5 weeks (B = 6.6, p = 0.04 and B = 2.5, p = 0.05).

Conclusion
n-3 Polyunsaturated FAs may beneficially affect quality of life, performance status and physical activity in patients with NSCLC undergoing multimodality treatment.
Introduction

Lung cancer is a leading cause of cancer death worldwide, causing approximately 1.2 million deaths per year (1). The two major types of lung cancer are non-small cell lung cancer (NSCLC) and small cell lung cancer, differentiated by cell type and biological behaviour. NSCLC accounts for 85% of all lung cancers (2).

Stage IV NSCLC has the shortest survival and is merely treated by palliative chemotherapy. Patients with localised or locoregional disease, such as stage III NSCLC, receive multimodality treatment with curative intent. This multimodality treatment includes concurrent chemoradiotherapy, followed by surgery (3–5).

Concurrent chemoradiotherapy increases the length and potential toxic effects of treatment as well as the rehabilitation and recovery process. This treatment frequently extends to 2 months, and the combined side effects can affect all areas of functioning, such as physical, psychosocial and nutritional status (3).

Yet, little is known about physical, psychosocial and nutritional status in lung cancer patients undergoing multimodality treatment. Anorexia and weight loss are frequently observed, which affects nutritional status and in turn might have an impact on the treatment toxicity, quality of life and survival (6–9).

Nutritional therapy in cancer patients aims to maintain or improve nutritional status and quality of life during cancer therapy, and to improve tolerance to treatment (9), but an increase of energy and nutrient intake in cancer has shown to be ineffective because of tumour-derived catabolism (9–11).

Research currently focuses on therapeutic agents, which modulate catabolism and appetite, for instance pharmaconutrients that are able to inhibit protein degradation and to stimulate protein synthesis.

In this respect, n-3 polyunsaturated fatty acids (FAs) from fish oil have presumable immune-modulating effects, partly caused by the formation of 3- and 5-series inflammatory mediators with a lower proinflammatory and immunosuppressive effect (12,13). In particular, eicosapentaenoic acid (EPA) has been shown to reduce the production of inflammatory cytokines and to treat tissue wasting in patients with cancer (12,13). Apart from immunemodulating effects of n-3 FAs and indications of preservation of body weight (14,15), one small trial documented an improved physical activity in a small group of patients with pancreatic cancer (16).

Up to now, a few randomised controlled trials showed promising effects of n-3 polyunsaturated FAs on quality of life and functional status in cancer patients receiving...
palliative care, without assessing physical activity (17,18).
The aim of this paper was to investigate the effects of a nutritional supplement containing n-3 polyunsaturated FAs on quality of life and functional status (performance status, handgrip strength and physical activity) in patients with stage III NSCLC undergoing multimodality treatment.

Subjects and methods

Patients
We included 42 patients with histological or cytological proven stage IIIa-N2 or IIIb NSCLC. Patients 18–80 years of age were included if they were eligible for multimodality treatment, and if their life expectancy was 43 months. Patients who had undergone surgery, chemotherapy or radiotherapy during the previous month and patients with oedema, ascites, severe comorbidities, or using high-dose corticosteroids or fish oil supplements during the previous month were excluded. Multimodality consisted of concurrent chemoradiotherapy; for the description of the treatment schedule, we refer to our previous publication.

Study design
This study is set up as a randomised, double-blind, placebo-controlled trial. We prescribed two packages per day of either a protein- and energy-dense oral nutritional supplement containing n-3 polyunsaturated FAs (ProSure, Intervention) or an isocaloric control oral nutritional supplement (Ensure, Control) during 5 weeks of chemoradiotherapy. Details on the oral nutritional supplements that are displayed have been previously described (19). The Medical Ethics Committee of the VU University Medical Center Amsterdam approved the trial and all patients provided written informed consent. We continuously monitored compliance with the study supplements and adverse events. We assessed quality of life, Karnofsky Performance Status and physical activity at baseline, after 3 and 5 weeks. Patients performed handgrip strength tests at baseline and every week for 5 weeks.
Effects of n-3 PUFAs on quality of life in stage III NSCLC

**Random assignment**

The pharmacist randomised patients, stratified by chemotherapy schedule, in blocks of four to the intervention (I) group (n = 20) or control (C) group (n = 20). Independent employees prepared numbered batches of study supplements in the pharmacy. After inclusion of a patient, the pharmacist assigned a batch number. This batch was delivered to the patient concerned. Patients, investigators and study personnel were blind to the treatment group allocation (19).

**Compliance with study supplements**

We used two methods to evaluate compliance with study supplements. First, patients were instructed to record supplement intake in a compliance diary. Second, plasma phospholipids EPA levels were assessed at baseline and after 5 weeks, as an objective indicator of study supplement intake, as described previously (19).

**Quality of life**

Patients filled out a self-administered questionnaire, the EORTC-QLQC30, a multidimensional validated cancer-specific measure that includes global health status and quality of life, functional and symptom scales (20).

Global health status implies the patient’s own judgment of health status and quality of life. Physical function score expresses the capacity to perform normal daily activities. Other functional scales include role function (the capacity to perform work, daily activities, hobbies or other leisure activities), cognitive function, and emotional and social function.

EORTC-QLQC30 subscales were calculated according to the EORTC-QLQC30 manual and vary from 0 to 100. A high score for a functional or quality of life scale represents a high level of functioning or quality of life. A high score for a symptom scale represents a high level of problems.

The investigator recorded the Karnofsky Performance Status, a valid and widely used instrument to quantify the functional status of cancer patients. The Karnofsky Performance Status ranges from 0 to 100, with a higher score indicating a better ability to carry out normal daily activities and work (21,22).

**Handgrip strength**

Handgrip strength was measured in the nondominant hand using a hydraulic hand dynamometer (Baseline; Fabrication Enterprises, White Plains, NY, USA). The patient
performed the test in sitting position, with the shoulder adducted and neutrally rotated, elbow flexed at 90°, forearm and wrist in neutral position. Patients were instructed to perform two maximal isometric contractions. Patients took brief pauses between measurements. The maximal value was recorded to the nearest 0.5 kg, and the mean of two measurements was used to compare with age- and sex-dependent reference values for handgrip strength (23). If patients were unable to perform handgrip strength with their nondominant hand, handgrip strength of the dominant hand was measured. Through the entire study period, measurements were performed with the same hand.

**Physical activity**
Physical activity in daily life, assessed by accelerations of the hip, was measured with the physical activity monitor (PAM) accelerometer (model AM101, 28 190 g, 59 x 43 x 10mm³; PAM B.V., Doorwerth, The Netherlands). The PAM produces a single index score on its display, which accumulates during the day and is a proxy measure of total daily physical activity. Every 3 points of the physical activity score reflects about 10 min of walking (24). Moreover, the PAM has the ability to register minutes of low and moderate intensity physical activity. Low intensity physical activity corresponded with small in-house movements; moderate intensity corresponded with walking (25). The reliability and validity of the PAM accelerometer has been tested in a laboratory setting and has shown results similar to the MTI Actigraph for estimating energy expenditure in walking and stair walking. The PAM was calibrated on a shaking device (2.1 kHz) prior to the study. Patients were instructed to wear the PAM for 7 consecutive days at the following time points: after inclusion (before starting with study supplements) and during the third and fifth week of the study. PAM data were included if the PAM was worn during at least 3 full days during the week before admission to the hospital; average daily PAM scores and minutes of low and moderate intensity were calculated.

**Adherence to chemoradiotherapy protocols**
The investigator registered acute, nonscheduled hospital admissions, adherence to chemoradiotherapy protocols and chemotherapy delay, using medical records from the departments of pulmonary diseases and radiation oncology.
Statistics
The primary end point of this study and the sample size calculation on body weight have been described previously (19). The statistical power of remaining parameters was calculated by entering the expected sample size of 20 and the average within-group differences from baseline to 5 weeks. With a significance level of 0.05, the statistical power of quality of life parameters was 66% (global health status) and 97% (physical function), respectively, and the statistical power of Karnofsky Performance Status, handgrip strength and physical activity were 51, 25 and 55, respectively.
Differences between groups for patient characteristics at baseline for nominal and ordinal variables were analysed by χ² tests. For continuous variables, differences between groups at baseline were analysed by linear regression analysis with sex as covariate.
The primary analysis of the effect of n-3 FAs was performed on an intention-to-treat basis of all patients as randomised and allocated to the I or C group.
Generalised estimating equation, a longitudinal linear regression technique, to account for the dependency of the observations in time, was used to analyse the effects of intervention over time for continuous variables. Adjustments were made by addition of baseline values and sex as covariates. Independent dummy variables for group (I or C group) and for separate time points (week 1, 2, 3, 4 and 5) were entered into the generalised estimating equation model. Absolute differences between I and C groups were expressed as B. The generalised estimating equation method is suitable for designs with unequally spaced time intervals (26,27). We used an exchangeable correlation structure to analyse the data, using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The exchangeable correlation structure assumes the correlation within different time points and is equal for all time points (28). P-values < 0.05 were considered as statistically significant.

Results
We included 40 eligible patients with stage III NSCLC, 21 men and 19 women, with median age 57.8 years (range 39-80). In all, 16 patients had stage IIIa NSCLC and 24 patients had stage IIIb NSCLC.
The I group consisted of more men than the C group (p = 0.001). Other baseline characteristics did not differ between the I and C groups. Quality of life variables did not differ between groups at baseline after adjustment for sex (Table 1).
We assessed 40 patients (I: n = 20, C: n = 20) at baseline, 35 patients (I: n = 15, C: n = 20) after 3 weeks and 33 patients (I: n = 14, C: n = 19) after 5 weeks.
Table 1: General and baseline characteristics of 40 patients with stage III NSCLC

<table>
<thead>
<tr>
<th></th>
<th>I (n = 20)</th>
<th>C (n = 20)</th>
<th>(p^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F), n (%)</td>
<td>4 (20%)</td>
<td>15 (75%)</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.4 ± 12.0</td>
<td>57.2 ± 8.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Stage of disease, n (%)</td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>IIIa</td>
<td>9 (45%)</td>
<td>7 (35%)</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>11 (55%)</td>
<td>13 (65%)</td>
<td></td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 ± 4.1</td>
<td>23.0 ± 2.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Weight loss previous month (%)</td>
<td>-0.3 ± 2.4</td>
<td>-1.5 ± 4.7</td>
<td>0.56</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77.1 ± 14.6</td>
<td>64.7 ± 7.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>31.3 ± 9.8</td>
<td>26.1 ± 7.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Karnofsky Performance Status</td>
<td>84.0 ± 11.4</td>
<td>80.5 ± 10.0</td>
<td>0.84</td>
</tr>
<tr>
<td>60</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>4 (20%)</td>
<td>8 (40%)</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>4 (20%)</td>
<td>4 (20%)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>8 (40%)</td>
<td>7 (35%)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>EORTC QLQ C30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global health status</td>
<td>60.2 ± 24.7</td>
<td>53.8 ± 21.7</td>
<td>0.81</td>
</tr>
<tr>
<td>Functional scales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Function</td>
<td>73.6 ± 20.2</td>
<td>68.3 ± 20.8</td>
<td>0.78</td>
</tr>
<tr>
<td>Role function</td>
<td>50.9 ± 39.0</td>
<td>50.0 ± 63.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Emotional function</td>
<td>70.6 ± 21.8</td>
<td>64.3 ± 29.9</td>
<td>0.73</td>
</tr>
<tr>
<td>Cognitive Function</td>
<td>81.6 ± 14.6</td>
<td>71.7 ± 26.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Social function</td>
<td>63.9 ± 30.4</td>
<td>55.8 ± 28.8</td>
<td>0.59</td>
</tr>
<tr>
<td>Physical activity a Day score (PAM activity score)</td>
<td>7.2 ± 8.7</td>
<td>5.7 ± 5.0</td>
<td>0.84</td>
</tr>
<tr>
<td>Low intensity physical activity (min/d)</td>
<td>46.5 ± 51.5</td>
<td>45.8 ± 27.7</td>
<td>0.73</td>
</tr>
<tr>
<td>Moderate intensity physical activity (min/d)</td>
<td>48.0 ± 67.9</td>
<td>31.0 ± 33.2</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; C, control; EORTC QLQ, European Organisation for Research and Treatment of Cancer-Quality of Life Questionnaire; I, intervention; NSCLC, non-small cell lung cancer. Results are presented as mean ± SD, unless stated otherwise. \(p\)-value of difference between groups, with sex included as covariate in the regression model. *\(p < 0.01\)
Five patients of the I group dropped out within 3 weeks. Reasons for dropout were withdrawal of consent (n = 3), disease progression (n = 1) or the occurrence of an adverse event. Compared with patients who did not drop out within 3 weeks, those five patients had a comparable Karnofsky Performance Status, global health status, physical function and stage of disease at baseline.

Compliance with study supplements
Consumption of study supplements during chemoradiotherapy was approximately one package a day in both the I and C groups (P > 0.05). After 5 weeks, plasma phospholipid concentrations of EPA and docosahexaenoic acid of the I group were significantly higher than those of the C group. For detailed results on compliance, we refer to our previous publication (19).

Quality of life
Patients in the I group performed in general better on quality of life scores than patients in the C group (Table 2). After 3 weeks, the I group had a higher Karnofsky Performance Status (B = 5.3, p = 0.04) than the C group. After 5 weeks, Karnofsky Performance Status did not differ between groups. After 5 weeks, the I group showed a significantly better global health status (B = 12.2, p = 0.04), physical function (B = 11.6, p < 0.01), cognitive function (B = 20.7, p < 0.01) and social function (B = 22.1, p = 0.04) on the EORTC-QLQC30 subscales than the C group. In addition, the I group reported less nausea/vomiting (B = -16.0, p = 0.04) and less financial problems (B = -9.5, p = 0.04) than the C group after 5 weeks. Other functional or symptom scales of the EORTC-QLQC30 did not significantly differ between groups at any time point.

Physical activity level
Physical activity scores of patients are depicted in Table 2 and Figure 1. Mean daily physical activity score in the patient population amounted 6.3, which is considerably lower than in healthy people (approximately 20) (24). In both groups, at least two-thirds of patients were able to wear the PAM accelerometer, during 5.4 ± 2.4 days at baseline, 5.9 ± 2.2 days during week 3 and 7 ± 3.3 days during week 5, with no significant differences between the I and C groups. Most often mentioned reasons for not wearing the PAM were forgetfulness, taking the PAM off when taking a short bed rest and difficulties with wearing a visible device.
Table 2: Quality of life, physical activity and handgrip strength after 3 and 5 weeks for the I and C groups with stage III NSCLC

<table>
<thead>
<tr>
<th></th>
<th>Week 3</th>
<th></th>
<th>Week 5</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
<td>p</td>
</tr>
<tr>
<td>EORTC QLQC30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global health status</td>
<td>0.4</td>
<td>0.95</td>
<td>12.2</td>
<td>0.04*</td>
</tr>
<tr>
<td>Functional scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Function</td>
<td>-1.7</td>
<td>0.76</td>
<td>11.6</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Role function</td>
<td>-14.9</td>
<td>0.21</td>
<td>17.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Cognitive Function</td>
<td>-0.1</td>
<td>0.99</td>
<td>20.7</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Emotional function</td>
<td>-8.6</td>
<td>0.25</td>
<td>6.8</td>
<td>0.30</td>
</tr>
<tr>
<td>Social function</td>
<td>2.0</td>
<td>0.87</td>
<td>22.1</td>
<td>0.04*</td>
</tr>
<tr>
<td>Symptom scales</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>4.8</td>
<td>0.57</td>
<td>0.5</td>
<td>0.95</td>
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<tr>
<td>Pain</td>
<td>2.4</td>
<td>0.79</td>
<td>-2.7</td>
<td>0.79</td>
</tr>
<tr>
<td>Nausea / vomiting</td>
<td>-9.9</td>
<td>0.06</td>
<td>-16.0</td>
<td>0.04*</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>-10.4</td>
<td>0.12</td>
<td>-8.7</td>
<td>0.46</td>
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<tr>
<td>Loss of appetite</td>
<td>-5.0</td>
<td>0.64</td>
<td>-2.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Insomnia</td>
<td>16.1</td>
<td>0.16</td>
<td>13.0</td>
<td>0.33</td>
</tr>
<tr>
<td>Constipation</td>
<td>-1.4</td>
<td>0.86</td>
<td>6.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>-5.2</td>
<td>0.62</td>
<td>1.2</td>
<td>0.90</td>
</tr>
<tr>
<td>Financial problems</td>
<td>-6.0</td>
<td>0.29</td>
<td>-9.5</td>
<td>0.04*</td>
</tr>
<tr>
<td>Karnofsky Performance Status</td>
<td>5.3</td>
<td>0.04*</td>
<td>7.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day score (PAM activity score)</td>
<td>6.6</td>
<td>0.04*</td>
<td>2.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Low intensity physical activity (min/d)</td>
<td>19.4</td>
<td>0.52</td>
<td>-4.0</td>
<td>0.85</td>
</tr>
<tr>
<td>Moderate intensity physical activity (min/d)</td>
<td>26.0</td>
<td>0.26</td>
<td>4.9</td>
<td>0.64</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>1.8</td>
<td>0.15</td>
<td>1.8</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Abbreviations: B, difference between I and C groups (analysed by generalised estimating equations, corrected for baseline value and sex), B > 0 implies a higher change in the I group than the C group; C, control; EORTC-QLQ, European Organisation for Research and Treatment of Cancer - Quality of Life Questionnaire; I, intervention. *Week 3: n = 13 (I) and n = 17 (C), week 5: n = 8 (I) and n = 13 (C). †Week 3: n = 12 (I) and n = 16 (C), week 5: n = 10 (I) and n = 16 (C). *p < 0.05.
Effects of n-3 PUFAs on quality of life in stage III NSCLC

Figure 1: Physical activity (daily PAM score) over time for the I and C groups. Values are mean ± s.d., baseline: n = 12 (I), n = 16 (C); week 3: n = 13 (I) and n = 17 (C); week 5: n = 8 (I), n = 13 (C). *p < 0.05, difference between the I and C group (analysed by generalised estimating equations, with baseline value and sex as covariate).

During week 3 and 5, the I group tended to have a considerably higher daily physical activity score (B = 6.6, p = 0.04 and B = 2.5, p = 0.05, respectively). There were no differences between the I and C groups in minutes of low and moderate intensity activity during week 3 and 5 (Table 2).

Handgrip strength
At baseline, handgrip strength of the study population was on average 92.2 ± 21.2% of age- and sex-specific reference values and not significantly different between the I and C groups. Over time, handgrip strength did not significantly differ between the groups (Table 2).

Adherence to chemoradiotherapy protocols
The number of patients with chemotherapy delays was four in the I group and two in the C group (p = 0.48). One patient in the C group needed a chemotherapy dose reduction. The number of patients with nonscheduled hospital admissions was 9 in the I group and 10 in the C group (p = 0.87). There were some patients who needed more than one nonscheduled hospital admission (I: n = 1: 3, C: n = 1: 2, n = 1: 3).
In both groups, there were various reasons for hospital admission, most often fever or nausea, vomiting and dehydration after chemotherapy. Other reasons were disease progression, pneumothorax, PEG-tube placement, depression, thrombosis and overall malaise.

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Discussion

This randomised controlled trial investigated effects of an oral nutritional supplement containing n-3 FAs on quality of life and functional status in patients with NSCLC during multimodality treatment. Observed differences between intervention and placebo groups suggest some beneficial effects of the oral nutritional supplement containing n-3 polyunsaturated FAs on quality of life and physical activity. These findings correspond with positive effects of n-3 FAs on nutritional status in the same patient population (19).

FAs are important constituents of immune cell membranes and precursors of prostanoids and leukotrienes. In contrast to n-6 FAs, n-3 FAs downregulate the production of pro-inflammatory cytokine production and cachectic factors. As a result, n-3 FAs might reduce anorexia, REE, muscle degradation and weight loss in cancer patients (29 – 32).

Maintenance of body weight and fat-free mass is expected to improve quality of life (9). Previous studies showed malnutrition to be associated with a reduced quality of life in patients with head and neck (8,33) and colorectal cancer (34) and in patients with radiotherapy for different types of cancer (35). Lung cancer patients with weight loss showed more symptoms, chemotherapy delay, anaemia and fewer symptomatic responses to chemotherapy than those without weight loss (6). Ravasco et al. (8) were the first to show beneficial effects of nutritional counselling on nutritional status, quality of life and outcome in head and neck cancer patients during radiotherapy.

To achieve immune modulation and effects on nutritional status and quality of life, patients’ compliance with n-3 FA supplementation is essential. Plasma phospholipids’ EPA levels and compliance diaries showed that the overall compliance with the study oral nutritional supplements was lower than expected. There was no significant difference between the I and C groups in the number of study supplements consumed, despite the lower prevalence of nausea and vomiting in the intervention group. Causes for suboptimal compliance of the study supplements, as mentioned by patients of both the I and C groups, were anorexia, palatability and early satiety, and patients’ preference to consume normal oral food rather than oral nutritional supplements. As suboptimal compliance with n-3 FA supplements has been reported in other studies, this confirms the need to develop more feasible methods of n-3 FA supplementation for cancer patients, either or not combined with other methods to enhance energy and protein intake.

In addition, some patients showed increased plasma phospholipids’ EPA levels at baseline. A number of control patients showed high levels after 5 weeks, indicating abnormal n-3 FA intake from fish or fish oil capsules. As a result, we might have observed smaller effects of the n-3 FA oral nutritional supplements.
To our knowledge, no other studies reported beneficial effects of n-3 FAs on quality of life in cancer during multimodality treatment. In the current study, observed effects on quality of life variables amounted 10-20 points on a 0-100 scale, indicating clinical significant improvements (40). Because of the uneven distribution of sex in the I and C groups, these differences might be partly caused by sex, for example, when men are known to be less critical towards their physical status than women. However, after correcting for sex, the I group showed a statistically significant higher physical function and cognitive function than the C group, and tendencies towards a higher global health status and social function.

The physical function score expresses the capacity to perform normal daily activities, such as to carry a heavy bag, to be able to go for a walk, to be bedridden during daytime and to be dependent on help with washing, dressing or eating. The I group also showed a substantially better cognitive function, expressed by reported concentration (for example, when watching television or reading a newspaper) and memory capacity. Polyunsaturated FAs are of importance in human brain development and n-3 FA status might influence cognitive function. A number of studies showed positive effects of docosahexaenoic acid supplementation on cognitive function in Alzheimer disease, elderly and children.

Mechanistically, these phenomena have been explained by the anti-inflammatory action of docosahexaenoic acid (36). Further on, reported problems with family life or social activities in the group receiving n-3 FAs were substantially lower during chemoradiotherapy. The intervention group also reported less nausea/vomiting and financial problems. These subjective findings are unlikely caused by n-3 FAs and may be caused by selection bias or by chance.

Physical activity is an important indicator of quality of life and performance status in patients with cancer (37,38). Gibney et al. (39) showed a reduced physical activity level in a small group of patients with small cell lung cancer. Beneficial effects of n-3 FAs on physical activity level have been shown in one small randomised controlled trial in pancreatic cancer patients (16). In line with the literature, we also found a reduced physical activity in patients with stage III NSCLC. After 5 weeks of chemoradiotherapy, physical activity in the group receiving the oral nutritional supplement containing n-3 FAs increased, whereas physical activity in the C group remained the same.

Although the statistical power of this study is limited, this is the first randomised controlled clinical trial indicating effects on both objective and subjective functional and quality of life parameters. n-3 Polyunsaturated FAs may beneficially affect quality of life and functional status in patients with NSCLC undergoing multimodality treatment. Yet,
more research is required to confirm these findings and to investigate the dose-response effect of n-3 FAs during cancer treatment.

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Should n-3 polyunsaturated fatty acids be prescribed in patients with cancer cachexia?

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Abstract

Existing guidelines on nutrition support in patients with cancer cachexia state limited evidence for the beneficial effects of n-3 polyunsaturated fatty acids (PUFAs) on clinical outcome. In order to report on the latest evidence for n-3 PUFAs in cancer cachexia, we conducted a systematic literature review of randomized controlled trials and meta-analyses, comparing effects of oral or enteral supplementation of n-3 PUFAs in cancer patients receiving chemotherapy, radiotherapy, surgery or palliative care. In PubMed®, EMBASE and the Cochrane library, search terms on cancer, n-3 PUFAs and clinical outcome parameters (nutritional status, morbidity, mortality, quality of life) were entered on April 27 2012, using limits for adults, humans and English language. The quality and evidence of the retrieved publications were appraised by an expert team of Australian and Dutch dieticians and nutritionists, using the ADA grading system. Fifteen RCTs and four systematic reviews were retrieved, of which one meta-analysis. Nine RCTs were of 9 of positive quality, 5 of neutral quality and 1 of negative quality, and performed in patients with various types of cancer. Fair evidence shows supplementation of n-3 PUFAs appears to be safe and may improve quality of life and physical activity in patients with cancer. However, supplementation of n-3 PUFAs does not improve energy or protein intake, appetite or survival and does not reduce postoperative complications. The evidence for the effect on body weight, fat-free mass and performance status remains inconclusive. In summary, supplementation of n-3 PUFAs may have some positive effects in patients with cancer.
Introduction and purpose

Cancer cachexia, a complex metabolic syndrome associated with underlying illness, characterized by an increased inflammatory status and loss of muscle mass with or without loss of fat mass, is highly prevalent among patients with cancer (1-5). This syndrome is a result of complex alterations in carbohydrate, lipid and protein metabolism (6), caused by inflammatory mediators such as cytokines and tumor-derived catabolic drivers. Proteolysis-inducing factor (PIF) is produced by the tumor and induces protein catabolism (7). As a result of the acute phase response, the liver shows an increased protein turnover for the production of inflammatory mediators, using muscle mass to release amino acids.

Changes in lipid metabolism in cancer include a reduction of lipogenesis, with unchanged whole body lipolysis and mobilization of fatty acids from fat tissue. Alterations in glucose metabolism are reflected by glucose intolerance and insulin resistance (7). Thus far, conventional nutritional support has been limited in its ability to stabilize body weight and maintain fat-free mass in patients with cachexia. Pharmaceutical interventions sometimes improved appetite, body weight and quality of life, but weight gain mostly consisted of fat mass (8;9).

N-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), seem to be promising agents to treat cancer cachexia. A dose of around 2 g of EPA per day (alone or combined with docosahexaenoic acid, DHA) appears to decrease the production of pro-inflammatory cytokines and PIF, and is associated with stabilization of body weight and probably fat-free mass (10). This has been shown in animal studies and in non-randomized human trials in pancreatic cancer patients (11;12). However, randomized controlled trials (RCTs) show contradictory results (13-15). This may be due to issues related to study limitations, such as the disease severity, confounding factors, and non-adherence with n-3 polyunsaturated fatty acids supplements. Also, study designs and outcome parameters differ in terms of supplementation dosage, comparison with control or other agents, and outcome parameters. Body weight is the most frequently used primary outcome measure, but may be biased by fluid retention. Another important issue is the heterogeneity in assessment of cachexia and weight loss in clinical studies.

In the past, several nutrition guidelines have addressed the issue of the prescription of EPA in patients with cancer cachexia. Overall, these guidelines conclude there are some indications for the beneficial effects of n-3 polyunsaturated fatty acids on body weight and physical function in cancer patients, but no effects on survival (16-18). These guidelines only included studies that were published before 2006. We published a systematic review
and included studies published until April 2011 (19). Since then there have been several new studies published in this field. Therefore the aim of this systematic review is to report the latest evidence for the prescription of n-3 polyunsaturated fatty acids in patients with cancer cachexia and provide recommendations for use in clinical practice.

**Materials and Methods**

*Patient characteristics*

This systematic review involved adult (≥ 18 y of age) patients with cancer. Only studies rated to have the highest level of evidence, i.e. RCTs, systematic reviews and meta-analyses, were included.

*Literature search*

A literature search was performed on April 27 2012, using three databases: PubMed© (start date 1948), EMBASE (start date 1986), and the Cochrane Library (start date 2005). Medical subject headings (MeSH or Emtree) and free text words for n-3 polyunsaturated fatty acids, EPA, cancer and clinical outcome parameters (body weight, fat-free mass, morbidity, mortality, length of stay and quality of life) were used to select relevant publications (Table 1). Any oral or enteral administration of n-3 PUFAs was included: (fish oil) capsules, oral nutritional supplements (ONS) or tube feeding containing n-3 PUFAs. Studies investigating multiple immune-enhancing compounds (e.g., arginine, glutamine, nucleotides, and n-3 FAs) or studies with concurrent use of appetite stimulants were excluded. The literature search was limited to RCTs in adult human subjects, which were available in English. Non-placebo controlled studies and single arm studies were excluded. In case a study was reported in more than one publication, the publication reporting at least one relevant outcome variable was included (Figure 1). Two members of the expert committee (BvdM and MvB) assessed the eligibility of publications on n-3 PUFAs in cancer patients by reviewing the title and abstract, and included eligible studies.

*Study quality and strength of the evidence*

BvdM composed an expert team of Australian and Dutch dieticians and nutritionists during an International Cancer Technology Transfer fellowship from the UICC in Brisbane, Australia. The aim of the expert team was to appraise the included studies, and to compose evidence statements and recommendations to be used for clinical practice. Two independent members of the expert team appraised the quality of individual studies.
using the Quality Criteria Checklist of the online accessible Evidence Analysis Library of the American Dietetic Association (ADA) (20). The checklist includes four relevance questions that address applicability to diietetic practice, and ten validity questions that address scientific soundness, most importantly on potential selection bias, randomization and blinding procedures and the validity and reliability of outcome parameters. Study quality was rated as positive (+), neutral (ɔ) or negative (-). No authors reviewed their own papers.

Following the quality appraisals, all team members assessed the strength of the evidence, using the ADA additional levels of evidence and grades for recommendations for developers of guidelines (20). The ADA grading system for recommendations has been developed to assist guideline developers in assessing the entire body of evidence and indicating the strength of each guideline recommendation. According to the ADA system, RCTs and meta-analyses obtain a class ‘A’ evidence. The ADA grades of recommendation, I Good, II Fair, III Limited, IV Expert Opinion, V Not Assessable, were used to evaluate the quality of studies and consistency of findings across studies (20).

Members of the expert team reached consensus on the strength of the evidence and the evidence statements for effects on outcome parameters. The team resolved conflicting appraisals by discussion and consensus.

**Results**

*Included studies*

Fifteen RCTs in relation to n-3 polyunsaturated fatty acid administration in patients with cancer were retrieved (Table 2). The quality of studies differed: nine studies were rated as positive quality (13;14;21-27), five as neutral quality (28-32), and one as negative quality (33).

Five studies included patients with unintentional, self-reported, weight loss of more than 5% (13;14;25;29) or more than 10% (23). Two studies excluded either patients who reported ≥ 10% of unintentional weight loss over the previous 3 months (24) or patients with severe malnutrition, as assessed by the Subjective Global Assessment (SGA) tool for malnutrition (30). One study did not report details on nutritional status (28), and the remaining studies only reported on the percentage of patients who had malnutrition at baseline (8 to 50%) without selecting patients with severe weight loss. However, these studies used various methods to identify malnutrition (see Table 2) (21;22;26;27;31).

N-3 PUFAs were supplemented via (fish oil) capsules (0.3 – 4 g EPA) (14;21;24;28;29;32;33), oral nutritional supplements (ONS) (2-2.3 g EPA)(13;23;25;27;30)
or tube feeding (2.3 – 6 g EPA) (26;31). One study prescribed ONS pre-operatively and tube feeding postoperatively (2.3 g EPA)(22).

Fish oil capsules
This paragraph describes the design of the seven RCTs supplementing n-3 PUFAs by fish oil capsules (Table 2).
In a positive quality RCT, 64 patients with generalized solid tumor types who did not receive anticancer treatment were randomized to receive 18 fish oil capsules daily until death. At assessments after 40 days, only 6.7% loss to follow-up occurred. The authors did not describe their randomization and blinding methods, and details on the intake of fish oil capsules were lacking (21).
In a small, neutral quality RCT, 16 patients undergoing chemotherapy and allogeneic bone marrow transplantation were randomized to receive oral EPA 3 times daily, or not. The authors did not describe the method of administration of EPA, dropout rates and patients’ adherence with the EPA intervention (28).
Bruera and colleagues randomized 91 patients (46 to fish oil and 45 to placebo) with advanced cancer, more than 5% weight loss and anorexia, to receive 18 fish oil or placebo capsules daily during 2 weeks. They observed non-adherence with the capsules and amended the protocol to a minimum of 6 capsules a day. Patient dropout rate was 31%. A major limitation of this neutral quality study is the duration as two weeks may have been too short to reach effects on nutritional parameters and quality of life (29).
In a large positive quality double-blinded study, 429 patients with gastrointestinal or lung cancer without anticancer treatment were randomized to receive 2 g or 4 g EPA by a diester or a placebo. Reported intake appeared to be > 80% in both groups. After 8 weeks, 50% of the patients were lost to follow-up for various reasons (death, withdrawal of consent, adverse event)(14).
In a small, positive quality double-blinded study, 33 patients with advanced non-small cell lung cancer undergoing chemotherapy were randomized to receive 4 capsules (510 mg EPA and 340 mg DHA), or placebo capsules. After 66 days, 18% of patients were lost to follow-up. The percentage of n-3 PUFAs in plasma and erythrocyte content increased in the intervention group, confirming the consumption of study supplements (24).
Another small neutral quality non-blinded study randomized 23 patients with colorectal cancer, starting with chemotherapy. Intervention patients were offered four fish oil capsules per day (total 600 mg EPA + DHA), the control group did not receive any supplements. After 9 weeks, 22% of patients were lost to follow-up. The authors did not report patients’ adherence with fish oil capsules (32).
Bonatto and colleagues performed a 8-week non-blinded intervention of 2 g fish oil per day in 38 patients receiving chemotherapy after cancer surgery. This study was rated to be of negative quality; the authors did not describe patient characteristics, inclusion and exclusion criteria, randomization methods, and adherence with fish oil capsules (33).

**Oral nutritional supplements containing n-3 PUFAs**

We identified five RCTs investigating effects of ONS containing n-3 PUFAs in patients with cancer, of which the design will be described in the following section.

In a large positive quality double-blinded study, 200 unresectable pancreatic cancer patients who had lost more than 5% of weight over the previous six months were randomized to receive n-3 PUFA containing ONS or an isonitrogenous control ONS. In this study, a high loss to follow-up (40.5%) occurred (13). In a small subgroup of this study in unresectable pancreatic cancer patients, Moses and colleagues investigated resting energy expenditure (REE) by indirect calorimetry, as well as total energy expenditure (TEE) by doubly labeled water; 9 patients of the n-3 PUFA group and 15 patients (more women) in the control group. The mismatch in sample size was due to the larger double-blinded study of Fearon and colleagues. Overall, this manuscript was rated as of positive quality (25).

Two positive quality studies investigated n-3 PUFA containing ONS interventions in patients with lung cancer: Guarcello and colleagues compared the effects of n-3 PUFA containing ONS to an isonitrogenous control ONS. Only within-group changes in time were described, no differences between intervention and control groups. After 60 days, almost 50% of patients were lost to follow-up, due to non-adherence or clinical deterioration. Blinding and randomization methods were not described in the manuscript (23).

Van der Meij and colleagues compared the effects of an n-3 PUFA containing ONS to an isocaloric control ONS in a double blinded RCT. Dropout rate was 18% after 5 weeks, and appeared to be higher in the n-3 PUFA group. Adherence with the study supplements was lower than reported in other studies, around 1 can/day (others: 1.5 – 2 cans/day). Plasma phospholipids EPA at baseline and after five weeks were also reported (27).

A small non-blinded RCT of negative quality compared the effect of n-3 containing ONS to dietary counseling in patients with stage IV colorectal cancer, who were not severely malnourished. This trial was limited by its size (n = 13), the nature of the control arm, and the abundance of between-group comparisons (30).
Enteral nutrition

Three studies investigated enteral nutrition containing n-3 PUFA around cancer surgery (Table 2).

A positive quality double-blinded RCT in oesophageal cancer patients administered n-3 PUFA containing ONS 5 days pre-operatively, and n-3 PUFA containing tube feeding during 21 days postoperatively. Optimal intake and tolerance of ONS and tube feeding was documented in both groups, and dropout rate was 24.3%. Only within group differences for body weight and fat-free mass were described, with no comparisons between intervention and control groups (22).

A neutral quality study performed a relatively short (7 days) postoperative intervention with n-3 PUFA containing enteral nutrition in 50 patients with upper GI cancer. Thirty percent of intervention and control patients dropped out, because enteral nutrition goals were not reached. Double blinding and randomization was not thoroughly described in this manuscript (31).

One recent publication reported on a large, high quality, double-blinded RCT in 195 patients undergoing oesophagogastric surgery; 7 days before and after surgery, n-3 PUFA containing enteral nutrition or an isonitrogenous control enteral nutrition was administered. A third group only received standard enteral nutrition during 7 days postoperatively, which could not be blinded. Only 46.7% of patients reached the postoperative feeding goals, because of tolerance issues and complications (26).

Strength of the evidence

Appetite

It is suggested that n-3 PUFAs reduce the inflammatory response, thereby improving appetite. In five studies, n-3 PUFAs did not affect appetite when compared to controls (van der Meij 2012 +, Fearon 2003 +, Trabal ø, Fearon 2006 +, Bruera ø) (total n = 858) (13;14;27;29;30). One positive quality study found an improvement in appetite over time (30 d, though not after 60 d) in patients with lung cancer receiving n-3 ONS, but not in control patients (n = 46, Guarcello +) (23).

Recommendation:
- N-3 PUFA supplementation does not improve appetite (grade II)

Energy and Protein intake

Energy intake was reported in seven studies (n = 443, 5 positive and 2 neutral quality) and
Should n-3 PUFAs be prescribed in patients with cancer cachexia?

Protein intake in six studies (n = 356; 5 positive quality and 1 neutral quality). There was no significant increase in energy (29) or both energy and protein intake with n-3 PUFAs (capsules or ONS) compared to a control supplement (n=373, Fearon 2003+, van der Meij 2010+, Finocchiaro+, BrueraØ, TrabalØ)(13;24;27;29;30). One study reported within-group clinically meaningful improvements in energy and protein intake with an n-3 PUFA ONS, but not in the group receiving the control ONS (+ 700 kcal/d and + 20 g protein/d vs. +170 kcal/d and +4.5 g protein/d) (n = 46 Guarcello+) (23), and one study reported improved protein intake in patients receiving the n-3 PUFA containing ONS (+ 27 g/d vs. control + 4 g/d) (n = 24 Moses+) (25).

Recommendation:
- There is no evidence that n-3 PUFA supplementation influences energy and protein intake (grade II)

Body weight

Results from twelve studies show equivocal effects on stabilization or improvement of body weight after supplementation of n-3 PUFAs in cancer patients: five studies did not find significant differences between n-3 and control groups (n = 570, 1 neutral quality, 4 positive quality)(13;21;25;26;29); one study observed a tendency for body weight maintenance in the n-3 PUFA group receiving 2 g EPA by diester emulsion, as compared to groups receiving 4 g EPA or a placebo emulsion (n = 518, Fearon 2006+) (14); three studies observed a significant body weight maintenance in the n-3 PUFA group vs. a control intervention (n = 91, van der Meij 2010+, TrabalØ, Bonatto-)(27;30;33); three studies demonstrated a within-group weight maintenance over time in the n-3 PUFA group (n = 102, SilvaØ, Guarcello+, FinochiaroØ)(23;24;32).

Recommendation:
- The effects of n-3 PUFA supplementation on body weight maintenance are inconclusive (grade II).
**Fat-free mass**

Five studies measured the effect of an n-3 PUFA ONS on fat-free mass. Three studies did not observe difference for fat-free mass as compared to a control intervention (n = 742, Fearon 2006 +, Fearon 2003 +, Moses +)(13;14;25). One study observed a better maintenance of fat-free mass in patients with lung cancer receiving n-3 ONS, as compared to a control ONS (n = 40, van der Meij 2010 +) (27), and one study observed a maintenance of fat-free mass over time in patients receiving n-3 PUFA containing ONS and enteral nutrition around oesophageal cancer surgery (n = 70, Ryan +) (22).

Recommendation:
- The effects of n-3 PUFA supplementation on fat-free mass are inconclusive (grade II)

**Karnofsky Performance Status**

Two large studies reported no improvements in Karnofsky Performance Status with n-3 PUFAs (n = 605 Fearon 2006 + Bruera ∅)(14;29). However two smaller positive quality studies (n = 104 van der Meij 2012 + Gogos +)(21;34) reported improvements in Karnofsky Performance Status.

Recommendation:
- The effects of n-3 PUFA supplementation on Karnofsky Performance Status are inconclusive (grade II)

**Quality of life**

ONS containing n-3 PUFAs improved global health status, physical, cognitive and social function compared to a control intervention in a small study (n = 40, van der Meij 2012 +)(34); another small study only observed a significant improvement in social function in the n-3 PUFA group, not in other quality of life parameters (n = 13, Trabal ∅)(30); one large study found a trend for improved physical function in patients receiving 2 g EPA by a diester emulsion, compared to 4 g EPA or a placebo (n = 518 Fearon 2006 +)(14), and one small study found reduced tiredness to be correlated with the n-3 PUFA dose (n = 87, Bruera ∅)(29). A small study observed a within-group improvement of functional status and symptom scores over time in the n-3 PUFA group (n = 46 Guarcello +)(23). A large positive trial did not find significant differences for quality of life parameters (n = 200, Fearon 2003 +)(13).
Recommendation:
- N-3 PUFA supplementation has modest beneficial effects on some aspects of quality of life (grade II).

Physical activity level
Two small studies investigated effects of n-3 PUFA containing ONS on parameters of physical activity (n = 64, Moses +, van der Meij 2012 +)(25;34).
In one study in pancreatic cancer patients, TEE (measured by doubly labeled water) increased after 8 weeks in the group receiving the n-3 PUFA containing ONS, not in the control group (25). The second study was performed in lung cancer patients. Physical activity assessed by an accelerometer was higher in patients with lung cancer receiving n-3 PUFA ONS during 5 weeks of chemoradiotherapy (34).

Recommendation:
- N-3 PUFA supplementation appears to have beneficial effects on physical activity in patients with cancer (grade III).

Complications
In one small neutral quality study, n-3 PUFA capsules reduced complication rate in Bone Marrow Transplant (BMT) patients (n = 16 Takatsuka ☐) (28). Three studies investigated the effects of a single n-3 PUFA intervention in the absence of multiple immune enhancing compounds around cancer surgery. One study observed a significant reduction in infections in the n-3 PUFA group (n = 50, Kenler ☐) (31), contrary to a large study which did not observe differences for infectious complications (n = 195, Sultan +) (26). Furthermore, there were no effects of n-3 PUFAs (by oral nutritional supplements and/or tube feeding) on major complications (n = 248, Ryan +, Sultan +) (22;26).

Recommendations:
- N-3 PUFAs (by oral nutritional supplements or tube feeding) do not reduce complication rate in cancer patients undergoing surgery (grade II)
- N-3 PUFA capsules may reduce complication rate in BMT patients (grade III)

Survival
There are seven studies that have reported on the effects of n-3 PUFA supplementation on survival. Three large RCTs of positive quality (n = 913, Fearon 2003 +, Fearon 2006 +, 2009 +,
Sultan +)(13;14;26), and two small RCTs did not find improvement of survival after supplementation of n-3 PUFAs, either via capsules, ONS or enteral nutrition (n = 120, Kenler ⊙, Ryan +)(13;14). One study found significantly longer survival in the group receiving n-3 PUFA capsules (n = 64, Gogos +) (21). Another study demonstrated improved survival in patients who received n-3 PUFA capsules around BMT, but this study had very small numbers (n = 16, Takatsuka ⊙) (28).

Recommendation:
- N-3 PUFA supplementation does not improve survival (grade II)

**Safety and GI tolerance**

Other important issues include the safety and tolerance of n-3 PUFA supplementation. Of the 15 retrieved studies, 10 reported on tolerance or adverse events during supplementation of n-3 PUFA.

Among the seven studies supplementing n-3 PUFA by capsules (n = 661), two studies observed gastrointestinal side effects in the n-3 PUFA group slightly more frequently than in the control group (P-value not reported) (n = 120, Bruera ⊙, Finocchiaro +)(24;29). Another study reported adverse events in n-3 PUFA and control groups; no serious adverse events were related to the study capsules (n = 518, Fearon 2006 +)(14). Furthermore, in one small study, no side effects related to fish oil capsules were reported (n = 23, Silva ⊙)(32).

In studies applying n-3 PUFA containing ONS (5 studies, n = 299), 2 studies reported adverse events in both groups, and no serious adverse events related to n-3 PUFA (n = 240, Fearon 2003 +, van der Meij 2010 +)(13;27). Another study reported excellent tolerance of the n-3 PUFA containing ONS (n = 46, Guarcello +)(23). Others reported chemotherapy delays in both n-3 PUFA and control groups, but numbers were too small to test for statistical significance (n = 53, Trabal ⊙, van der Meij 2012 +)(30;34).

In studies using enteral nutrition in upper GI surgery patients, GI complaints and intolerance to the enteral nutrition were similar in n-3 PUFA and control groups (n = 315, Ryan +, Sultan +, Kenler ⊙)(22;26;31). One study reported minor GI complaints (no serious adverse events) in both n-3 and control groups (n = 70, Ryan +) (22), another study observed slightly more GI complaints in the control group (P-value not reported, n = 50, Kenler ⊙)(31). In one study, only 50% of overall the study population reached the aimed feeding rate owing to problems with tolerance and/or complications, such as diarrhoea, ileus, nausea, vomiting or bloating (n = 195, Sultan +)(26).
Recommendations:
- N-3 PUFA supplementation by capsules or ONS appears to be safe to administer in cancer patients receiving chemo(radio)therapy or palliative care (grade II)
- Intolerance to enteral nutrition around upper GI cancer surgery does not appear to be related to n-3 PUFAs (grade II).

Meta-analyses and systematic reviews
One Cochrane systematic review evaluated the effectiveness of EPA in cancer cachexia. This meta-analysis included five trials published until February 2005 (involving 587 participants), and compared outcomes on weight, quality of life and adverse events. It was concluded that by that time, there was insufficient data to establish whether oral EPA was better than placebo. In addition, comparisons of n-3 PUFAs containing ONS in the presence of an appetite stimulant (megestrol acetate) provided no evidence that n-3 PUFAs improve weight maintenance, quality of life or survival (35). A systematic review of RCTs published until October 2006 drew the same conclusion, but did not perform meta-analyses (36). A systematic review of Colomer and colleagues included 17 studies published between 1996 and 2006. A panel of experts established the evidence and suggested that administration of n-3 FA in doses of at least 1.5 g EPA/day for a prolonged period of time is associated with an improvement in clinical, biological and QoL parameters (37).

A recent systematic review included 38 RCTs, uncontrolled studies and case series published until June 2010. This review found evidence of a net benefit of n-3-fatty acids on cachexia in advanced cancer only in low quality trials, and no evidence of a clear benefit in studies of high quality. Moreover, adverse effects of n-3 PUFA supplementation (such as abdominal discomfort and fish belching) impact on quality of life and dose escalation. This led to a weak negative GRADE recommendation (38).

In summary, conclusions from meta-analyses and systematic reviews were equivocal and did not consistently recommend supplementing n-3 PUFAs in patients with cancer.

Discussion
This literature review gives an up-to-date overview on the available evidence for the prescription of n-3 PUFAs in patients with cancer. We conclude that fair evidence shows supplementation of n-3 PUFA appears to be safe and may improve quality of life and physical activity. However, supplementation of n-3 PUFA does not influence energy or protein intake, appetite, or survival in cancer patients. Effects on body weight, fat-free
mass and performance status are equivocal: around half of studies find beneficial effects while others do not show differences compared to control interventions. Moreover, fair evidence shows that supplementation does not reduce postoperative complications. These conclusions were drawn after a systematic qualitative evidence analysis of studies published until April 2012, carried out by an international expert team. In order to objectively evaluate effects of n-3 PUFAs in cancer, a meta-analysis on this subject is required, but the variability in study designs makes it yet impossible to carry out meta-analyses. Also, some authors report between group differences whereas others only report within group differences.

The 15 available RCTs differ in terms of patient populations, in- and exclusion criteria, intervention strategies, and study durations. The administered dose ranged from 300 mg to 6 g of EPA, and in some studies, especially the studies with fish oil capsules, patient adherence with study supplements was not documented. In addition, the study populations are heterogeneous in terms of nutritional status and cachexia. Some studies selected patients on the degree of weight loss; others did not apply an inclusion criterion on nutritional status or cachexia. Some studies did not have adequate statistical power: around half of the studies were small (10 studies included 60 or fewer participants). The large studies had a high dropout rate (around 50%): this may also have biased the results.

Apart from immune-modulating effects of n-3 PUFAs in patients with cancer cachexia, additional energy and protein are required for body weight maintenance and synthesis of lean tissue. The effect of n-3 PUFAs combined with additive energy and protein on nutritional status is expected to be larger than the effect of a single n-3 PUFA intervention.

Apart from the study of Trabal and colleagues, all included studies provided comparable amounts of energy and protein and nutritional counseling in both intervention and control groups. As a result, these studies investigated the pure effect of n-3 PUFAs, provided that both groups consumed a comparable amount of n-3 PUFAs by normal foods. None of the studies described the consumption of fish or other foods containing n-3 PUFAs, such as walnuts containing ALA. Alternatively, only a few studies checked the plasma phospholipid EPA concentration as a measure of n-3 PUFA intake.

Study design is a limiting factor when addressing the evidence of n-3 PUFAs in cancer patients. The adherence to the study intervention was not always monitored; suboptimal adherence to n-3 PUFA supplements could have resulted in a lack of effect. Future trials should consider a choice of supplementation format (capsules or liquid), or the use of n-3 PUFA-containing enteral or parenteral nutrition, in order to improve patients’ adherence. The outcome parameters also differ between studies. Body weight is widely used as outcome parameter, but is unreliable in case of ascites or oedema and gives no
information on body composition. Even so, bio-electrical impedance analysis to measure fat-free mass is unreliable in patients with cancer. CT image analysis or DEXA is preferred as the method to precisely quantify skeletal muscle (39),(40). Ultimately, positive effects on body composition should be translated to improvements of quality of life. A few RCTs observed positive effects of n-3 PUFAs on both body weight and quality of life; the latter is more relevant to patients with cancer (23;27;30;34). Still, we need more large RCTs confirming this relationship.

Conventional nutritional interventions for cancer patients have limited effects on clinical endpoints or the quality of life during chemotherapy (41) or palliative care (42). If supplementation of n-3 PUFAs does improve cancer cachexia parameters, such as body weight and quality of life, the effect is relatively small. Therefore, more benefits are expected from combination treatments including anti-catabolic and orexogenic agents and n-3 PUFAs. In a 5-arm RCT in patients with cachexia, the combination regimen that included the administration of an appetite stimulant, oral nutritional supplementation with EPA and DHA, L-carnitine, and thalidomide was shown to be the most effective treatment in terms of lean body mass, REE and fatigue (43).

Another promising intervention to improve fat free mass and physical function is physical exercise training with resistance training. Accumulating evidence suggests beneficial effects of physical exercise training during chemotherapy, but a combination intervention of training and nutritional counseling or nutritional supplementation has yet to be studied. Rogers and colleagues are currently conducting an open label, prospective RCT on EPA, the COX-2 inhibitor celecoxib, combined with leucine supplementation and resistance training (44).

Nutritional interventions are often offered in patients with nutritional issues and weight loss; prophylactic nutritional intervention is not standard. New insights suggest that early nutritional intervention in patients with early stage cachexia could be more effective than interventions in patients with advanced cancer cachexia, but to our knowledge, no studies on this topic have been published (45). Recently, a proposal for the definition of cancer cachexia has been published, and attention has been paid to the identification of patients with ‘pre-cachexia’ as a probable indication to start nutritional intervention (9;46). However, definitions of cachexia and pre-cachexia need to be validated and further refined.
Conclusion

This review helps clinicians to decide on the prescription of n-3 PUFAs in patients with cancer cachexia. There is increasing evidence that n-3 PUFAs are safe to administer to cancer patients, and can improve quality of life and physical activity. However the evidence for maintenance of body weight and fat-free mass remains inconsistent. Research should focus on early intervention and on multimodal treatment strategies with attention to the adherence and feasibility of n-3 PUFA supplementation methods.
Should n-3 PUFAs be prescribed in patients with cancer cachexia?

Figure 1: Flow chart literature search

Search result: 491

PubMed: 241
EMBASE: 250

Potential studies (title or abstract):
EMBASE: 34
PubMed: 62

Duplicates: 91

Excluded Pubmed: 52
Reasons for exclusion:
- non-RCTs: 23
- no enteral supplementation of n-3 FA: 9
- no clinical outcome parameters: 5
- multiple immune enhancing compounds and/or use of appetite stimulants: 11
- no cancer: 4
- in vivo/in vitro: 0
- no adult: 0

Excluded EMBASE: 29
Reasons for exclusion:
- non-RCTs: 4
- no enteral supplementation of n-3 FA: 13
- no clinical outcome parameters: 0
- multiple immune enhancing compounds and/or use of appetite stimulants: 9
- no cancer: 3
- in vivo/in vitro: 0
- no adult: 0

Studies included: 10 RCTs
Table 1: Example of search strategy in PubMed®; effects of enteral supplementation of n-3 PUFA on clinical outcome in cancer

<table>
<thead>
<tr>
<th>PubMed® Search</th>
<th>Medical subject headings [MeSH] and keywords</th>
<th>Limits: human and English</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>n-3 PUFA</td>
<td></td>
<td>41215</td>
</tr>
<tr>
<td></td>
<td>&quot;Eicosapentaenoic Acid&quot;[MeSH] OR &quot;Fish Oils&quot;[MeSH] OR &quot;Fatty Acids, Omega-3&quot;[MeSH] OR &quot;Docosahexaenoic Acids&quot;[MeSH] OR &quot;Fatty Acids, Unsaturated&quot;[MeSH] OR eicosapentaenoic OR docosahexaeno* OR (fatty acid*) OR EPA[tiab] OR MaxEPA[tiab] OR DHA[tiab] OR (fish* AND oil*) OR omega3* OR omega-3* OR (omega 3*) OR (cod oil*) OR (marin oil*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2</td>
<td>Cancer</td>
<td></td>
<td>844623</td>
</tr>
<tr>
<td></td>
<td>&quot;Neoplasms&quot;[MeSH Terms] OR neoplasm* OR malignan* OR cancer OR carcino* OR tumor OR tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#3</td>
<td>Oral or enteral nutrition</td>
<td></td>
<td>132837</td>
</tr>
<tr>
<td></td>
<td>enteral* OR supplement* OR sip OR feed OR formula* OR liquid OR tube OR nasogastric OR nasojejunal OR nasoduodenal OR gastrostomy OR jejunostomy OR Enteral Nutrition&quot;[MeSH]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#4</td>
<td>Nutritional status</td>
<td></td>
<td>168621</td>
</tr>
<tr>
<td></td>
<td>&quot;cachexia&quot;[MeSH] OR cachexi* OR cachectic OR wasting OR &quot;weight loss&quot;[MeSH] OR (weight loss) OR (weight gain) OR &quot;body weight&quot;[MeSH] OR &quot;body composition&quot;[MeSH] OR appetite OR &quot;Energy Metabolism&quot;[Mesh] OR energy OR REE OR TEE OR hypermetabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#5</td>
<td>Morbidity</td>
<td></td>
<td>1119366</td>
</tr>
<tr>
<td></td>
<td>&quot;Postoperative Complications&quot;[MeSH] OR complications OR complication* OR &quot;morbidity&quot;[MeSH] OR morbidit*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#6</td>
<td>Mortality</td>
<td></td>
<td>487681</td>
</tr>
<tr>
<td></td>
<td>&quot;mortality&quot;[MeSH Terms] OR &quot;hospital mortality&quot;[MeSH Terms] OR mortalit* OR death* OR survival OR &quot;survival&quot;[MeSH Terms]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#7</td>
<td>Length of stay</td>
<td></td>
<td>66749</td>
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<tr>
<td></td>
<td>length of stay OR LOS OR &quot;length of stay&quot;[MeSH Terms]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#8</td>
<td>Quality of life</td>
<td></td>
<td>104200</td>
</tr>
<tr>
<td>#9</td>
<td>#4 OR #5 OR #6 OR #7 OR #8</td>
<td></td>
<td>1583988</td>
</tr>
<tr>
<td>#10</td>
<td>#1 AND #2 AND #3 AND #9</td>
<td></td>
<td>246</td>
</tr>
</tbody>
</table>
Should n-3 PUFAs be prescribed in patients with cancer cachexia?

Table 2: Summary of randomized controlled trials on the role of EPA in patients with cancer

<table>
<thead>
<tr>
<th>Author Year</th>
<th>Study Design</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gogos et al 1998 (21)</td>
<td>Non-blinded RCT Positive</td>
<td>To investigate the effect of n-3 PUFA plus vitamin E on the immune status and survival of well-nourished and malnourished patients with generalized malignancy.</td>
<td>64 patients with mixed solid tumor types (prevalence of malnutrition at baseline: 50%)</td>
<td>40 d E: n=30 18 g/d fish oil capsules (Max-EPA, 3.06 g EPA + 2.07 g DHA) C: n=30 18 g/d placebo capsules</td>
<td>4 patients (6.7%) dropped out of the study because of poor adherence, Survival – increased in E group only (p&lt;0.025 compared with C) Other – No effect of fish oil on albumin or transferrin. No toxicity of fish oil except for mild abdominal discomfort and transient diarrhea</td>
<td>n-3 PUFA seemed to prolong survival and improve KPS in malnourished patients with generalized malignancy.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study Design Quality</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
</table>
| Takatsuka 2001 (28) Non-blinded RCT Neutral | To assess the effectiveness of prophylactic oral EPA therapy in preventing complications in patients who received bone marrow transplantation | n = 16 patients undergoing chemotherapy and allogeneic bone marrow transplantation from unrelated donors (details on nutritional status not provided) | 21 d before, to 180 d after bone marrow transplantation | Survival rate was significantly higher in the group given EPA and EPA significantly reduced the complications of BMT. | Zero dropouts reported  
Energy intake – NA  
Protein intake – NA  
Weight – NA  
FFM – NA  
Functional capacity – NA  
Quality of Life – NA  
Survival – higher survival rate in E group (E: n = 0 died vs. C: n=5 died) (p < 0.01)  
Other – reduced complications in E group (E: n = 3 Graft-versus-Host Disease, n = 4 no complications vs. C: n = 6 Graft-versus=Host Disease, n = 4 thrombotic microangiopathy, n = 4 CMV disease) |  

| Bruera 2003 (29) Double-blinded RCT Neutral | To determine whether high doses of fish oil, administered over 2 weeks, improve 87 advanced cancer patients (> 5% weight loss) | 2 wk  
E: n = 30 fish oil capsules (mean EPA 1.8 g)  
C: n = 30 placebo | 60 patients completed the study (drop-out 31%)  
27 patients (31%) did not complete the study  
Energy intake – ↑51 kcal E vs. ↓57 kcal C (NS) | No effects on outcome parameters after 2 weeks of n-3 PUFA supplementation |  


Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study Design Quality</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruera 2003 (29) (continued)</td>
<td></td>
<td></td>
<td>capsules</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>symptoms in patients with advanced cancer.</td>
<td></td>
<td>Protein intake – NA Weight – ↑0.03 kg E vs. ↓0.89 kg C NS FFM – NA Functional capacity – KPS ↑ 10.0 E vs. ↓ 6.9 C (NS) Quality of Life – NA Survival – NA Other – Appetite, tiredness, nausea, wellbeing NS. Same number of patients with gastrointestinal complaints in both groups (E: n = 5, C: n = 5)</td>
<td>Conclusion. Non adherence with protocol in both groups 10% controls high EPA levels High dropout rate due to intolerance of fish oil</td>
</tr>
<tr>
<td>Fearon 2006 (14) Double-blind</td>
<td></td>
<td>To compare EPA diethyl ester with placebo in cachectic cancer patients for effects on (≥ 5% weight loss)</td>
<td>8 wk</td>
<td>At week 8, 270 patients remained (drop-out 48%) Energy intake – NA Protein intake – NA Weight – ↑1.2 kg (2 g EPA) vs. C; ↑0.3 kg vs. Placebo (4 g EPA),</td>
<td>The results indicate no significant benefit from single agent EPA in the treatment of</td>
</tr>
<tr>
<td>Author Year</td>
<td>Study population</td>
<td>Intervention</td>
<td>Outcomes</td>
<td>Conclusions</td>
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</tr>
<tr>
<td>Fearon 2006 (14) (continued)</td>
<td>weight and fat free mass in patients with advanced cancer</td>
<td>C: n = 171: placebo EPA: 0.9 vs. C; 4 g EPA: -0.1 vs. C NS Functional capacity – KPS, weakness: NS Quality of Life – 2 g EPA: ↑4.3 physical functioning; 4 g EPA: -3.4 (p = 0.04) Survival – 2 g EPA: 155 d; 4 g EPA: 142 d; C: 140 d NS Other – appetite, albumin, CRP, nausea, vomiting, diarrhea NS, supplements were well tolerated, adverse events NS, serious adverse events not related to supplements</td>
<td>p = 0.066</td>
<td>cancer cachexia.</td>
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</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study Design Quality</th>
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<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finocchiaro 2011 (24) Double-blinded RCT Positive</td>
<td>To investigate the effect of EPA and DHA vs. placebo on inflammatory condition, and oxidative and nutritional statuses</td>
<td>n = 33 patients with lung cancer (patients with ≥10% weight loss over the previous 3 months were excluded)</td>
<td>66 d E: n = 19 4 fish oil capsules (510 mg EPA + 310 mg DHA) C: n = 14 4 olive oil capsules (850 mg)</td>
<td>27 patients completed the study (E: n = 13, C: n = 14), adherence was good Energy intake – NS between groups Protein intake – NS between groups Weight – E increase of 3.4 kg (p &lt; 0.05 vs. baseline), C stable, NS between groups FFM – NA Functional capacity – NA Quality of life – NA Survival – NA Other – albumin, thyroxine-binding prealbumin and transferrin: NS between groups, CRP and IL-6 after 66 d lower in E than C group (p &lt; 0.05)</td>
<td>Increase in body weight in the n-3 group; reduction in inflammatory index-es and oxidative status. Small sample size, no data on FFM or quality of life</td>
</tr>
</tbody>
</table>
## Conclusions

- Fish oil prevented the decline in neutrophil number and function and decreases of body weight during chemotherapy.
- Beneficial effects on nutritional status of the fish oil supplement.
- No control intervention;

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonatto 2012</td>
<td>To investigate the effect of fish oil in patients receiving chemotherapy following surgery for removal of a tumour</td>
<td>n=38 patients tumors at various sites (predominantly gastrointestinal) Prevalence of malnutrition at baseline not reported</td>
<td>8 wk E: n = 19 fish oil capsules 2 g/d (0.3 g EPA + 0.4 g DHA) C: n = 19 no capsules</td>
<td>Zero dropouts reported EPA and DHA in polymorphonuclear cells increased significantly and AA decreased significantly after 8 wk. Energy intake – NA Protein intake – NA Weight – E + 1.7 kg, C – 2.5 kg (P &lt; 0.002 for difference between groups) FFM – NA Functional capacity – NA Quality of life – NA Survival – NA</td>
<td>Fish oil prevented the decline in neutrophil number and function and decreases of body weight during chemotherapy.</td>
</tr>
<tr>
<td>Silva 2012</td>
<td>To check whether there is a change in the markers of inflammation and nutritional status of patients with colorectal cancer supplemented with</td>
<td>n = 23 patients with colorectal cancer in chemotherapy treatment (prevalence of malnutrition at baseline: 0 %, according to BMI, 52.2% lost &gt; 5%)</td>
<td>9 wk, during chemotherapy (starting at the first day of chemotherapy)</td>
<td>5 individuals did not complete the study (1 E, 4 C) Energy intake – NA Protein intake – NA Weight – difference between baseline and wk 9 E: no difference (p &gt; 0.05), C: reduction (p = 0.01) FFM – NA</td>
<td>Beneficial effects on nutritional status of the fish oil supplement.</td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study Design Quality</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silva 2012 (32)</td>
<td>2 g of fish oil, compared with the non-supplemented ones</td>
<td>26.1% lost &gt; 10%</td>
<td>E: n = 11 2 g fish oil capsules (4 caps, 200 mg EPA + DHA per capsule) C: n = 12 no capsules</td>
<td>Functional capacity – NA Quality of life – NA Survival – NA Other – E: decrease in CRP/albumin-ratio (p=0.005)</td>
<td>adherence with capsules and plasma fatty acids not reported</td>
</tr>
<tr>
<td>Fearon 2003 (13) Double-blinded RCT Positive</td>
<td>To compare a protein and energy dense supplement enriched with n-3 PUFA and antioxidants with an isocaloric isonitrogenous control supplement for effects on weight, FFM, dietary intake and quality of life in cachectic patients</td>
<td>200 untreated pancreatic cancer patients</td>
<td>8 wk E: n = 95 high protein and energy ONS + 2.2 g EPA + 0.9 g DHA C: n = 105 isonitrogenous control ONS</td>
<td>110 patients completed the study (drop-out 45%) Energy intake - ↑224 kcal E vs. 68 kcal C, NS; Sig ↑ E baseline to 8 wks only Protein intake - ↑15 g E vs. 6 g C, NS; Sig ↑ E baseline to 8 wks only Weight - ↓0.37 kg E vs. ↓0.25 kg C, NS; Sig change baseline to 8 wks E &amp; C; Wt ↑ correlated with intake cans E only FFM - ↑0.27 E vs. 0.12 C, NS; ↑ FFM correlated intake cans E only</td>
<td>Both E and C supplements attenuated weight loss. ↑ FFM in E only, post hoc analyses showed a dose response effect on weight, FFM and quality of life in cachexia?</td>
</tr>
<tr>
<td>Author Year Study Design Quality</td>
<td>Aim</td>
<td>Study population</td>
<td>Intervention</td>
<td>Outcomes</td>
<td>Conclusions Limitations</td>
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</tr>
<tr>
<td>Fearon 2003 (13) (continued)</td>
<td>with advanced pancreatic cancer.</td>
<td>n = 24 patients with advanced pancreatic cancer</td>
<td>8 wk</td>
<td>Functional capacity – NA Quality of Life – Global E vs. C, NS; Post hoc analysis ↑ QoL and ↑ wt only Survival – 142 days E vs. 128 days C, NS Other – ONSs were well tolerated, adverse events NS, serious adverse events not related to study supplements</td>
<td>compliant E patients. Non-adherence with protocol in both groups High dropout rate due to death</td>
</tr>
<tr>
<td>Moses 2004 (25) Double-blinded RCT Positive</td>
<td>To investigate the effects of an ONS containing n-3 PUFA on TEE, REE and PAL in home-living cachectic patients with advanced pancreatic cancer.</td>
<td>8 wk</td>
<td>5 patients did not complete the study (E: n = 3, C: n=2). Adherence: mean intake E: 1.9 cans/d, C: 1.5 cans/d (NS) Energy intake – increased significantly in E patients compared with baseline intake, trend when compared with C patients Protein intake – increased significantly in E patients compared with baseline intake, trend when compared with C patients Weight – NS FFM – NS</td>
<td>Administration in EPA-containing ONS was associated with an increase in PAL</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study population</th>
<th>Intervention</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moses 2004</td>
<td></td>
<td>n = 46 lung cancer patients undergoing chemotherapy</td>
<td>Energy intake = E: 7,700 kcal (p &lt; 0.05 vs. baseline) vs. C: 7,170 kcal</td>
<td>ONS containing n-3 PUFA seems effective in improving nutritional status and quality of life of lung cancer patients.</td>
</tr>
<tr>
<td>Guarcello 2007</td>
<td>(23)</td>
<td>(continued)</td>
<td>Powder intake = E: 14.4 g vs. C: 14.4 g</td>
<td>Functional capacity – NA, Quality of life – NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Study Design</th>
</tr>
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<tbody>
<tr>
<td>Non-blinded RCT</td>
<td>Non-blinded RCT</td>
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<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional capacity – NA</td>
<td>Functional capacity – NA</td>
</tr>
<tr>
<td>Quality of life – NA</td>
<td>Quality of life – NA</td>
</tr>
</tbody>
</table>

Other – RE, TE, and PAL of C patients did not change significantly; TE and PAL of E patients increased significantly; RE did not change; no significant differences between groups.

25 patients completed the study (dropout rate 45.7%).

60 d

Energy intake – E: ▲ 700 kcal (p < 0.05 vs. baseline)

Protein intake – E: ▲ 20 g (p < 0.05 vs. baseline)

Weight – Increase in E (7.09 kg (p < 0.05 vs. baseline), C: ▲ 0.0 kg)

FFM – NA

REE, TEE, and PAL of C patients did not change significantly; TEE and PAL of E patients increased significantly, RE did not change; no significant differences between groups.

Study design: Non-blinded RCT

Aim: To evaluate the influence of an EPA-enriched, energy-dense oral supplement on inflammatory and nutritional status, as well as on the quality of life of lung cancer patients under chemotherapy.

Study population: n = 46 lung cancer patients (> 10% weight loss over the previous 6 months).

Intervention: 60 d

Energy intake: E: ▲ 700 kcal (p < 0.05 vs. baseline) vs. C: ▲ 7,170 kcal

Protein intake: E: ▲ 20 g (p < 0.05 vs. baseline) vs. C: ▲ 14.4 g

Weight: Increase in E (7.09 kg (p < 0.05 vs. baseline), C: ▲ 0.0 kg)

FFM: NA

Conclusions: ONS containing n-3 PUFA seems effective in improving nutritional status and quality of life of patients.
Table 2 (continued)

<table>
<thead>
<tr>
<th>Author Year Study Design Quality</th>
<th>Aim</th>
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<th>Intervention</th>
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<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guarcello 2007 (23) (continued)</td>
<td></td>
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</tr>
<tr>
<td>van der Meij 2010 (27), 2012 (34) Double-blinded RCT Positive</td>
<td>To investigate the effects of an ONS containing n-3 PUFA on nutritional status and inflammatory markers in patients with stage III NSCLC undergoing undergoing</td>
<td>n = 40 patients with lung cancer undergoing chemoradiotherapy (prevalence of malnutrition at baseline: 20%)</td>
<td>E: n = 20 high protein and energy ONS + 2.02 g EPA + 0.92 g DHA C: n = 20 isocaloric control ONS</td>
<td>7 patients did not complete the study (dropout rate 17.5%). Adherence: mean dose ~1 pack/d (1.1 g EPA)</td>
<td>A protein- and energy-dense ONS containing n-3 PUFA beneficially affects nutritional status during multimodality treatment in patients with NSCLC.</td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
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<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>van der Meij 2010 (27), 2012 (34) (continued)</td>
<td>BMI &lt;18.5</td>
<td>multimodality, therapy. Secondary effect parameters included quality of life and functional status.</td>
<td></td>
<td>FFM – E less decrease vs. C, difference: 1.5 kg (p = 0.05), 1.9 kg (p = 0.02) after 3 and 5 wk&lt;br&gt;Functional capacity – KPS E †5.3 vs. C after 3 wk (p = 0.04)&lt;br&gt;Quality of life – Physical and cognitive function E †11.6 and †20.7 (P&lt;0.01) vs. C; Global health status E †12.2 and social function †22.1 vs. C (p = 0.04)&lt;br&gt;Survival – NA&lt;br&gt;Other – physical activity: E †6.6 (p = 0.04), †2.5 (p = 0.05) vs. C after 3 and 5 wk&lt;br&gt;REE: E ↓16.7% of predicted (p = 0.01), ↓4 kJ/kg (p = 0.07) vs. C after 3 wk</td>
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### Table 2 (continued)

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<tr>
<th>Author Year Study Design Quality</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
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<tr>
<td>Trabal 2010 (30) Non-blinded RCT Neutral</td>
<td>To assess the effect of an intervention with an EPA-ONS on chemotherapy tolerability in patients with advanced colorectal cancer (severely malnourished patients according to SGA were excluded)</td>
<td>n = 13 patients with stage IV colorectal cancer that were going to receive first line chemotherapy treatment</td>
<td>12 wk E: n = 5 high protein and energy ONS + 2 g EPA + 0.9 g DHA + dietary counseling C: n = 6 dietary counseling</td>
<td>2 patients (15.4%) did not complete the study. Adherence: mean dose 1.6 packs/d (1.6 g EPA) Energy intake – E group consumed on average 312 kcal more than C group (NS between groups) Protein intake – E group consumed on average 18 g protein more than C group (NS between groups) Weight – E group weight gain +4.94 kg vs. -1.17 kg (C), p = 0.045 FFM – NA Functional capacity – NA Quality of life – GHS/QoL scale: E: 3.33 vs. C: -6.94, NS; Role function: E: 13.33 vs. 2.78, NS; Social function: E: 16.67 vs. C: -13.89; p = 0.038. Fatigue: -4.44 vs. C: 11.11, NS; Pain: -10 vs. 2.78, NS;</td>
<td>Improvement in weight gain and some important domains of QoL in advanced colorectal cancer patients taking EPA-containing ONS plus dietary counseling. Small sample size, no EPA vs. control ONS</td>
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Table 2 (continued)

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<tr>
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<tr>
<td>Kenler 1996 (31) Double-blinded RCT Neutral</td>
<td>To compare the safety, gastrointestinal tolerance, and clinical efficacy of feeding an enteral diet containing n-3 PUFA/MCT vs. an n = 50 patients with upper gastrointestinal cancer n = 17 n-3 PUFA/MCT tube feeding (4.0 g EPA; 1.9 g DHA) n = 18 isocaloric, isonitrogenous 7 d postoperative 15 patients did not complete the study (dropout rate 30%)</td>
<td>Loss of appetite: E: 6.67 vs. C: -16.67, NS. Survival – NA Other – chemotherapy adherence: none of 5 E patients had to delay or stop their chemotherapy; 4 of 6 control patients experienced toxicity and interruption (NS); routine laboratory parameters: NS</td>
<td>Tube feeding containing n-3 PUFA after surgery appears to be safe, and to reduce gastrointestinal complications and infections.</td>
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Table 2 (continued)

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<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions/Limitations</th>
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<tr>
<td>Kenler 1996 (31)</td>
<td>Double-blinded RCT Neutral</td>
<td>isonitrogenous, isocaloric formula in patients undergoing major abdominal surgery for upper gastrointestinal cancer</td>
<td>Definition of malnutrition: MUAMC &lt;10th pct</td>
<td>control tube feeding</td>
<td><strong>Survival</strong> – mortality: NS  <strong>Other</strong> – number of infections, length of stay, nitrogen balance: NS  Fewer gastrointestinal complications in n-3 PUFA group (p = 0.053); 50% reduction in the total number of infections in n-3 PUFA group (p = 0.037); lower number of infected patients with more than one infection in n-3 PUFA group (p = 0.09).  Safety parameters (serum biochemistry, electrolytes, hematology, blood coagulation): NS</td>
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### Table 2 (continued)

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<tr>
<th>Author Year Study Design</th>
<th>Aim</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Conclusions Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryan 2009 (22) Double-blinded RCT Positive</td>
<td>To examine the effects of perioperative EPA enriched enteral nutrition on the metabolic, nutritional, and immuno-inflammatory response to esophagectomy, and on postoperative complications</td>
<td>n = 70 esophageal cancer patients (prevalence of mild, moderate or severe malnutrition at baseline: 63%, &gt;10% weight loss: 19%)</td>
<td>5 d pre-operative (ONS) to 21 days postoperative (tube feeding) E: n = 28 high protein and energy ONS or tube feeding + 2.3 g EPA + 1.0 g DHA C: n = 25 isocaloric control ONS or tube feeding</td>
<td>53 patients completed the study (drop-out rate 24%) Energy intake – postoperative day 10 to 21: E: 645 kcal vs. C 710 kcal NS Protein intake –NS Weight – E stable, C ↓ 1.8 kg. E n = 2 (8%) &gt;5% weight loss, C n = 10 (39%) comparison between groups: p = 0.03 FFM – E + 0.3 kg (p = 0.8 vs. baseline) vs. C -1.9 kg (p = 0.03 vs. baseline) Functional capacity – NA Quality of life –NA Survival –NA Other – major complications and gastrointestinal complaints (NS)</td>
<td>n-3 PUFA supplemented by early enteral nutrition is associated with preservation of FFM post-esophagectomy compared with a standard enteral nutrition. Pre-operative vs. Post-operative day 21 (P-value of comparison between time points)</td>
</tr>
</tbody>
</table>
### Conclusions

Limitations

N-3 PUFA supplement ed enteral nutrition did not affect immune function or clinical outcome following oesophagogastric cancer surgery.

References

20. American Dietetic Association. ADA Evidence Library, 3-1-2010. Ref Type: Internet Communication


25. Moses AW, Slater C, Preston T, Barber MD, Fearon RC. Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. Br J Cancer 2004;90:996-1002.


43. Mantovani G, Maccio A, MadeuU C, Serpe R, Massa E, Dessi M, Panfz E, Conto P. Randomized phase III clinical trial of
Should n-3 PUFAs be prescribed in patients with cancer cachexia?


Nutrition during trimodality treatment in stage III non-small cell lung cancer - not only important for underweight patients

BS van der Meij
EC Phernambucq
GM Fieten
EF Smit
MA Paul
PAM van Leeuwen

Journal of Thoracic Oncology 2011;6(9):1563-8
Abstract

Introduction
Trimodality treatment for stage III non-small cell lung cancer (NSCLC), consisting of chemoradiotherapy followed by surgery, is associated with treatment-related toxicity, malnutrition, and postoperative complications. The aim of this retrospective study was to investigate the predictive value of nutritional parameters on postoperative morbidity, mortality, and survival.

Methods
Patients with stage III NSCLC undergoing concurrent chemoradiotherapy followed by surgery in one center between 2003 and 2009 were included. Age, sex, forced expiratory volume in 1 second, body mass index, weight change, and surgical and pathological factors were recorded and related to the occurrence of postoperative complications/mortality, overall survival (OS), and progression-free survival.

Results:
Of 51 study patients, 17 (33%) had overweight (body mass index ≥ 25) at start of treatment and 20 patients (39%) were malnourished at hospital admission for surgery. Postoperative complications occurred in 25 patients (49%), 6 had major complications, and 2 died within 90 days after surgery, but no significant predictive factors were found. Overall, weight loss ≥ 5% during induction period was associated with shorter OS (p = 0.03), but especially overweight patients experiencing weight loss ≥ 5% during induction period (n = 7) had shorter OS (hazard ratio 4.63, p = 0.005; log-rank p = 0.04) and progression-free survival (hazard ratio 6.03, p = 0.007).

Conclusions:
This study indicates that malnutrition especially in overweight patients negatively influences survival outcomes of trimodality treatment for stage III NSCLC.
Introduction

The debate for optimal treatment of stage III non-small cell lung cancer (NSCLC) is mainly focused on the use of different modalities, i.e., chemotherapy with surgery and/or radiotherapy. However, survival still remains poor despite the use of several chemotherapy regimens, implementation of concurrent radiotherapy, and extensive restaging before surgery. In stage III NSCLC, the application of surgery after induction treatment is controversial because no survival benefit has been demonstrated in randomized clinical trials compared with chemoradiotherapy (CRT) alone (1,2). In our practice, stage III NSCLC patients without mediastinal lymph node involvement and those with N2/N3 disease and proven mediastinal downstaging after concurrent CRT are selected for surgery. In this situation, consideration is given to the risk of local recurrence and technical resectability of the primary tumor.

A minority of patients treated with upfront surgery for NSCLC have a poor nutritional status, which has been shown to be an independent risk factor for postoperative death and reventilation after lung resection (3). In addition, malnutrition in lung carcinoma is associated with advanced stage of disease and is a reason for careful and extensive staging (4). Perioperative nutritional support in malnourished surgical patients has been shown to reduce the risk of postoperative complications and death and is advocated by international guidelines (5). Apart from a negative effect on surgical morbidity and mortality, weight loss and catabolic state are associated with a negative impact on long-term oncological outcomes in patients with cancer, such as a reduced progression-free survival (PFS) (6–9). The mechanisms for this phenomenon are poorly understood, but immunologic processes may play an important role (10–13). However, it is unknown whether nutritional support can ameliorate this phenomenon. Because cisplatin-based CRT is associated with nausea, vomiting, diarrhea, anorexia, and esophagitis, patients undergoing trimodality treatment may become malnourished before surgery. Surgery after CRT is associated with a higher morbidity and mortality, and weight loss and malnutrition, which may occur during induction treatment, may be one of the causes (3,14). The importance of nutritional factors as an independent risk factor for outcome and long-term prognosis in patients operated after CRT is however poorly studied.

The objective of this retrospective study was to investigate nutritional status and weight change during induction CRT and to evaluate the influence of nutritional status variables on surgical morbidity/mortality and survival outcomes in stage III NSCLC patients receiving trimodality treatment.

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Patients and methods

Patient Selection

All consecutive patients with NSCLC who underwent induction treatment with cisplatin-based chemotherapy and concurrent thoracic radiotherapy followed by surgical resection from January 1, 2003, until December 31, 2009, in the VU University Medical Center Amsterdam were included in this study. NSCLC patients were selected for trimodality treatment on basis of one or more of the following criteria:

1. Pathologically proven mediastinal lymph node involvement (N2 or N3 disease), either by transbronchial fine needle aspiration and/or esophageal ultrasonography (endoscopic ultrasound-guided fine needle aspiration) or mediastinoscopy
2. Superior sulcus tumor (SST).
3. Consensus in the VU Medical Center Amsterdam multidisciplinary thoracic oncology meeting that the tumor should be staged as cT4 on basis of a combination of clinical signs (e.g., neurologic signs) and/or imaging studies such as computed tomography scan or magnetic resonance imaging (e.g., involvement of vertebra).
4. Eastern Cooperative Oncology Group performance status 0 and 1.
5. Ability to tolerate cisplatin-based chemotherapy.

Trimodality Treatment

Two different induction schemes were used: (1) one course of cisplatin and gemcitabine or pemetrexed followed by two courses of cisplatin-etoposide (course cycle of 3 weeks) combined with radiotherapy (daily fractions of 2 Gy to a total dose of 46–66 Gy); (2) six courses of weekly cisplatin docetaxel combined with radiotherapy (daily fractions of 1.8 Gy to a total dose of 45 Gy). The latter scheme was used in patients participating in a phase II study (NALT-6) (15). After CRT, restaging was performed using imaging studies and one or more of the abovementioned invasive staging techniques. When no residual mediastinal lymph node involvement was encountered and the primary tumor was deemed resectable, patients underwent a thoracotomy approximately 5 weeks after the end of induction treatment. If possible, a macroscopically complete resection of the tumor was performed, preferably by lobectomy, combined with an ipsilateral mediastinal lymphadenectomy. In rightsided tumors, lymph node stations 2, 4, 7, 8, and 9 were removed and in left-sided tumors stations 5, 6, 7, 8, and 9, together with hilar and intralobar lymph nodes. The bronchial stump was reinforced with a pedicled intercostal muscle flap in all patients. SSTs were typically approached by a high posterolateral incision
(Shaw-Paulson), and all other resections were performed through a standard posterolateral thoracotomy.

**Morbidity and Mortality**

Anesthetic risk was determined for all patients using the American Society of Anesthesiologists (ASA) score ([http://www.asahq.org/clinical/physicalstatus.htm](http://www.asahq.org/clinical/physicalstatus.htm)). Postoperative complications occurring within 90 days after surgery were recorded and classified for type and severity. The type of postoperative complications was subdivided into pulmonary, cardiac, infectious, or miscellaneous. Severity was classified as (1) minor (non–life-threatening, no need for reintubation, or admission to intensive care unit) or (2) major (potentially life-threatening, need for reintubation, and/or admission to intensive care unit) (16). Patients having both minor and major postoperative complications were classified as having major complications, although the nature of the minor complications was also recorded. A separate analysis was made of infectious complications, because previous studies have shown that these are especially related to nutritional status in abdominal and thoracic surgical patients (14,17). Information on type of resection, length of postoperative hospital stay, and 90-day postoperative mortality was recorded. Follow-up data included last visit, first recurrence, and date/cause of death. Overall survival (OS) and PFS were calculated from date of surgery to October 5, 2010, or date of last radiological follow-up, respectively.

**Nutritional Status**

Nutritional status was assessed at start of first chemotherapy course (“baseline”) by recall of recent involuntary weight loss and by measuring body mass index (BMI). BMI was calculated as the ratio of body weight (kg)/height (m²). Malnutrition was defined as ≥ 5% involuntary weight loss in the previous month (18) and/or underweight (BMI ≤ 18.5). Overweight was classified as BMI ≥ 25, according to the NIH classification of obesity ([http://www.nhlbi.nih.gov/health/public/heart/obesity/lose_wt/bmi_dis.htm](http://www.nhlbi.nih.gov/health/public/heart/obesity/lose_wt/bmi_dis.htm)).

During induction period, development of nutritional status was investigated by follow-up of body weight at start of week 6 after baseline (during CRT) and at hospital admission for surgery (surgery). Body weight, without shoes and wearing light clothing, was measured on a compact digital flat scale (SECA 708) to the nearest 0.1 kg at hospital admissions for chemotherapy and surgery. The percentages of weight change between pre-illness and baseline, baseline and during CRT, and baseline and surgery were calculated. Throughout treatment, nutritional status was monitored by the medical staff, and the dietician was
consulted whenever necessary. Energy requirements were estimated using the Harris Benedict 1984 equation including sex, age, body weight, and height (19). To estimate total energy requirements, 130% of predicted resting energy expenditure was applied (according to guidelines of the Dutch Malnutrition Steering Group) (20). Patients received enteral nutrition in case of malnutrition and/or intake failure. Tube feeding was indicated in case of (expected) oral intake < 75% of energy requirements for more than 3 days, combined with the inability to increase energy intake by oral food or sip feeds. Enteral nutrition was supplied by oral nutritional supplements or tube feeding, the latter via a nasogastric tube or a percutaneous endoscopic gastrostomy (PEG) tube. Patients participating in the NVALT-6 phase II study received a PEG tube before the start of CRT.

**Statistics**

Independent variables (age, sex, stage of disease [N2/N3 versus T4 or SST], ASA score, CRT schedule, forced expiratory volume in 1 second (FEV1), extent of lung resection, postoperative pathological tumor node metastasis (TNM) stage, BMI, and percentage of weight change) were related to the occurrence of postoperative complications, 90-day postoperative mortality, OS, and PFS. Associations between independent variables and postoperative complications were investigated by logistic regression analyses. To identify factors associated with OS and PFS, Cox regression and Kaplan-Meier analyses were carried out. Outcomes were adjusted for the following confounding factors in the regression model (based on a > 10% change of OR after adding a single factor): age, sex, stage of disease, chemotherapy scheme, pathological complete remission, and/or extent of lung resection. *p*-values less than 0.05 were considered statistically significant.

**Results**

**Patient and Treatment Characteristics**

Fifty-one patients were included (26 males), 26 patients with involvement of N2/N3 lymph nodes and 41 patients with a SST or T4 tumor. Median age at start of treatment was 57 years (range, 39–74 years). ASA score was I in 4 patients, II in 30 patients, and III in 17 patients. Patient characteristics are summarized in Table 1. Of 44 patients, results of pulmonary function tests at diagnosis were available. Average FEV1% of these patients was 87.5% (range, 33–126%). Forty-two patients (82%) generally received one course of cisplatin combined with gemcitabine or pemetrexed followed by two courses of cisplatin-etoposide. Nine patients (18%) received six weekly courses of cisplatin and docetaxel. Resections consisted of pneumonectomy (n = 4, 8%), bilobectomy (n = 3, 6%), lobectomy...
Table 1: Patient characteristics

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<th>Characteristic</th>
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</tr>
<tr>
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</tr>
<tr>
<td>Female</td>
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<tr>
<td>Age at surgery (years)</td>
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<td>&lt; 65 (n)</td>
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<td>≥ 65 (n)</td>
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<td>Baseline^a nutritional parameters</td>
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<td>Mean body weight (kg)</td>
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<td>18.0 – 40.3</td>
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<td>≥ 25 (overweight)</td>
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<td>Mean weight change (%)</td>
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<td>Normal weight – baseline^b</td>
<td>-1.03</td>
<td>-10.0 to 16.8</td>
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<td>Baseline^b – during CRT</td>
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<td>-15.4 to 8.5</td>
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<td>Baseline^b – admission for surgery</td>
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<td>-13.8 to 14.2</td>
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<td>Malnourished patients (n)</td>
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<td>During CRT</td>
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<td>At surgery</td>
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<td>II</td>
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^a at start of first chemotherapy. CRT, chemoradiotherapy; ASA, American Society of Anesthesiologists.
(n = 39, 77%), and 5 patients (10%) had a sublobar resection, because a more extended resection was impossible for functional reasons. Macroscopically complete resection was achieved in all patients. In three patients (6%), the resection margins were microscopically involved (R1 resection). These three cases were patients with a SST in whom the lateral chest wall was microscopically involved at the plane between the muscle fascia and the scapula. Finally, pathological complete remission was achieved in 15 patients (29%).

**Nutritional Status**

At baseline and during CRT, 12 and 19 patients were malnourished, respectively. Most patients (69%) maintained weight between CRT and time of surgery, but on average, a weight loss of 3.1% (range, -13.8 to 14.2) was observed for the period between baseline and surgery. In addition, at time of surgery, 20 patients were malnourished. Specifications on nutritional status and weight change are shown in Table 1.

Forty-two patients (83%) received one or more dietetic consultations. In 11 (22%) patients, a pretreatment PEG was inserted. During induction period, 9 patients received nasogastric tube feeding and 42 (83%) patients used oral nutritional supplements to increase their daily energy and nutrient intake.

**Postoperative Complications, Survival, and Cause of Death**

One or more postoperative complications occurred in 25 (49%) patients. Six patients had major complications and two patients died within 90 days after surgery. The cause of death in these two patients was acute respiratory distress syndrome (ARDS) after pneumonectomy in one and sepsis in the other. Age, FEV1, sex, type of resection, and percentage of weight change during induction period were not significantly associated with postoperative complications or infectious complications in univariate analysis (data not shown). The mean length of postoperative hospital stay was 11.4 days (range, 6–31 days). After a median follow-up of 36.9 months, median OS had not been reached. The mean OS was 46.7 months and mean PFS 37.1 months. None of the preoperative variables were significantly associated with OS or PFS in univariate analysis (Table 2). However, pneumonectomy was associated with shorter PFS (HR 4.91, p = 0.02) and complete pathological remission with a longer PFS (HR 0.26, p = 0.03). Weight loss ≥ 5% from baseline until surgery was associated with shorter OS (HR 2.80, p = 0.03).
Table 2: Associations between Independent Variables and Overall/Progression-Free Survival

<table>
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<tr>
<th>Variable</th>
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<th>Progression-free survival</th>
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<tr>
<td></td>
<td>HR</td>
<td>95% Cl</td>
<td>p</td>
<td>HR</td>
<td>95% Cl</td>
<td>p</td>
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<tr>
<td>Sex (male vs. female)</td>
<td>1.91</td>
<td>0.73 - 4.97</td>
<td>0.19</td>
<td>1.81</td>
<td>0.75 - 4.40</td>
<td>0.19</td>
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<td>Age (≥ 65 vs. &lt; 65 years)</td>
<td>1.16</td>
<td>0.46 - 2.94</td>
<td>0.75</td>
<td>1.21</td>
<td>0.51 - 2.90</td>
<td>0.66</td>
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<tr>
<td>Stage of disease (SST/T4 vs. N2/N3)</td>
<td>0.81</td>
<td>0.30 - 2.23</td>
<td>0.69</td>
<td>0.75</td>
<td>0.29 - 1.93</td>
<td>0.55</td>
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<tr>
<td>BMI at baseline (&lt; 25 vs. ≥ 25 kg/m²)</td>
<td>1.75</td>
<td>0.70 - 4.38</td>
<td>0.23</td>
<td>1.41</td>
<td>0.59 - 3.36</td>
<td>0.45</td>
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<tr>
<td>FEV1 (%)</td>
<td>1.00</td>
<td>0.98 - 1.03</td>
<td>0.97</td>
<td>1.00</td>
<td>0.97 - 1.02</td>
<td>0.66</td>
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<tr>
<td>Chemotherapy scheme (cisplatin-docetaxel vs. other)</td>
<td>1.21</td>
<td>0.46 - 3.22</td>
<td>0.70</td>
<td>0.67</td>
<td>0.24 - 1.90</td>
<td>0.45</td>
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<tr>
<td>ASA score (III vs. I/II)</td>
<td>1.17</td>
<td>0.49 - 2.80</td>
<td>0.72</td>
<td>0.64</td>
<td>0.26 - 1.53</td>
<td>0.31</td>
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<tr>
<td>Extent of lung resection (pneumonectomy vs. other)</td>
<td>2.32</td>
<td>0.55 - 9.73</td>
<td>0.25</td>
<td>4.91</td>
<td>1.40 - 17.2</td>
<td>0.02*</td>
</tr>
<tr>
<td>Completeness of resection (R0 vs. R1)</td>
<td>1.83</td>
<td>0.51 - 6.57</td>
<td>0.35</td>
<td>2.28</td>
<td>0.59 - 8.73</td>
<td>0.233</td>
</tr>
<tr>
<td>Complete pathological remission (yes vs. no)</td>
<td>0.32</td>
<td>0.10 - 1.08</td>
<td>0.07</td>
<td>0.26</td>
<td>0.08 - 0.88</td>
<td>0.03*</td>
</tr>
<tr>
<td>Weight loss ≥ 5% between baseline and surgery</td>
<td>2.80</td>
<td>1.10 - 7.13</td>
<td>0.03*</td>
<td>1.77</td>
<td>0.75 - 4.16</td>
<td>0.19</td>
</tr>
<tr>
<td>BMI ≥ 25 and ≥ 5% weight loss between baseline and surgery</td>
<td>4.63</td>
<td>1.58 - 13.6</td>
<td>0.005*</td>
<td>6.03</td>
<td>1.65 - 22.1</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

Cox regression for progression-free and overall survival, adjusted for confounding factors.

*p < 0.05

HR, hazard ratio; CI, confidence interval; SST, superior sulcus tumour; BMI, body mass index; FEV1, forced expiratory volume in 1 s; ASA, American Society of Anaesthesiologists; R0, complete resection; R1, microscopic residual disease.
Especially overweight patients who experienced a weight loss of ≥ 5% (n = 7) tended to have a shorter OS (adjusted HR 4.63, p = 0.005) and PFS (adjusted HR 6.03, p = 0.007). In addition, Figure 1 shows the Kaplan-Meier OS curve for last-mentioned group compared with the other study patients (log-rank p = 0.04). At the date of survival analysis, in total 22 patients (43%) have died of which 10 were noncancer-related death. Respiratory failure was the cause of death in seven of latter patients. Finally, the overweight patients who experienced a weight loss of ≥ 5% died of cancer (n = 2) or respiratory failure (n = 3), but only one of them died within 90 days after surgery (ARDS).

Figure 1: Kaplan Meier overall survival curve of overweight patients (BMI ≥ 25) experiencing ≥ 5% weight loss during induction period (n = 7) compared with other study patients (n = 44) (log-rank p = 0.04).

Discussion

In this study, a well-defined group of 51 stage III NSCLC patients underwent trimodality treatment, consisting of concurrent CRT followed by surgery. The nutritional status of patients was not only determined at baseline but also monitored continuously during CRT and thereafter until surgery. A high number of postoperative complications were observed, which is in line with other series of lung resections after induction CRT (1,2,21). However, most complications were minor, and postoperative mortality was lower than in
other studies (1,21). None of the preoperative risk factors, including nutritional status, were predictive for postoperative complications. In general, obese patients are thought to be at higher risk for postoperative complications due to more difficulty in mobilization, impaired wound healing, and atelectasis (22). However, several studies found that only morbidly obese patients have a higher risk of major complications or mortality after general surgical procedures and major intra-abdominal oncological resections. A low risk was observed for moderately obese patients (17). BMI has not been found to be a risk factor for complications of mortality in patients operated for esophageal cancer or lung cancer (23–25).

Several studies have indicated a higher risk for postoperative complications and death in malnourished patients (3,14,17,26). Although all patients in our study received intensive nutritional support during induction treatment, the percentage of malnourished patients at time of surgery was 39% compared with 24% at baseline, indicative of the toxicity of induction treatment with CRT. Malnutrition measured at different phases of the treatment, however, was not associated with outcome in terms of postoperative complications or mortality. This is in line with previous studies showing that nutritional support in malnourished surgical patients, as was given in our study group, reduces postoperative morbidity and mortality (5,14). Our study shows that in general, surgery can be performed safely after CRT despite worsening of the nutritional status during induction treatment, provided that patients receive adequate nutritional support throughout the treatment period.

Next to the observation that weight loss ≥ 5% during induction period predicted for shorter OS, a remarkable finding was that especially the combination of overweight and weight loss ≥ 5% during induction treatment predicted for both poor OS and PFS, suggesting that the development of malnutrition during induction CRT in overweight patients impairs not only surgical outcome but also long-term oncological outcome. Therefore, the nutritional status of (overweight) patients treated with CRT and surgery for NSCLC should be monitored throughout the entire treatment period and not only at the start of the treatment.

Obesity leads to central fat deposition, disordered energy use by cell mitochondria, especially in muscle and liver, and malfunctioning immune, coagulation, endothelial, and other systems (27). Patients with a high BMI, experiencing undesired weight loss, might also develop malnutrition. However, little is known about the consequences of malnutrition on body composition in obesity. Probably the relatively low fat-free mass in obese patients might result in altered metabolic pathways, which might influence the response to surgery after CRT and the hosts’ immune response to the malignant tumor. In
daily practice, recognition of malnutrition in patients with overweight, who do not appear to be malnourished at first sight, rarely takes place. Therefore, recognition by regular weighing during the induction period and treatment of malnutrition in patients with overweight or obesity may be beneficial.

The main limitation of this study is a small, selected study population. The toxicity of trimodality treatment makes careful patient selection imperative. As a result, statistical power is low and the importance of other risk factors might have been overlooked.

In conclusion, this study, focusing on the significance of nutritional status in stage III NSCLC patients undergoing trimodality treatment did not identify predicting factors for general postoperative complications. Despite nutritional support throughout the treatment period, especially overweight patients (BMI ≥ 25) who suffered from weight loss ≥ 5% during induction treatment had a significantly shorter OS and PFS. These findings indicate that (mal)nutrition during aggressive trimodality treatment is an important factor with potentially negative impact on outcomes in stage III NSCLC, not only in underweight patients but also in those with overweight at initial diagnosis.
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Nutritional support in patients with GVHD of the digestive tract: state of the art

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Abstract

An important complication of allo-SCT is GVHD, which commonly affects the skin, liver and digestive tract. Clinical symptoms of GVHD of the digestive tract (GVHD-DT) include excessive diarrhoea, abdominal pain and cramps, nausea and vomiting, gastrointestinal bleeding, dysphagia, and weight loss. Treatment is complicated and regarding nutritional support, only a few guidelines are available. Our aim was to critically appraise the literature on nutritional assessment, nutritional status and nutritional support for patients with GVHD-DT. Evidence shows that GVHD-DT is often associated with malnutrition, protein losing enteropathy, magnesium derangements, and deficiencies of zinc, vitamin B12 and vitamin D. Limited evidence exists on derangements of magnesium, resting energy expenditure, bone mineral density and pancreatic function, and some beneficial effects of n-3 polyunsaturated fatty acids and pancreatic enzyme replacement therapy. Expert opinions recommend adequate amounts of energy, at least 1.5 g protein/kg body weight, supplied by total parenteral nutrition in cases of severe diarrhoea. When diarrhoea is < 500 mL a day, a stepwise oral upgrade diet can be followed. No studies exist on probiotics, prebiotics, dietary fibre and immunonutrition in GVHD-DT patients. Future research should focus on absorption capacity, vitamin and mineral status, and nutritional support strategies.
**Introduction**

GVHD of the digestive tract (GVHD-DT) is one of the most challenging complications after allo-SCT for haematological malignancies. Although there are no standard criteria, a diagnosis of GVHD-DT may be established by means of histological findings of epithelial cell apoptosis with or without inflammation, epithelial sloughing and the exclusion of infectious causes (1–3). The incidence of GVHD-DT is estimated to be 10–60% of patients with GVHD (2,4). In addition to severe diarrhoea and vomiting, clinical symptoms are abdominal pain and cramps, nausea, gastrointestinal bleeding and dysphagia, which can lead to malabsorption, dehydration, severe electrolyte loss and weight loss (3) (Figure 1). Even with immunosuppressive treatment and symptom management, chronic and acute GVHD-DT can last for weeks or months and patients often need long-term admission to the hospital (5).

The standard treatment of GVHD consists of high-dose corticosteroids (5), which have profound effects on body composition by inducing alterations in substrate oxidation, thus leading to increased body fat at the expense of lean body mass (LBM). Apart from appetite improvement and weight gain, nutrition-related side effects of corticosteroids include fluid and sodium retention, hyperglycaemia, hypertriglyceridaemia, hypercholesterolaemia, muscle wasting and bone demineralization (6). Another common immunosuppressive treatment of GVHD is CYA, which is known to reduce food intake through nausea, vomiting, abdominal discomfort and disturbances in taste sensation. Other relevant side effects of CYA that may impact nutritional status include hyperglycaemia, hyperkalaemia, hypomagnesaemia, hyperlipidaemia, nephrotoxicity and neurotoxicity (6).

Overall, GVHD-DT is a complex condition with significant effects on nutritional status and quality of life (7). Accurate measurement of intestinal losses in patients with GVHD-DT can be difficult due to the large amounts of diarrhoea, faecal incontinence and mixing of urine and faeces. Nutritional assessment is also challenging, as many patients experience excessive fluid retention related to low serum albumin levels, masking body weight loss. Nutritional support is complicated by the severe gastrointestinal symptoms, and exact energy and nutrient requirements are unknown. Moreover, indications for total parenteral nutrition (TPN) are not well described, and recommendations on oral nutrition differ widely among caregivers. Our aim was to critically appraise all the available data on nutritional assessment and nutritional support in patients with GVHD-DT.
Methods

We searched the electronic database PubMed using medical subject headings and free text words. Search terms for nutritional assessment, nutritional status, nutritional support, pancreatic enzyme supplementation, probiotics and prebiotics, taste disorders, absorption capacity tests, immunonutrition and immunosuppressive medication were combined with search terms for GVHD, GVHD-DT, SCT, BMT and/or gastrointestinal diseases (Supplementary information). All searches were limited to studies, which were available in English and published from any date up to 1 March 2012. No age restrictions were applied. In addition, reference lists of included publications were inspected for references not retrieved by the database search.

To critically appraise the evidence, we used the classification of the Oxford Centre for Evidence-Based Medicine to assess the levels of evidence of individual studies (1a/b/c, 2a/b/c, 3a/b, 4 and 5) and the grades of recommendation (A, B, C and D) (Table 1) (8).
Table 1: Levels of evidence and grades of recommendation

<table>
<thead>
<tr>
<th>Level of evidence</th>
<th>Types of study</th>
<th>Grades of recommendation</th>
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<tr>
<td>1</td>
<td>Local and current random sample surveys (or censuses), or systematic review of RCTs</td>
<td>A: Consistent level 1 studies</td>
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<td></td>
<td>B: Extrapolations from level 1 studies</td>
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<tr>
<td>2</td>
<td>Systematic review of surveys that allow matching to local circumstances, or randomized trial or observational study with marked effect</td>
<td>B: Consistent level 2 studies</td>
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<td></td>
<td>C: Extrapolations from level 2 studies</td>
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<tr>
<td>3</td>
<td>Local non-random sample, or non-randomized controlled cohort / follow-up study</td>
<td>B: consistent level 3 studies</td>
</tr>
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<td></td>
<td>C: Extrapolations from level 3 studies</td>
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<tr>
<td>4</td>
<td>Case-series, or case-control studies, or historically controlled studies</td>
<td>C: Level 4 studies</td>
</tr>
<tr>
<td>5</td>
<td>Mechanism-based reasoning</td>
<td>D: Level 5 evidence</td>
</tr>
<tr>
<td>Any level</td>
<td></td>
<td>D: Troublingly inconsistent or inconclusive studies of any level</td>
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</table>

Abbreviation: RCT = randomised controlled trial

Results of the literature study

The following paragraphs describe the literature findings and evidence on nutritional status, intestinal failure, nutritional support, micronutrients, immunonutrition and probiotics, prebiotics, and dietary fibre in patients with GVHD. A summary of the available evidence on these topics is displayed in Table 2.

Nutritional status

Disease-related malnutrition is associated with loss of body weight and LBM, and affects morbidity and quality of life (9). Treatment-related toxicities and complications may affect nutritional status in the first year following allo-SCT. In patients with GVHD, diarrhoea, systemic inflammation, dysphagia, abdominal pain and anorexia often deteriorate nutritional status. These symptoms are accompanied by an inadequate energy and nutrient intake (10).

In the literature, follow-up of parameters of nutritional status in allo-SCT and GVHD
patients has been described in a number of prospective studies, retrospective analyses and case reports; study populations range from \( n = 2 \) to \( n = 2220 \).

Two studies showed a deterioration of nutritional status after allo-SCT (11,12). A large prospective study of high quality showed allo-SCT patients had a lower LBM index than healthy controls, at 6 and 12 months up to 6 years post transplant. In particular patients with chronic (c)GVHD had a significantly lower LBM index, and steroid treatment was associated with a lower LBM index and a higher body fat mass index (12). In a small prospective study (\( n = 47 \) patients who underwent allo-SCT), around 50% of patients experienced diarrhoea at median of 10 days after allo-SCT; diarrhoea had a profound negative effect on nutritional status and well-being, despite the administration of TPN in the majority of those patients (11).

A few studies investigated nutritional status in patients with GVHD, and looked at differences between subgroups with limited and extensive (or active and inactive) GVHD (6,10,13–15). The study populations and nutritional parameters were heterogeneous, which makes it hard to draw conclusions on the prevalence of malnutrition in patients with GVHD, and study quality was moderate to poor (most were small retrospective studies).

Jacobsohn and colleagues retrospectively observed malnutrition (BMI (body mass index) < 21.9) in 43% and severe malnutrition in 14% (defined as BMI < 21.9 and 18.5) in 93 patients with cGVHD. Patients with active, ongoing cGVHD had lower BMIs than patients with inactive cGVHD. In this study, symptoms thought to contribute to weight loss, such as odynophagia and oral sensitivity, were not related to weight loss (13). In a large retrospective review of 192 children and adults 1 year post allo-SCT, nutrition-related problems (weight changes, oral sensitivity, xerostomia and so on.) were more frequent in patients with GVHD than in patients without GVHD. The incidence of weight loss was 33% in patients with extensive cGVHD and 19% in patients with limited cGVHD, but the amount of weight loss per patient was not recorded. Weight gain also occurred, in respectively 28 and 34% of patients with limited and extensive cGVHD. Although this study was relatively large, it only estimated the occurrence of nutrition-related problems and weight loss; differences between patients with GVHD and those without GVHD were not statistically analysed (10).

Lee and colleagues retrospectively found steroid myopathy in 41% of 70 patients with acute (a)GVHD using high-dose steroids (14). In another small retrospective study in 18 children with extensive cGVHD, nine patients with multi-organ involvement experienced severe weight loss (20.9% of their body weight), and required salvage therapy beyond steroids and CSA. In contrast, patients with one organ system involved experienced a
lower decrease in body weight (5% of their body weight). In this study, weight loss preceded clinical symptoms of cGVHD (15). In a small cross-sectional study, Zauner and colleagues investigated resting energy expenditure (REE) and substrate oxidation in 13 patients with chronic extensive GVHD. Patients’ average body weights were significantly lower than the body weights before allo-SCT (difference of ~10 kg, p < 0.05) (16).

These typical GVHD symptoms were also described in a case series of two patients with GVHD of both the skin and the digestive tract. Both patients experienced side effects of immunosuppressive treatment and difficulties to reach adequate nutritional intake. They lost > 10% of their usual body weight (6).

In summary, small retrospective studies and case series indicate that malnutrition and weight loss frequently occur in patients with GVHD (grade B); malnutrition and weight loss appear to be more severe in patients with extensive GVHD compared with patients with limited GVHD (grade C). GVHD and steroid treatment are associated with a decline in lean body mass (grade C). The prevalence of malnutrition in patients with GVHD-DT, as well as the relationship between weight loss and symptoms, such as diarrhoea and oral sensitivity, require further investigation.

**Intestinal failure**

*Diarrhoea and protein-losing enteropathy*

Diarrhoea is the main complaint of GVHD-DT, and the aetiology can be multi-factorial. Possible causes of diarrhoea include villous atrophy, mucosal ulceration, secretory dysfunction, osmotic factors, rapid passage, pancreatic insufficiency and side effects of medication. Diarrhoea in patients with GVHD-DT is often green, mucoid and watery (18), and stool volumes can exceed 2 L a day (1,19). This may result in dehydration, loss of electrolytes, protein (‘protein-losing enteropathy’) and fat, and intolerance to oral and enteral feeding. In GVHD-DT of the upper digestive tract, in particular the stomach, complaints include nausea, vomiting, anorexia and/or food intolerance, but not necessarily include diarrhoea (4,20).

Small prospective studies and case series in patients after allo- SCT investigated the extent of diarrhoea and protein-losing enteropathy.

In a case series of 40 patients with cGVHD and persistent gastrointestinal (GI) symptoms, moderate diarrhoea (500 mL/day to 1 L/day or 3 to 5 times/day) occurred in 39% of patients and severe diarrhoea (> 1 L/day or > 5 times/day) in 48% of patients with GVHD-DT (2).

Two small prospective studies investigated protein-losing enteropathy by measuring alpha
1-antitrypsin in 24-h stool samples. Papadopoulou and colleagues observed protein-losing enteropathy in 91% of 27 patients with diarrhoea after allo-SCT. In this study, protein-losing enteropathy was more severe in patients with GVHD, compared with other causes of diarrhoea (11). Others showed that in patients after allo-SCT (n = 25), stool alpha 1-antitrypsin significantly increased in those developing GVHD DT, whereas in patients not developing GVHD-DT, stool alpha 1-antitrypsin returned to below normal values (20). In summary, small studies suggest that diarrhoea is prevalent in at least 39% of patients with cGVHD (level C). Protein-losing enteropathy (defined by increased alpha 1-antitrypsin in stool samples) occurs after allo-SCT, especially in patients with GVHD-DT (level B).

Maldigestion
Maldigestion caused by liver and exocrine pancreatic insufficiency could also have a role in GVHD-DT (21). Evidence of GVHD of the pancreas has been found on autopsy and in experimental models of GVHD (22–24) but pancreatitis in allo-SCT patients could also be caused by drugs like azathioprine, cyclosporine, glucocorticoids and l-asparaginase (21). Loss of pancreatic exocrine function leads to poor output of digestive enzymes, such as lipases, proteases and carbohydrases. Symptoms of exocrine pancreatic insufficiency include chronic diarrhoea, steatorrhoea, fatigue, abdominal pain, weight loss and flatulence (25). Faecal fat excretion above the upper limit of normal has been described in 5–8% (2,26) of patients with GVHD-DT, and in 5% of patients with cGVHD (10). Overall in patients two years post allo-SCT, the probability of developing steatorrhoea was somewhat lower (3.3%) (27).

A number of small retrospective studies and case reports investigated pancreatic function in patients with GVHD-DT. In a retrospective review of 30 long-term survivors of allo-SCT, Cgvhddt occurred in nine patients (30%), and pancreatic atrophy in five patients (16.7%); GVHD involving the liver and digestive tract occurred significantly more frequent in patients who experienced pancreatic atrophy (28). Akpek and colleagues described four case reports of patients with a clinical presentation of malabsorption. In these patients, radiologic and endoscopic studies did not show evidence of GVHD-DT, and pancreatic insufficiency was ascribed to chronic pancreatitis (21). One retrospective analysis in 40 patients with cGVHD showed exocrine pancreatic insufficiency in two out of 15 patients experiencing GI symptoms > 1 year after the diagnosis of cGVHD. The authors did not specify their definition of pancreatic insufficiency (2).

Pancreatic enzyme substitution may be indicated when faecal fat excretion exceeds 15 g/day, resulting in steatorrhoea and/or when weight loss is present (29); several case
reports of patients with aGVHD or cGVHD documented good effects of pancreatic enzyme replacement therapy on GI symptoms (2,21,25,30,31).

Apart from assessment of pancreatic exocrine function, investigation of small intestinal function is pivotal in patients receiving induction chemotherapy and allo-SCT. As the small bowel is by far the largest source of circulating citrulline, this amino acid is thought to be an attractive biomarker of enterocyte function (32–34).

In a large retrospective study (n = 163), plasma citrulline was determined three times a week around allo-SCT to estimate intestinal damage. The authors found a striking pattern of inflammatory response coinciding with the occurrence of plasma hypocitrullinaemia for patients receiving myeloablative conditioning; there was a strong correlation between plasma citrulline and C-reactive protein, and between intestinal damage and the occurrence of bacteremia and acute lung injury, but not between plasma citrulline and aGVHD after allo-SCT (35). The authors also compared the plasma citrulline assay with sugar permeability tests in ten patients receiving myeloablative therapy, and showed plasma citrulline earlier detects maximum intestinal damage as well as recovery of the intestinal damage than sugar permeability tests (36). Yet, no studies on plasma citrulline in patients with GVHD have been published.

In summary, these small studies show that pancreatic atrophy may occur after allo-SCT, especially in patients with GVHD-DT (grade C), and so does exocrine pancreatic insufficiency in patients with GVHD-D (grade C). Pancreatic enzyme replacement therapy may decrease GI symptoms in patients with aGVHD or cGVHD (grade D). Citrulline may be a good biomarker of enterocyte function in allo-SCT patients, but future studies on the significance of citrulline in patients with GVHD are required (grade C).

**Nutritional support**

**Route of administration**

Side effects of chemotherapy and complications around SCT can induce nutrition-related symptoms such as nausea, mucositis and anorexia. These symptoms can significantly decrease the ability to tolerate oral nutrition and to meet nutritional needs (37). Nutritional support by enteral or parenteral nutrition is therefore essential around SCT. Because of severe mucositis, bleeding risks and excessive vomiting, TPN is often applied in clinical patients. However, there is a debate about whether enteral nutrition should be favoured over TPN (38,39). The enteral route, if tolerable and clinically possible, may be preferred for maintaining digestive function and mucosal barrier, and prevents bacterial translocation from the digestive tract (40). In two small non-randomized studies, patients
who received enteral nutrition around allo-SCT less often developed aGVHD than those who received parenteral nutrition, and they showed better outcomes in terms of survival (41,42).

Patients with GVHD-DT often do not tolerate oral or enteral nutrition. In the acute phase with severe diarrhoea (> 1 L/day), nil by mouth during days to weeks (or even months) is required to alleviate gastrointestinal complaints, and nutritional support consists of TPN. When the volume of diarrhoea has decreased (typically < 500 mL/day), oral food is restarted, but certain foods may be better tolerated than others (19,37,43). General guidelines of the Seattle Cancer Care Alliance recommend specific upgrade diets for patients with GVHD-DT, with limited amounts of fats, fibre, lactose, acidic items and GI irritants, which are stepwise introduced (19).

Only one prospective study investigated effects of this stepwise upgrade diet in 18 patients with GVHD-DT; in this study, the stepwise upgrade diet was compared with a historic control cohort (n = 17) receiving nil by mouth and TPN, for effects. In the stepwise upgrade diet group, there was a significantly slower decrease of laboratory parameters (serum total protein and albumin; p < 0.001). Changes in body weight were not significantly different between groups. The oral diet appeared to be safely applicable to patients suffering from GVHD-DT, and recovery to a normal diet tended to be shorter (31 vs.38 days, p = 0.09) in the stepwise upgrade diet group. This study was limited by the small sample size, comparison with a historic control group, and the definition of recovery to a normal diet was unclear (17).

In brief, guidelines recommend TPN and nil by mouth in patients with 4500 mL of diarrhoea (level D). With regard to the introduction and type of oral nutrition in patients with GVHD-DT, the oral upgrade diet appears to be safe (level C). More research on nutritional support in patients with GVHD-DT is required.

**Energy requirements**

Weight loss in patients with GVHD-DT could hypothetically be a consequence of increased REE. In many disease states, REE is not markedly different from the predicted REE, between 100–107% (44). However, some diseases, in particular sepsis, trauma and burns, cause a clinically relevant increase in REE of 40 to 80% (44). In cachectic cancer patients, REE is elevated in some patients and decreased in other patients (45). It is generally suggested that energy requirements during the early phase of SCT are 50–70% above REE (46,47).

In theory, patients with active cGVHD can be in a hyper metabolic state for months or
even years. This hyper metabolic state is likely a response to inflammatory cytokines (TNF-α, IL-1 and IL-6) or alterations in levels of norepinephrine and glucagon (15,16) One small cross-sectional study compared REE by indirect calorimetry of 13 patients with chronic extensive GVHD of the skin, mucocutaneous membranes, lung, eyes and liver, with REE of healthy age-, sex-, height- and weight-matched controls, and showed a small increase in REE (1.9 kcal/kg/day or 133 kcal in a person of 70 kg) and alterations in fat and carbohydrate oxidation rates. The effects of glucocorticoids and CYA on energy metabolism were not investigated in this study, although these drugs could modify energy metabolism. Probably, this study was too small and patients too heterogenous to draw conclusions on REE. In addition, physical activity level, body composition and use of medication was not documented (16).

It is expected that energy expenditure in patients with GVHD is increased, but apparently little is known about this topic. Thus, grade C evidence suggests small, clinically irrelevant, increases in REE in patients with chronic extensive GVHD, but more research is required.

**Protein requirements.**

The World Health Organization states that the accepted safe value for intake of protein is 0.83 g/kg body weight/day for healthy subjects of both sexes, all ages and all body weights (within the acceptable range) (48) The maximum capacity of protein synthesis on whole body level is reached with a protein intake of 1.5 g/kg/day in healthy volunteers as well as in critically ill patients (9).

Patients with GVHD-DT probably have increased protein requirements, but human studies on this topic have not been performed until now. Chronic use of high-dose of corticosteroids contributes to an increased need for protein (6). In addition, GVHD-DT patients lose protein by mucosal exudation because of an abnormal or inflamed mucosal surface (11,49).

Expert opinions and reviews (level D) on nutritional support in SCT recommend 1.8–2.5 g/kg/day in case of malnutrition and severe complications (that is, GVHD), and 1.5–1.8 g/kg/day if no severe malnutrition and only mild complications are present (43,47).

**Micronutrients**

As patients with GVHD-DT experience nutritional and metabolic derangements, requirements for micronutrients might alter, and so does micronutrient status. Due to diarrhoea, malabsorption and inadequate food intake, GVHD-DT patients are at high risk
of being in a deficient state for electrolytes and vitamins. In the literature, vitamin D, B12, zinc, magnesium and iron have been discussed in relationship with allo-SCT and GVHD.

**Vitamin D**
Reduced bone mineral density is a well-known complication of SCT and probably relates to treatment with steroids and/or vitamin D deficiencies.

Four small (n = 9 to n = 79) studies in various study populations described the occurrence of vitamin D deficiency and/or bone loss after allo-SCT. In a cross-sectional study in 79 long-term (43 years) survivors of allo-SCT, bone loss occurred in 73.4% of patients. The risk of bone loss was higher in older patients, but was independent of sex, treatment, diagnosis, TBI and GVHD (50). One small prospective study in patients with cGVHD who were treated with corticosteroids and CYA showed that six out of eight patients had a decrease in bone mineral density one year after transplantation. These patients also had low serum levels of 1.25-dihydroxycholecalciferol (1.25(OH)2D3) (51). Two other prospective studies investigated vitamin D status after allo-SCT. A study in 67 children who had been treated for cGVHD found a high percentage of vitamin D deficiency or insufficiency (80.6%) (52). A small study (n = 48) found a more significant decrease in 25(OH)D3 than in the active metabolite 1.25(OH)2D3 at engraftment after BMT, and significant lower serum levels of 25(OH)D3 and 1.25(OH)2D3 in patients with GVHD grade 3 and 4 (53). This means that there could be a deficiency in transforming vitamin D3 in the liver or in the kidneys.

In summary, these studies provide fair evidence for the occurrence of vitamin D deficiencies in patients with GVHD (grade B), and for the occurrence of bone loss after allo-SCT, in particular in patients with GVHD (grade C). In general, treatment of reduced bone mineral density consists of supplementation of calcium and vitamin D, in combination with biphosphonates. Supplementation of the active metabolites 1.25(OH)2D3 or 25(OH)D3 could result in a superior effect on bone density and contribute to modulation of GVHD (54,55).

**Vitamin B12**
Regarding water-soluble vitamins, only vitamin B12 has been discussed in the literature. GVHD of the stomach affects the production of intrinsic factor, and GVHD of the small intestinal mucosa produces long-term impairment of vitamin B12 absorption (25).

One small prospective study in 26 patients found a severely reduced absorption of vitamin B12 shortly after allo-BMT, which was ascribed to the effects of the conditioning regime,
resulting in crypt cell degeneration. Vitamin B₁₂ further decreased in six patients with cGVHD, of which only two patients had obvious clinical symptoms of GVHD-DT. Probably, bowel function was also impaired in those with GVHD involving other sites (56). Monitoring vitamin B₁₂ status appears necessary in patients with GVHD (grade C).

**Zinc**

Zinc requirements may also change in cases of GVHD-DT, as chronic diarrhoea and malabsorption may lead to faecal losses of zinc and zinc deficiencies. Zinc has an important role in wound healing and taste acuity, and is important for maintaining the integrity of mucosal defence against intestinal infection (57,58). In clinical practice, zinc status is measured by plasma zinc and alkaline phosphatase, but the gold standard is to assess zinc concentrations in hair or in tissue, such as liver or blood cells. Only one prospective study documented zinc deficiency in 67% of 47 children following BMT, especially in those with diarrhoea (grade C) (11). Dysgeusia (abnormal taste or taste alterations) is a common side effect of cancer treatment (radiotherapy and/or chemotherapy) and may be prevented or treated by zinc supplementation, but so far, the evidence on supplementation of zinc in general cancer patients is limited (59–61). Although the evidence is limited, expert opinions recommend supplying increased zinc (and vitamin C) for patients with GVHD, following the requirements of patients with wounds (grade D) (6). Other expert opinions suggest supplementation of zinc (up to 3 times daily 45mg ZnSO₄) is safe and effective to improve taste disorders in cancer patients (grade D) (59).

**Trace elements**

Only a few publications on minerals and trace elements (magnesium and iron) have been found in GVHD. Grade C evidence suggests magnesium derangements in patients using immunosuppressive medication; common side effects are hypomagnesaemia, but there have also been several case reports of GVHD patients that endured hypermagnesaemia. A magnesium-containing cathartic and a magnesium-containing antacid seemed to be the cause. Both led to a rapid rise in serum magnesium, possibly due to an increased permeability of the inflamed digestive tract and dehydration (60,61).

Finally, iron overload is common in SCT patients (62). Causes include enhanced iron absorption due to anaemia, and multiple blood transfusions before and after allo-SCT. Allo-SCT may contribute to the dysregulation of iron homoeostasis by way of hepatic and intestinal injury due to GVHD. According to case series, iron overload appears to be less
prevalent among Auto-SCT patients compared with allo-SCT patients (62). Iron may also increase the risk of GVHD through its tendency to cause direct organ toxicity; alternatively, it might decrease the risk of GVHD through its ability to impair host immune responses (63). Therefore, expert opinions recommend using iron-free multivitamin/mineral supplements in patients with GVHD (6).

**Immunonutrition**

Immunonutrition is a special form of enteral nutrition containing specific nutrients. The most commonly used and researched are n-3 fatty acids, glutamine, arginine and nucleotides. Targets for immunonutrition are mucosal barrier function, cellular defence and local or systemic inflammation.

**N-3 polyunsaturated fatty acids**

N-3 polyunsaturated fatty acids downregulate the inflammatory response by suppressing the production of inflammatory cytokines and vascular endothelial damage, and by improving overall immune function. The n-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid produce beneficial effects through alterations in membrane structure and function, and gene transcription. Gamma-linolenic acid, the essential n-3 polyunsaturated fatty acid, appears to act additively with eicosapentaenoic acid and docosahexaenoic acid in decreasing acute inflammation and organ injury (64). Meta-analyses showed favourable effects of an enteral nutrition containing a high concentration of n-3 fatty acids on mortality, secondary infections and length of hospital stay in ICU patients with sepsis, acute respiratory distress syndrome and systemic inflammation response syndrome (64). Systemic inflammation response syndrome has also been linked to the aetiology of GVHD (65,66), and grade C evidence suggests beneficial effects of eicosapentaenoic acid: in a small prospective study, supplementation of eicosapentaenoic acid around BMT was associated with a reduction of inflammatory cytokines (IL-10, TNF-α and IFN-γ), reduced vascular endothelial dysfunction, higher survival rate (67) and a lower grade of acute colonic GVHD (68).

**Glutamine, arginine and nucleotides**

The nonessential amino acid glutamine is a precursor for nucleotide synthesis; rapidly dividing cells, such as enterocytes, are most likely to suffer from a shortage of glutamine.
During stress and trauma, endogenous production of glutamine may become insufficient, while consumption of glutamine by lymphocytes and enterocytes is increased. In experimental and clinical studies, supplementation of glutamine has been demonstrated to restore the integrity of the gastrointestinal mucosa and decrease bacterial translocation during cancer treatment (64). Glutamine not only modulates the immune system’s function in the digestive tract but may also promote intestinal healing and reduce the severity of mucositis and GVHD (69). However, clinical studies on enteral or parenteral supplementation of glutamine show unequivocal effects. A systematic Cochrane review concluded oral glutamine may reduce mucositis, days of opioids and GVHD. Based on two studies, parenteral glutamine may reduce clinical infections and positive cultures (70).

Arginine is a semi-essential amino acid, obtained from dietary sources and by endogenous synthesis via the urea cycle. Under nonstressed conditions, arginine contributes to adequate wound healing, enhanced immune response and stimulation of various anabolic hormones. Arginine is also a unique substrate for the production of nitric oxide (71). The use of high arginine containing diets in critically ill patients is controversial; the increased nitric oxide production caused by arginine supplementation may lead to increased tissue injury, and trigger cardiovascular collapse in patients with sepsis and systemic inflammation response syndrome. Arginine also appears to increase the inflammatory response and to counteract the reduction of the inflammatory response by n-3 fatty acids. On the other hand, sepsis is an arginine deficient state, and the decreased production of nitric oxide exacerbates the microvascular injury, suggesting benefits of arginine supplementation. Other studies showed that arginine has no adverse effects on haemodynamic effects in patients with septic shock (64). Some data suggest that giving glutamine and arginine together may be beneficial during intestinal inflammation, in particular on inflammatory response and digestive tract barrier function (72).

Animal studies found an association between arginine, nitric oxide production and GVHD: in vivo administration of human arginase-1 resulted in L-arginine depletion and significant GVHD reduction (73) and administration of L-arginine after injection of endotoxin blocked nitric oxide production in the intestine (74). Treatment of GVHD mice with arginine abrogated GVHD-associated enteropathy and reduced lymphocytic infiltration in the intestinal epithelium (75).

Nucleotides have a role in cellular proliferation and immune modulation. They serve as building blocks for DNA, RNA and ATP, and can be newly synthesized or salvaged. Parenteral supplementation of nucleotides leads to increased immune responsiveness, decreased bacterial translocation and fewer episodes of graft rejection (71). Nucleotides
might also act as prebiotics and facilitate the proliferation of beneficial microflora. Another possible effect from nucleotide supplementation is mitigation of endotoxin-induced mucosal damage. This mechanism may lead to reduced bacterial translocation (71).

Glutamine, arginine and nucleotides could have beneficial effects on the prevention or treatment of GVHD, but up to now no human studies on supplementation of these nutrients have been performed.

**Probiotics, prebiotics and dietary fibre**

The normal enteric bacterial flora influence a variety of intestinal functions and have a key role in nutrition, in maintaining integrity of the epithelial barrier and in the development of mucosal immunity (76). In SCT and GVHD-DT patients, an impaired mucosal barrier resulting from the conditioning regimen and inflammation may lead to increased bacterial endotoxin translocation to the circulation. In most centres, prophylactic antibiotic treatment before and during transplantation is therefore used.

Probiotics are live microbial food supplements with beneficial effects on human health (77). The rationale behind supplementing probiotic microorganisms is that they alter the composition of the intestinal microflora and improve the mucosal barrier. So far, no studies on probiotics in humans with GVHD have been published. In one study, the use of probiotics in aGVHD in mice was studied. In this study, treatment of recipient mice with L. rhamnosus GG (a probiotics strain) significantly reduced mortality and gave a reduced GVHD score after SCT (78). However, a problem with probiotics is that no two probiotics are the same. Different species, even different strains, may have vastly different and even contrasting effects (76). Another important concern regarding probiotics in SCT patients is their potential to induce bacteraemia.

Prebiotics are non-digestible food ingredients, which beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria strains in the colon. Bacteria stimulated by prebiotics have the potential to improve host health (77). Prebiotic compounds, such as dietary fibre and resistant starches, can be fermented in the colon, producing short chain fatty acids, which are the primary source of fuel for colonocytes. These short-chain fatty acids may enhance wound healing in the colon. In patients with inflammatory bowel disease, dietary fibre showed clinical benefits because of maintenance of remission and reduction of colonic damage (79). No studies on probiotics, prebiotics and dietary fibre in SCT or GVHD patients have been published, and guidelines even recommend limited amounts of dietary fibre in patients with GVHD-DT
Nutritional support in patients with GVHD-DT

(grade D) (19). For probiotics, no recommendations were found.

Discussion

The aim of this review was to critically appraise the available literature on nutritional assessment, malabsorption, energy and nutrient requirements, and nutritional support in patients with GVHD-DT.

GVHD-DT is a complicated condition with many consequences for nutritional status, resulting in many questions about nutritional support. This review shows that for a lot of topics on nutrition in GVHD-DT, evidence is lacking. Most studies that were discussed in this review involved small patient populations. The lack of proper, well-designed studies in patients with GVHD-DT makes it hard to compare various outcomes. Transplantation procedures, conditioning regimes and (immunosuppressive) treatments are changing continuously.

This review shows that studies on nutritional support in patients after allo-SCT, in particular with GVHD-DT, are scarce. The literature mainly consists of small studies, neither retrospective nor prospective, and expert opinions. Overall, we propose the following recommendations for clinical practise:

Recommendations for monitoring post allo-SCT patients

1. It is crucial to monitor nutritional issues and to assess body weight on a regular basis (at least once a week).
2. Correct measured body weight for the estimated amount of fluid retention, and consider follow-up of the mid upper arm circumference as a marker of nutritional status.
3. Promote oral or enteral nutrition if the digestive and haemostatic function allow, in order to prevent mucosal atrophy in patients with TPN.
4. Assess serum magnesium, 25 OH vitamin D₃, vitamin B₁₂ and zinc during the first months after allo-SCT, and supplement if concentrations are below reference values.
5. If micronutrient intake does not meet requirements, supplements containing vitamins, minerals and trace elements at 100% of the recommended daily allowance are recommended, but without iron in order to prevent iron overload.
6. The safety and efficacy of immune-modulating nutrients (glutamine, arginine, nucleotides and n-3 polyunsaturated fatty acids) have not been proven for patients post allo-SCT.
7. Prebiotics and probiotics should be avoided because of the lack of evidence and the
probable risk of infectious complications.

**Recommendations for nutritional support for patients with GVHD-DT (in addition to above mentioned recommendations)**

1. Vitamin D₃ and calcium supplementation is recommended to minimize bone demineralization due to chronic steroid use.
2. In case of exocrine pancreatic insufficiency, consider pancreatic enzyme replacement therapy (~30 000 U of lipase per meal).
3. Measure faecal fat loss, protein-losing enteropathy (stool nitrogen or alpha 1-antitrypsin) and REE in patients with persistent diarrhoea.
4. In cases of severe gastrointestinal failure (diarrhoea > 500 mL/day), TPN is recommended.
5. Provide at least 1.5 g/kg/day of protein or 1.8–2.5 g/kg/day in patients with protein-losing enteropathy, as well as adequate amounts of energy (REE plus the amount of energy required for physical activity and faecal energy losses).
6. When diarrhoea is < 500 mL/day, oral foods can be introduced, for example using a stepwise oral upgrade diet.

These recommendations are composed of conclusions from the available literature. To adequately treat patients with GVHD-DT, more research on micronutrient status, energy and protein requirements, immunonutrition, pancreatic enzyme replacement therapy, and application of citrulline tests is required. Also, there is need for intervention studies on various nutritional support strategies and administration routes in patients with GVHD-DT.

In clinical practice, the optimal timing and amount of oral or enteral nutrition is unclear, and definitions and cutoff points for intestinal failure in GVHD-DT do not exist. Up to now, the amount of diarrhoea is used to classify the severity of GVHD-DT, but it is hard to quantify the amount of diarrhoea in this patient population.

In conclusion, GVHD-DT is associated with malnutrition, malabsorption, and deficiencies of vitamin D, vitamin B₁₂, zinc and magnesium. Multidisciplinary treatment for patients with GVHD-DT, by nurses, haematologists, dieticians and nurse practitioners, should include early nutritional assessment, nutritional support and follow-up of micronutrient status.
Table 2: Summary of the literature on nutritional status in patients with GVHD following allo-SCT

<table>
<thead>
<tr>
<th>Topic</th>
<th>Level of evidence</th>
<th>Grade of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GVHD and steroid treatment are associated with a decline in lean body mass (Kyle et al.(12): level 3, Lee et al.(14): level 3)</td>
<td>C</td>
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<tr>
<td></td>
<td>Relationship between weight loss and symptoms (for example, diarrhoea and oral sensitivity): inconsistent findings (Lenssen et al.10: level 3, Papadopoulou et al.(11): level 3, Jacobsohn et al.(13): level 3)</td>
<td>B</td>
</tr>
<tr>
<td>Intestinal failure</td>
<td>Malabsorption Diarrhoea is prevalent in at least 39% of patients with cGVHD (Akpek et al.(2): level 4)</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>Protein-losing enteropathy leads to significant protein loss in patients after allo-SCT, especially in those with GVHD-(DT) (Papadopoulou (11): level 3, Weisdorf et al.(49): level 3)</td>
<td>B</td>
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<tr>
<td></td>
<td>Patients with recent or active aGVHD or cGVHD may respond well to pancreatic enzyme replacement therapy (Akpek et al.(2), Akpek et al.(21), Maringhini et al.(25), Anderson et al.(30), Jurges et al.(31): level 4)</td>
<td>C</td>
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<tr>
<td></td>
<td>Citrulline may be a good biomarker of enterocyte function in allo-SCT patients; future studies in patients with GVHD are required (van der Velden et al.(35): level 3, Lutgens et al.(36): level 3)</td>
<td>C</td>
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Table 2 (continued)

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<thead>
<tr>
<th>Topic</th>
<th>Level of evidence</th>
<th>Grade of recommendation</th>
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<tr>
<td><strong>Nutritional support</strong></td>
<td></td>
<td></td>
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<tr>
<td>Route of administration</td>
<td>Expert opinion suggests TPN is indicated for patients with diarrhoea 4500mL (Rzpecki et al.(37), Martin-Salces et al.(43): level 5)</td>
<td>D</td>
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<td></td>
<td>The Seattle oral upgrade diet appears to be safely applicable to patients suffering from GVHD-DT (Imataki et al.(17): level 3, Flowers et al.(19): level 5)</td>
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<tr>
<td>Energy requirements</td>
<td>Apparent small increases in REE in patients with chronic extensive GVHD (Zauner et al.(16): level 4)</td>
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<tr>
<td>Protein requirements</td>
<td>Protein requirements in patients with GVHD-DT are increased; experts propose 1.8–2.5 g/kg for patients with severe complications (such as GVHD) (Muscaritoli et al.(47): level 5, Martin-Salces et al.(43): level 5)</td>
<td>D</td>
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<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
<td></td>
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<tr>
<td>Vitamin D</td>
<td>Vitamin D deficiencies are prevalent in patients with GVHD (Duncan et al.(52): level 3, Kreutz et al.(53): level 3, Stern et al.(51): level 3)</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Patients after allo-SCT are at risk of bone loss, in particular patients with GVHD (Stern et al.(51): level 3, Savani et al.(50): level 4)</td>
<td>C</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc deficiency is prevalent in patients following allo-SCT, especially in patients with diarrhoea (Papadopoulou (11): level 3) Supplementation of zinc may be considered: as in patients with wounds (Roberts et al.(6): level 5), zinc supplementation to improve taste: up to 3 times daily 45mg ZnSO4 (Kelly et al.(58): level 5)</td>
<td>C</td>
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<tr>
<td>Magnesium</td>
<td>Hypo- and hypermagnesaemia can occur in patients using immunosuppressive medication (Jaing et al.(80): level 4, Leong et al. (81): level 4)</td>
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<td>Miscellaneous</td>
<td>Human studies on the availability of other micronutrients in patients with GVHD are not available.</td>
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### Table 2 (continued)

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<td></td>
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<tr>
<td>Glutamine, arginine, nucleotides</td>
<td>Up to now, no human studies on glutamine, arginine or nucleotides on the prevention or treatment of GVHD have been performed</td>
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<tr>
<td>n-3 PUFAs</td>
<td>EPA around BMT could reduce inflammatory cytokines (IL-10, TNF-a and IFN-g), reduce vascular endothelial dysfunction, the grade of acute colonic GVHD and improve survival rate (Takatsuka et al.(67): level 3, Takatsuka et al.(68): level 3)</td>
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<tr>
<td>Probiotics, prebiotics, dietary fibre</td>
<td>No human studies have been found concerning prebiotics, probiotics or dietary fibre in SCT or GVHD patients</td>
<td>-</td>
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<tr>
<td></td>
<td>Clinical recommendation to use limited amounts of dietary fibre in patients with GVHD-DT (Flowers et al.(19): level 5)</td>
<td>D</td>
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</table>

Abbreviations: GVHD-DT = GVHD of the digestive tract; a = acute; c = chronic; DT = digestive tract; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid.

### Acknowledgements

We would like to thank Marieke CE Schoordijk for sharing her experience and reviewing the conceptual article, and Kelly Duin for carrying out data collection and explorative literature research during her Master fellowship in VU Health and Sciences.
### Supplementary Information: Search Terms

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<th>Vitamin D</th>
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Feasibility of intermittent fish oil infusions in outpatients with Graft-versus-Host disease of the digestive tract

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OJ Visser
JAE Langius
M Hacquebard
PAM van Leeuwen

Submitted for publication
Abstract

N-3 polyunsaturated fatty acids from fish oil may have immune-modulating effects in Graft-versus-Host Disease of the digestive tract (GVHD-DT). The objective of this pilot study was to investigate feasibility, safety and effects on fatty acid composition of plasma lipids and white blood cells (WBC) following intermittent fish oil infusion in outpatients with chronic GVHD-DT. Four outpatients received intermittent infusion of a 10% fish oil emulsion (Omegaven®) during 4 hours, at day 1 (1.5 mL/kg), 3 (2.25 mL/kg) and 5, 8, 10 and 12 (3 mL/kg). At baseline and consecutive visits, fatty acid composition of plasma triglycerides (TG), plasma phospholipids (PL) and WBC, serum TG concentrations, routine laboratory tests as well as adverse events were monitored. During the fish oil infusions, serum TG increased, but decreased 2 h after termination of infusion. In 3 patients, the dose of Omegaven needed to be reduced. EPA was incorporated into plasma PL, plasma TG and WBC as of 2 days after the first infusion; peak levels of EPA were reached at the final infusion or 2 days after. In conclusion, intermittent fish oil infusions result in incorporation of EPA in plasma and WBC but can be complicated by a reversible increase in serum triglycerides.
Introduction

Allogeneic haematopoietic stem cell transplantation (allo-SCT) is a commonly used modality in the treatment of haematological malignancies. A significant, but rare complication of this treatment is Graft-versus-Host Disease (GVHD), when donor T cells mediate cytotoxic damage to host target organs, such as the skin, liver or digestive tract. The prevalence of GVHD is 30 – 40% after myeloablative allo-SCT and at least 50% after reduced intensity conditioning stem cell transplantation (RIC) (1;2).

Two main categories of GVHD are recognized, acute and chronic GVHD (1). By definition, acute GVHD presents within the first 100 days after the conventional myeloablative allo-SCT and chronic GVHD emerges 100 days or longer after allo-SCT. The distinction between chronic and acute GVHD after RIC appears to be less clear.

When GVHD involves the digestive tract (GVHD-DT), symptoms include abdominal pain and cramps, nausea, gastrointestinal bleeding and dysphagia, as a result of inflammation and crypt cell degeneration (3;4). These symptoms can cause malabsorption with severe fluid and electrolyte losses and weight loss. Treatment of chronic GVHD-DT often requires long-term use of immunosuppressive agents (e.g. corticosteroids), supplementation of fluids, electrolytes and artificial nutrition, mostly in the outpatient setting. N-3 polyunsaturated fatty acids (PUFA) from fish oil are claimed to have immune-modulating effects and may attenuate graft-versus-host responses. In animals, diets rich in fish oil resulted in diminished graft versus host responses (5). Takatsuka and colleagues described the effect of oral administration of fish oil during conventional bone marrow transplantation in humans. Fish oil capsules, containing 1.8 g/24 h of EPA, were administered from 3 weeks before transplantation up to 180 days after transplantation, while a matched control group did not receive fish oil. The results showed a lower complication rate and better overall survival in the EPA group, and at the time of maximum GVHD symptoms, the EPA group showed significantly lower levels of TNF-α, IFN-γ and IL-10 compared to the control group (6). As shown in another study by Takatsuka and colleagues, oral supplementation of fish oil could also have immune-modulating effects in patients with GVHD-DT (7). Thus far, comparable clinical studies are not available. Given the degree of intestinal failure in patients with chronic GVHD-DT, parenteral supplementation of fish oil might be preferable over oral or enteral supplementation. However, little is known about feasibility and safety of intermittent infusion of n-3 PUFA in the outpatient setting.

The objective of this pilot study was to investigate feasibility, safety and dose-response effects on fatty acid composition of plasma lipids and WBC following intermittent infusion
of n-3 PUFA in outpatients with chronic GVHD-DT.

**Materials and methods**

This study was conducted as a pilot study in outpatients with chronic GVHD-DT after RIC allo-SCT of a human leukocyte antigen (HLA) identical sibling. The Medical Ethics Committee of the VU University Medical Center Amsterdam approved of the protocol, and written informed consent was obtained from all patients.

**Patients**

Patients with GVHD-DT at least grade B International Bone Marrow Transplant Registry (IBMTR) histopathological proven by biopsies, at least 3 weeks after initial conditioning of GVHD-treatment, using steady levels of immunosuppressive medication and low dose corticosteroids (<40 mg/24 h prednisone), n-6 PUFA intake of at least 50 g (n-6/n-3 PUFA ratio of at least 2:1 as recommended (9;10)), WHO performance status < 3, fasting triglyceride level < 7 mmol/L and age 18 to 75 years, were asked to participate. Patients were excluded if they had active CMV disease, thrombocytes < 50 x 10⁹ /L, cardiac dysfunction New York Hearth Association (NYHA) classification II-IV, renal failure (creatinine clearance < 40 mL/min), uncontrolled infections, or when they were HIV-positive or allergic to fish or egg protein.

**Fish oil infusions**

The infusion schedule was designed to gradually reach an average dose of at least 2 g EPA per day in the second infusion week (6;7;11). For this purpose, a 10% fish oil emulsion (Omegaven®, Fresenius Kabi) was infused intermittently via a peripheral venous catheter. The fatty acid composition of Omegaven is displayed in Supplementary Table 1. The aim was to infuse 1.5 mL/kg at day 1, 2.25 mL/kg at day 3 and 3 mL/kg of Omegaven at day 5, 8, 10 and 12, during 4 hours. The infusion volume was calculated according to actual body weight, rounded off to tens. The estimated weight of oedema and/or ascites was deducted and in case of obesity (BMI > 30), body weight at BMI of 22.5 was used.

**Feasibility and safety parameters**

We measured vital signs (blood pressure, body temperature and heart rate) before infusion, and every hour, from the start of infusion until 2 hours after infusion.
Assessments of blood pressure and heart rate were performed using the Maxi Stabil 3® (Welch Allyn, Skaneateles Falls, NY USA); body temperature was measured in the axillae by a digital thermometer (Thermoval rapid®, Hartmann, Heidenheim, Germany). During infusion, a nurse inspected the injection site of the peripheral venous catheter. The physician recorded and classified adverse events according to Common Toxicity Criteria for Adverse Events (CTCAE v3.0)[12] during infusion and at all study visits. Routine laboratory tests were performed at baseline and once a week thereafter.

During the first 3 infusions, plasma elimination of Omegaven was evaluated by measuring serum triglycerides at t = 0 h, t = 2 h, t = 4 h and t = 6 h. The triglyceride half-life was calculated from these plasma triglyceride concentrations, and corrected for pre-infusion concentration (t = 0 h). For the remainder of the study, non-fasting triglyceride levels were analysed.

Haemostasis was evaluated by the bleeding score (CTCAE v3.0 haemorrhage / bleeding) (12) and PT and APTT tests. After the final infusion, follow-up measurements were taken twice a week, during 2 weeks.

Considerations for dose escalation or study discontinuation

The dose of Omegaven was increased according to the protocol, if no adverse events were observed, and if serum triglyceride concentrations 2 hours after infusion were < 10 mmol/L and the infusion-related increase was < 4 mmol/L. If not, the dose of the consecutive infusion was reduced by 0.75 mL/kg.

In case of a fish oil related adverse event exceeding CTCAE level 3 (12), haemostatic abnormalities (defined as APTT > 50 s, PT > 1.4 INR), local intolerance (e.g. erythema, oedema, swelling at injection site), or the decision to stop GVHD treatment because of therapy resistant GVHD and/or deteriorating clinical performance, the study in the concerning patient was discontinued.

Clinical parameters

Once a week, patients filled out the Edmonton Symptoms Assessment System (ESAS), a tool that was developed to assist in the assessment of nine symptoms that are common in palliative care patients: pain, tiredness, drowsiness, nausea, lack of appetite, depression, anxiety, shortness of breath, and wellbeing. Patients were instructed to rate the severity of each symptom on a 0 to 10 scale, where 0 represented absence of the symptom and 10 represented the worst possible severity. The sum of the scores for all symptoms is defined as the symptom distress score (13).
The course of GVHD was scored once a week by standardised instruments (8;14).

**Fatty acid composition of plasma lipids and WBC**

At baseline and every study visit, blood samples were collected into 7 mL glass tubes containing disodium EDTA (1 mg/mL). Plasma was immediately separated from blood cells by low speed centrifugation at 228 g for 10 min (10°C) and stored for fatty acid composition analyses. A dextran sedimentation procedure was used to isolate WBC from RBC. In brief, after the addition of Tris (pH 8.4) and 10% of a Dextran solution (50 mg/L), tubes were gently mixed and incubated at 37°C for 20 min to allow the red blood cells to settle. The WBC-rich supernatant was collected (with care to avoid disturbance of the RBC layer) and centrifuged at 1430 g for 5 min. After which WBC pellet was resuspended in Tris and again centrifuged. Finally, WBC pellet was resuspended in 1 mL of Tris and used for fatty acid composition analyses.

Analysis of fatty acid composition of plasma lipids and WBC were performed by Nutrisub (Bruxelles), in collaboration with Prof. Y.A. Carpentier, using gas chromatography as described by Richelle and colleagues (15).

**Statistics**

Data analysis was performed using SPSS for Windows, Release 15. Descriptive statistics were carried out for evaluation of parameters at each time point.

**Results**

We screened 12 patients with GVHD-DT complaints after allo-RIC of an HLA-matched sibling; 4 of them deceased, 3 patients were not willing to participate, and 1 patient was not suffering from GVHD-DT, but diagnosed with an oesophageal ulcer. The 4 remaining eligible patients were included. General characteristics of patients are displayed in Table 1; Table 2 summarises the administered doses of Omegaven per patient. Results of serum triglycerides and PUFA concentrations of plasma and WBC are depicted in Figure 1 to 5.
Table 1: Baseline characteristics of 4 patients with chronic GVHD-DT grade B

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
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<td>59</td>
<td>63</td>
<td>55</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
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<tr>
<td>Diagnosis</td>
<td>MM</td>
<td>MM</td>
<td>MDS</td>
<td>MM</td>
</tr>
<tr>
<td>Conditioning treatment</td>
<td>TBI (2 Gy)</td>
<td>Fludarabine, Cyclofosfamide</td>
<td>Fludarabine, TBI (2 Gy)</td>
<td>TBI (2 Gy)</td>
</tr>
<tr>
<td>Allo-RIC donor</td>
<td>HLA-matched sibling</td>
<td>HLA identical sibling</td>
<td>HLA identical sibling</td>
<td>HLA identical sibling</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L) at baseline</td>
<td>2.9</td>
<td>2.1</td>
<td>4.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>82.0</td>
<td>74.7</td>
<td>68.4</td>
<td>96.8</td>
</tr>
<tr>
<td>Diarrhoea (mL/24 h)</td>
<td>1000</td>
<td>500</td>
<td>500-1000</td>
<td>700</td>
</tr>
<tr>
<td>GVHD Liver</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>GVHD Skin</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>102</td>
<td>95</td>
<td>34</td>
<td>70</td>
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<td>Lipase (U/L)</td>
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</tr>
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<td>ALAT (U/L)</td>
<td>94</td>
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<td>ASAT (U/L)</td>
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<td>GGT (U/L)</td>
<td>776</td>
<td>300</td>
<td>1139</td>
<td>307</td>
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<tr>
<td>AP (U/L)</td>
<td>165</td>
<td>177</td>
<td>212</td>
<td>99</td>
</tr>
</tbody>
</table>

MM, multiple myeloma; MDS, myelodysplastic syndrome; TBI, total body irradiation; RIC, reduced intensity conditioning stem cell transplantation; HLA, human leukocyte antigen; GVHD, Graft-versus-Host Disease; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma glutamyl transferase; AP, alkaline phosphatase.

\^Reference values: amylase: < 100 U/L, lipase: < 70 U/L, ALAT: < 50 U/L, ASAT: < 45 U/L, GGT: < 45 U/L, AP: < 125 U/L
Patient 1

The first participant was a 60-γ-old male with stage III multiple myeloma (MM), also affecting L4 [plasmocytoma]. One month after allo-SCT, he developed GVHD of the skin, liver and digestive tract, in spite of prophylactic treatment. Donor chimerism by that time was almost 100%. After 2 months on high dose prednisone treatment, chronic GVHD remained, with 3 to 9 stools daily (approximately 1000 mL/24 h), hypo-albuminemia, severe oedema (approximately 3 L), insulin-dependent diabetes mellitus, and 7.9% involuntary weight loss. Resting energy expenditure (REE), assessed by indirect calorimetry, was 1912 kcal/24 h, and total energy intake was approximately 3000 kcal/24 h, of which 50% was delivered by nasogastric tube feeding. His total dietary fat intake was 150 g/24 h, of which 3 g of n-3 PUFA.

The patient received respectively 1.5, 2.25, 3, 3, and 3 mL/kg of Omegaven (Table 2). Omegaven appeared to be well tolerated, but after the first 3 infusions, the patient experienced continuous hyperglycaemia, which also led to dehydration. By that time, blood levels of cyclosporine were too high (364 and 479 μL at day 5 and 8, reference value 150-300 μg/L), and cyclosporine dosage was decreased. The hyperglycaemias were treated by adaptation of the insulin dosage algorithm. The 6th infusion of Omegaven was not administered, because of severe dyspnoea and clinical deterioration, and diagnosis of bronchiolitis obliterans and aspergillusosis of the lungs, classified as a complication of immune-suppressive treatment and GVHD.

Laboratory tests showed anaemia, leucopenia and increased levels of ALAT, ASAT, GGT and AP at baseline and throughout the study, probably caused by GVHD of the liver. Blood amylase concentrations were slightly above reference values throughout the study, and lipase concentrations remained below reference values. With regard to haemostasis/bleeding, the patient reported grade 1 hematoma and grade 2 petechiae at all time points, and PT and APTT remained within reference ranges. The patients’ perceived symptom distress scores at day 1, 8, 15 and 22 were respectively 7, 44, 20, and 31.

Patient 2

Subsequently, a 59-γ-old male with MM, in complete remission 2 γ after allo-SCT, was included. At enrolment, he had insulin-dependent diabetes mellitus, chronic GVHD of the skin, liver and digestive tract, and sicca syndrome. The chronic GVHD was treated by a low dose of prednisone (5 mg/24 h). Gastrointestinal complaints consisted of 4 fatty stools daily, and a diarrhoea volume of approximately 500 mL/24 h. REE was 1697 kcal/24 h, and

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daily oral intake approximately 1700 kcal and 69 g of fat (of which 2 g of n-3 PUFA), without the use of ONS, tube feeding or TPV. The patients’ body weight was 10.4% below his usual body weight. He had minimal oedema.

After inclusion, the patient received 6 infusions of Omegaven, in accordance with the protocol. The patient experienced minimal adverse events: slight RR increases during the first infusion, grade 1 hematoma and grade 1 petechiae at all time points, and grade 1 nosebleeds at the last study visit. His triglyceride levels remained within the described ranges. Laboratory tests showed increased levels of ALAT, ASAT, GGT and AP at baseline and throughout the study. Blood amylase concentrations were slightly above reference values throughout the study, and lipase concentrations remained rather low. PT and APTT remained within reference ranges throughout the study. Bleeding time was 6.5 min at baseline and at day 15, after having received all Omegaven infusions, bleeding time was 7 min. Perceived symptom distress scores at day 1, 8, 15 and 22 were respectively 5, 7, 4, and 3.

**Patient 3**

The third patient was a 63 y-old male with myelodysplasia (MDS), GVHD of the skin and digestive tract, onset 10 wk after allo-SCT, and donor chimerism almost 100%. Treatment of GVHD consisted of high dose prednisone and intensive supportive care. At enrolment, the patient had chronic GVHD with stools 5-7 times daily (diarrhoea volume 500 to 1000 mL/24 h). He had lost a substantial amount of body weight over the previous 6 months (24.2%). REE was 1500 kcal/24 h, and oral intake, supported by 2 oral nutritional supplements (600 kcal) daily, contained approximately 2150 kcal/24 h and 83 g of fat (of which 2 g of n-3 PUFA).

During the first infusion of Omegaven (1.5 mL/kg), systolic blood pressure increased by 20 to 35 mm Hg, and heart rate decreased by 25 beats/min. Serum triglycerides increased from 11.0 to 13.4 mmol/L during infusion, and were > 10 mmol/L at t = 6 h. At day 3, the dose was reduced to 0.75 mL/kg. During this infusion, systolic blood pressure increased by 20 mm Hg, and triglycerides exceeded 10 mmol/L at t = 6 h; therefore, we discontinued the study and switched to the follow-up schedule. During the first follow up week, the patient reported more gastrointestinal complaints and fatigue/malaise, and in the second follow-up week, he developed a pressure ulcer on the rump, increased abdominal pain and abdominal discomfort. He reported grade 1 hematoma throughout the study; ALAT, ASAT, GGT and AP fluctuated around levels beyond upper limit of reference values. Blood amylase and lipase concentrations remained far below reference values throughout the study. His perceived symptom distress scores were 37, 49, and 28 at day 1, 8, and 15.
Patient 4
The fourth patient was a 55-y old male with MM, who was in complete remission after allo-SCT, with 100% donor chimerism. Nine months post allo-SCT, he experienced severe diarrhoea and vomiting; GVHD of the digestive tract was histopathologically proven by biopsies. Due to weight loss and intolerance for oral and enteral nutrition, total parenteral nutrition was started. Within 2 weeks, the gastrointestinal complaints reduced and the patient shifted to oral nutrition and nasogastric tube feeding.
At enrolment, the patient had diarrhoea (2-4 stools daily, approximately 700 mL/24 h) and a weight loss of 19.3% in the previous 6 months. REE was 2001 kcal/24 h, and his oral intake, supported by 2 oral nutritional supplements (600 kcal), contained around 3700 kcal/24 h and 173 g of fat (of which 5 g of n-3 PUFA).
The patient received the first dose of Omegaven according to the protocol (145 mL), but due to hypertriglyceridemia during and after infusion, the dose was reduced to 70 mL at day 3 and 5. Adverse events included grade 1 hematoma throughout study and grade 1 hypertension (and reduced heart rate) at day 1, 3 and 5, which was successfully treated by one dose of Norvasc (5 mg) at day 5.
In the second week, the patient was admitted to the hospital with cyclosporine intoxication; he accidentally used 3x5 mg/24 h instead of 3x1 mg/24 h of cyclosporine, and experienced nausea and trembling. Liver function tests showed considerable increases. For this reason, it was not possible to continue the infusions. In the first follow-up week, he was dizzy, tired, hyperglycaemic, oedematous, anorexic, and depressive. Blood amylase and lipase concentrations fluctuated below reference values throughout the study. His perceived symptom distress scores were 28, 39, 54, and 54 at respectively day 1, 8, 15, and 22.

Serum triglycerides
Figure 1 displays the individual curves of serum triglycerides during the study. As expected, serum triglycerides increased during infusion of Omegaven. Triglyceride half-life varied between patients, and depended on the administered dose of Omegaven. Higher amounts of Omegaven resulted in higher serum triglycerides and a longer half-life.
At day 1, serum triglyceride concentrations returned to baseline at t = 6 h. After dose increases, triglyceride concentrations at t = 6 h were slightly higher than baseline at day 3, and at day 5, half-life was roughly reached at t = 6 h. On subsequent visits (2 to 3 days later), serum triglycerides had returned to individual baseline concentrations. Triglyceride half-life was 42, 69, 72 and 1040 minutes in the 4 consecutive patients at day 1. The
Intermittent fish oil infusions in outpatients with GVHD-DT

extreme high half-life in the fourth patient can be explained by the extreme small decrease (0.3 mmol/L) after the first 2 hours following infusion. At day 3 and 5, triglyceride half-life varied from respectively 84 to 129 minutes and 101 to 132 minutes at day 5.

Figure 1. Serum triglycerides concentration in 4 patients with GVHD-DT during intermittent fish oil infusions

Fatty acid composition of plasma lipids and WBC
Figures 2 to 5 display the incorporation of EPA in plasma PL and WBC. In all patients, WBC and plasma PL and TG fatty acid analyses showed dose-dependent increases in the concentrations of EPA and DHA and decreases of AA/EPA ratios as of 2 days after the first infusion of Omegaven (data of DHA and plasma TG not shown). Peak concentrations of EPA in plasma PL and TG as well as WBC were reached either at the final infusion or the first follow-up assessment (2 days after the final infusion) (Figure 2 and 4). Peak DHA in plasma PL and TG were reached during follow-up, after 2 to 8 days (data not shown). Likewise, the ratio of arachidonic acid (AA)/EPA of plasma PL, plasma TG and WBC decreased until the final infusion (Figure 3 and 5). In 2 patients, the AA/EPA ratio of plasma PL, plasma TG and WBC progressively increased during the follow-up period. In these patients, EPA concentrations during follow-up decreased to almost zero, while AA concentrations remained stable, which could be related to infections or general clinical deterioration. After the final infusion, washout of EPA was seen after 5 to 13 days in plasma TG and PL, and after 2 to 13 days in WBC. For DHA, washout from plasma and WBC
appeared to be longer, and varied from 5 to more than 13 days. Fatty acid concentrations of plasma TG showed comparable curves as those of plasma PL.

Table 2: Administered amounts of Omegaven and EPA per patient

<table>
<thead>
<tr>
<th>Day</th>
<th>Infusion dose</th>
<th>Patient</th>
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<th>2</th>
<th></th>
<th>3</th>
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<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>Omegaven (mL)</td>
<td>125</td>
<td>110</td>
<td>100</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protocol (mL/kg): 1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EPA (g)</td>
<td>(1.6-3.5)</td>
<td>(1.4 - 3.1)</td>
<td>(1.3 - 2.8)</td>
<td>(1.8 - 4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Omegaven (mL)</td>
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<td>170</td>
<td>50</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protocol (mL/kg): 2.25</td>
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<td>2.25</td>
<td>0.75</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EPA (g)</td>
<td>(2.3 - 5.2)</td>
<td>(2.1 – 4.8)</td>
<td>(0.6 – 1.4)</td>
<td>(0.9 – 2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Omegaven (mL)</td>
<td>245</td>
<td>225</td>
<td>0</td>
<td>70</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Protocol (mL/kg): 3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0.75</td>
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</tr>
<tr>
<td></td>
<td>EPA (g)</td>
<td>(3.1 - 6.9)</td>
<td>(2.8 - 6.4)</td>
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<td>(0.9 – 2.0)</td>
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<td>8</td>
<td>Omegaven (mL)</td>
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<tr>
<td></td>
<td>Protocol (mL/kg): 3</td>
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<td>0</td>
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<tr>
<td></td>
<td>EPA (g)</td>
<td>(3.1 - 6.9)</td>
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<tr>
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<td></td>
<td>Protocol (mL/kg): 3</td>
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<tr>
<td></td>
<td>EPA (g)</td>
<td>(3.1 - 6.9)</td>
<td>(2.8 - 6.4)</td>
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<td>12</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protocol (mL/kg): 3</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EPA (g)</td>
<td>0</td>
<td>4.6</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|     | Total EPA (g) | 21.3 | 24.0 | 3.1 | 5.8 |
|     |              | (13.1 - 9.5) | (14.8 - 33.3) | (1.9 - 4.2) | (3.4 - 8.0) |
|     | Total EPA (g/24 h) | 2.1 | 1.7 | 1.0 | 1.2 |
|     |              | (1.3 – 3.0) | (1.1 - 2.4) | (0.6 - 1.4) | (0.7 - 1.6) |
Intermittent fish oil infusions in outpatients with GVHD-DT

Figure 2: EPA relative weight content (%) in plasma phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions

Figure 3: Arachidonic acid (AA)/ eicosapentaenoic acid (EPA) ratio in plasma phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions

Figure 4: EPA relative weight content (%) in white blood cell (WBC) phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions

Figure 5: Arachidonic acid (AA)/ eicosapentaenoic acid (EPA) ratio in white blood cell (WBC) phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions
Discussion

This pilot study investigated the feasibility and dose-response effects of intermittent infusion of Omegaven in patients with chronic GVHD-DT, and shows the rate of incorporation and washout of n-3 PUFA in plasma and WBC after infusion of various doses of fish oil. Although only a small group of patients was able to participate, this pilot study obtains useful information on n-3 PUFA administration in a complex patient group, suffering from malabsorption and nutritional issues. The idea of n-3 PUFA administration in patients with GVHD originated from the supposed immune-modulating effects, which could translate into clinical benefits with regard to nutritional status, morbidity and mortality. Animal and human studies showed incorporation of n-3 FA to be associated with a reduction of pro-inflammatory cytokines and PGE2 after supplementation of at least 2 g of EPA per day in healthy subjects (16;17), patients with cancer (18-20), sepsis (21) and ARDS (22-24). Supplementation of a lower dose of EPA did not modulate immune function in humans (25-27). Small clinical studies from a Japanese group showed the relationship of GVHD and inflammation, and the probable beneficial effects of EPA (6;7). Apart from these studies, clinical trials supplementing n-3 PUFA in patients with GVHD-DT have not yet been performed.

A major concern in patients with GDVH-DT is the intestinal malabsorption of nutrients (3;28;29), also resulting in a reduced intestinal absorption of n-3 PUFA, when supplemented by oral or enteral nutrition. Parenteral administration of n-3 PUFA is not hindered by malabsorption and appeared to be a better option for patients with GVHD-DT. These patients are often in need of intensive medical support, and need to visit the outpatient day-care unit twice a week or are admitted to the hospital. Therefore, it appeared to be possible to carry out intermittent fish oil infusions in outpatients 3 times a week. To administer an average infusion dose of 2 g EPA per day, dose escalation to 4 g EPA per 4-h infusion was required. To reach this amount in 4 hours, an infusion rate above the recommended rate described in the SPC (summary of product characteristics) was required.

In the current pilot study, 2 out of 4 patients reached the average dose of 2 g EPA per day, and the protocol could be followed in only 1 patient. After Omegaven infusions, the concentrations of EPA in plasma and WBC increased, and the AA/EPA ratio in plasma and WBC phospholipids decreased. The increase of EPA in plasma PL in this patient (around 2%) was comparable with the increase resulting in immune-modulation in earlier studies (30), but was lower than the increase reached in other studies in cancer patients (20;31).
In patient 2, monocytes were isolated to explore the incorporation and washout of n-3 PUFA from these cells. In this patient, we observed an increase of monocytes EPA after 2 weeks of Omegaven infusions, and washout of EPA from monocytes was more extended than washout from plasma and WBC; after 2 weeks, baseline concentrations were not yet reached.

The incorporation of n-3 PUFA into plasma and cell membranes has been demonstrated in healthy subjects and various patient populations. The incorporation in cancer patients after enteral supplementation was thought to be at least 3 to 4 weeks (19;32), but an increasing number of studies show the incorporation to occur within a day (30;33;34). Depending on the frequency of blood sampling, incorporation within one or more days was also demonstrated after parenteral supplementation of n-3 PUFA in patients with sepsis and around surgery (31). We took blood samples 2 days after the first infusion of Omegaven, and found incorporation of n-3 PUFA in WBC and plasma phospholipids.

The infusion rate in this pilot study (1.5 to 3 ml/kg in 4 h; 0.38 to 0.75 ml/kg/h) was beyond the maximal rate as recommended by the producer of Omegaven (0.5 ml/kg/h). In addition, all patients had increased serum triglycerides (> 2 mmol/L) at baseline. One patient had serum triglycerides beyond the recommended level (4.7 mmol/L, recommended maximum level: 3 mmol/L).

Although triglycerides significantly increased during infusions, these increases were reversible within a few hours. Elevations in serum triglycerides could also be explained by other factors, such as GVHD of the liver. For example, the progressive increases of serum triglycerides in patients 3 and 4 occurred more than a week after their last Omegaven infusion, and were unlikely to be related to Omegaven. In addition, hyperlipidemia is a common side effect of cyclosporine, and was seen in 2 patients (3 out of 4 patients used cyclosporine). Moreover, inflammation is associated with alterations in lipid metabolism and a reduced expression of lipoprotein lipase (LPL), an enzyme that hydrolyses triglycerides into lipoproteins (35). All patients experienced moderate to severe infectious complications as a result of GVHD-DT. This could also explain hypertriglyceridemia at baseline, and the reduced clearance of triglycerides during infusion. Hypertriglyceridemia can lead to pancreatitis, and may interfere with blood tests, and a serious complication of rapid fat infusion is the 'fat overload syndrome', characterized by sudden elevation of the serum triglyceride level associated with fever, hepatosplenomegaly, abnormal platelet function and bleeding disorders, and variable end-organ dysfunction.

The hazards of transient hypertriglyceridemia (during a few hours) have not been well described; in a small group of infants, rapid infusions of a fish oil emulsion were well tolerated and no fat overload syndrome was observed (36). Nevertheless, infusing fatty
Chapter 9

acid at a slower rate appears to be preferable. Because our patients had a normal oral intake, we choose to supplement n-3 PUFA by a parenteral lipid emulsion, and the only option to do so was the 10% fish oil emulsion (Omegaven). Omegaven, a 10% n-3 PUFA emulsion with a high amount of phospholipids, is known to have a slow triglyceride clearance, and EPA and DHA may enter cells as triglycerides or partial glycerides within emulsion particles, and not as free fatty acids (9;37). The use of a 20% n-3 lipid emulsion, which contains a lower amount of phospholipids, would already allow for more efficient triglyceride clearance (38). Even more, emulsions with the lipid being a mix of MCT, soybean oil, olive oil and fish oil appear to have an increased plasma elimination as compared to the standard soybean oil emulsion (37;39;40).

Other documented side effects of Omegaven infusion are anaemia, leukocytopenia, liver enzyme abnormalities, and hyperglycaemia, and are related to metabolic overload. We observed continuous hyperglycaemia in a patient with already known insulin-dependent diabetes, and temporary increased blood pressures during infusion, together with decreased heart rates. These adverse events were rated as mild, and could be adequately treated by insulin and Norvasc.

Administration of a high amount of n-3 PUFAs can also cause a prolonged bleeding time and an inhibited platelet aggregation. We monitored bleeding and haemostats lab, and did not observe clinically significant changes after infusion of Omegaven. More importantly, two patients also showed clinical deterioration due to complications associated with GVHD-DT or medicine use. Another issue was the experienced burden for the patients with GVHD-DT to visit the outpatient clinic 3 days a week, as compared with their normal schedule of 2 days a week.

Adequate treatment of GVHD-DT and participation in an intensive pilot study appears to be complicated by serious illness, fatigue and unexpected events. Intermittent intravenous supplementation of a 10% fish oil emulsion at a high infusion rate appears to be not feasible in this patient population. For patients with total parenteral nutrition, the continuous use of a formula containing n-3 PUFA would be an option, although clinical efficacy in this patient population has not yet been established.

In conclusion, intermittent fish oil infusions result in incorporation of EPA in plasma and WBC but can be complicated by reversible increases in serum triglycerides, which may be caused by GVHD-related liver failure. Clinical implementation of n-3 PUFA administration in outpatients who do not tolerate or absorb enteral nutrition, such as patients with short bowel syndrome or GVHD-DT, deserves more extensive research, focusing on safety,
Intermittent fish oil infusions in outpatients with GVHD-DT

clinical and immunological effects of n-3 PUFA infusion, either or not as part of total parenteral nutrition.

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General discussion
Chapter 10

General discussion

The studies described in this thesis investigated the effects of enteral and parenteral fish oil supplementation on clinical outcomes in two high-risk patient populations; patients with non-small cell lung cancer undergoing concurrent chemoradiotherapy, and those with Graft-versus-Host Disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). Complementary studies explored the presence and prognostic value of weight loss and cachexia at diagnosis of lung cancer, as well as the nutritional issues and nutritional support strategies in patients with Graft-versus-Host Disease. This chapter will summarize the main findings and discuss methodological considerations and interpretations in the light of the existing literature. The discussion will conclude with recommendations for future research and clinical practice.

Main findings and comparison with the literature

Weight loss and cachexia in patients with cancer

Despite attempts to reach consensus in the field on the definition of cachexia, many definitions are circulating (1-7). To our knowledge, we were the first who applied two consensus-based frameworks in patients at diagnosis of stage III non-small-cell lung carcinoma (NSCLC) (Chapter 3). These frameworks include diagnostic criteria to diagnose cachexia, such as a certain degree of weight loss, muscle wasting, inflammation and anorexia.

This study showed that almost 50% of the patients with stage III NSCLC showed signs of precachexia or cachexia, despite the relatively low amount of weight loss (2, 8-11) and good performance status. Consistent with earlier findings (5, 11, 12), cachexia appeared to be associated with a reduced quality of life and shorter survival. In contrast with others, we did not observe any significant associations between (pre)cachexia and physical function, other quality of life subscales (5, 13, 14), fatigue (13), resting energy expenditure (REE) (8, 15-19), physical activity (15, 16), or bioelectrical phase angle (20). This could be due to the limited stage of disease, and to the small sample size and low statistical power. Patients with stage III NSCLC at the start of concurrent chemoradiotherapy are not representative for general cancer patients. Only patients with a good performance status and no severe co-morbidities are eligible to receive this intensive treatment. Still, our findings are relevant for the understanding and definition of the cancer cachexia.

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syndrome, and suggest that patients who are not recognised as malnourished or weak may yet experience signs of precachexia.

Our study showed that the two frameworks differ in terms of included parameters and cut-off points, and consequently diagnose cachexia in different patients. Due to the lack of a gold standard, it was not possible to tell which framework was most valid. As more effects are expected for early nutritional treatment (before cachexia and refractory cachexia develop), a framework which includes staging of cachexia appears needs to be developed and validated in large populations of cancer patients.

Apart from malnutrition and cachexia, sarcopenia also plays a role in patients with cancer, and there has been little research on this topic to date. Sarcopenic obesity is defined as the increase in fat mass and reduction in lean mass, seen in aging, physical inactivity and illness (21). Patients with sarcopenic obesity can be easily overlooked in case of moderate weight loss, as they do not look severely malnourished or cachectic. Results of our retrospective study in patients with stage III NSCLC showed that weight loss during chemoradiotherapy, in particular in patients with a BMI greater than 25 kg/m², was associated with a shorter progression-free and overall survival (Chapter 7). This poor outcome could be explained by the existence of ‘sarcopenic obesity’, but as only data on body weight and height were available, not on fat mass and lean mass, sarcopenia could not be investigated in this study.

The impact of fish oil on clinical parameters

Fish oil supplementation is a relatively new area of clinical nutrition. Although the immune-modulating and anti-cachectic effects have been shown in preclinical studies (22-24) and time series (25-28), clinical studies in patients with cancer did not consistently show clinical benefits. So far, it remains unclear when and to whom caregivers should supplement n-3 PUFAs from fish oil. Therefore, we performed comprehensive literature studies on the clinical effects of n-3 PUFAs in three patient groups with an inflammatory state (Chapter 2 and 6). We conducted a number of consecutive intervention studies in patients with NSCLC and GVHD (Chapter 4, 5 and 9).

The main conclusion of the systematic literature review in Chapter 2 was that there is evidence to advise the oral or enteral supplementation of n-3 PUFAs in cancer patients and in critical care patients. Parenteral supplementation of n-3 PUFAs around surgery also showed beneficial effects on clinical outcome. In addition, we carried out a more up-to-date literature study in patients with cancer. Evidence analysis in collaboration with Australian experts showed that oral or enteral supplementation of n-3 fatty acids appears
to be safe and may have positive effects on quality of life and physical activity. However, the evidence for the beneficial effect on body weight, fat free mass and performance status turned out to be inconclusive after adding studies that were recently published (Chapter 6). These literature studies help clinicians to decide on the supplementation of fish oil in the clinical setting, and justify the enteral supplementation of n-3 PUFAs in critical care patients and the parenteral supplementation of n-3 PUFAs around surgery. However, qualitative reviews could be judged as expert opinions. The best and most objective way to review the evidence is by meta-analyses. Meta-analyses confirmed the beneficial effects of parenteral fish oil in surgical patients (29, 30) and enteral fish oil in patients with acute respiratory distress syndrome (ARDS) (31). Up-to date meta-analyses of studies in patients with cancer are not available.

Two chapters of this thesis report on an RCT on the effects of a protein-and energy dense oral nutritional supplement containing n-3 PUFAs in patients with stage III NSCLC. Supplementation of the oral nutritional supplement containing n-3 fatty acids during multimodality treatment beneficially affected body weight, fat free mass and REE (Chapter 4). Besides the effects on nutritional status, the intervention group had a higher quality of life, performance status and physical activity than the control group (Chapter 5). Dietary energy and protein intake of the intervention group appeared to be higher at one time point (after 4 weeks). No effects could be demonstrated for mid upper arm circumference, handgrip strength and inflammatory markers. The lack of effects on inflammatory parameters could be explained by the moderate inflammatory status of these patients.

The positive results of this small study in patients with lung cancer contribute to the evidence of beneficial effects of n-3 PUFAs in patients with cancer. In addition to other positive studies in patients with lung cancer (32-34), we suggest fish oil supplementation is warranted in patients with stage III lung cancer who show signs of (pre)cachexia. For patients with other stages of lung cancer as well as for patients with other types of cancer, the evidence from clinical studies remains inconclusive.

Thus far, the prophylactic effects of n-3 PUFA supplementation around allogeneic stem cell transplantation as well as the effects of n-3 PUFAs on the treatment of GVHD are understudied. A few small studies documented positive effects of n-3 PUFAs on inflammatory markers and GVHD (35-38). As patients with GVHD of the digestive tract often depend on parenteral nutrition, we investigated intermittent parenteral supplementation of fish oil in a pilot study in the outpatient clinic (Chapter 9). Parenteral supplementation of fish oil resulted in rapid incorporation of EPA and DHA in plasma and
WBC (within two days). The levels of incorporation of n-3 PUFAs into plasma and WBC, and the washout rate after cessation of infusions were comparable with other studies (39). However, this first study investigating effects of intermittent infusions of n-3 lipid emulsion in patients with chronic GVHD-DT, immune suppression, liver failure and thrombocytopenia, showed to be labour-intensive and not feasible to apply to a larger group of patients. If future research shows that n-3 PUFAs have beneficial effects in patients with GVHD of the digestive tract, they need to be infused at a lower dose, or administered by oral or enteral nutrition to avoid serum hypertriglyceridemia and hyperglycaemia.

**N-3 fatty acid status**

Immune-modulation by n-3 PUFAs can only be reached when sufficient amounts of n-3 PUFAs are incorporated into immune cells. Therefore, we also investigated effects of n-3 PUFA supplementation on n-3 fatty acid status in patients with severe inflammation. Studies on the dietary intake of fatty acids often assess fat intake by self-administered food frequency questionnaires, but these are inaccurate to measure the individual fatty acid status. Alternatively, various biological compartments, such as whole blood, blood cells, plasma, and adipose tissue reflect the fatty acid status (40). The systematic review described in Chapter 2 investigated the incorporation of n-3 PUFAs in phospholipids of blood and tissues.

In patients with cancer, undergoing surgery, or receiving critical care, supplementation with EPA (either or not combined with DHA) resulted in incorporation into plasma phospholipids. Depending on the supplemented dose of n-3 PUFAs, concentrations of EPA in plasma phospholipids reached values of ~2 to ~7%. The exact dose-response relationship for the incorporation of n-3 PUFAs in different blood and tissue compartments remains unclear: study designs were heterogeneous, and assessed n-3 PUFA status in different tissues (e.g. phospholipids, white blood cells, red blood cells) and units (e.g. micromole/L, percent of total fatty acids or weight%). However, it was concluded that n-3 PUFAs are incorporated into plasma phospholipids within a few days after oral, enteral or parenteral supplementation, and that concentrations in plasma phospholipids return to baseline within a few days after cessation of supplementation. For blood cells, the incorporation and washout appear to be slower. This information is valuable for clinical practice; to date, it was thought that oral or enteral supplemented n-3 PUFAs need a few weeks to be incorporated, and the exact duration of washout was unknown.

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**Inflammation**

In Chapter 4, we showed small increases of plasma phospholipids' EPA in patients with NSCLC using oral nutritional supplements containing n-3 PUFAs during chemoradiotherapy, and serum IL-6 and CRP were negatively correlated with plasma EPA levels in patients who had an increase of at least 1.5% in plasma phospholipids EPA. Others observed improvements in inflammatory markers after supplementation of n-3 PUFAs in patients during cancer surgery (44), chemotherapy or palliative care (28, 34, 45). Inflammation, fatigue and muscle wasting are strongly related and therefore it is interesting that studies in patients with cancer demonstrated the relationship between low plasma n-3 PUFAs and muscle wasting. Murphy and colleagues showed that plasma n-3 PUFAs are depleted in patients with NSCLC with sarcopenia, and those with maximal muscle loss during chemotherapy had lower plasma concentrations of EPA than patients who were gaining muscle (46). The relationship between a high plasma EPA and the maintenance of muscle mass was also observed in a post-hoc dose response analysis of an RCT in patients with pancreatic cancer. This study found a net gain of weight, muscle mass and quality of life in patients who consumed sufficient amounts of n-3 PUFAs (47).

**Nutritional support in patients with Graft-versus-Host disease**

The number of allogeneic stem cell transplantations provided to patients with haematological malignancies is increasing annually, and so does the number of patients with GVHD. Patients with GVHD encounter nutritional issues and need optimal nutritional support, but the existing knowledge on this topic is limited (48-51). For this reason, we reviewed the literature on the nutritional issues and nutritional support strategies in patients with GVHD following allogeneic stem cell transplantations in Chapter 8. In Chapter 9, we described four case studies of patients with chronic GVHD-DT who participated in a feasibility study on intermittent fish oil infusions. These patients had lost substantial amounts of body weight (7.9 to 24.2%), and were receiving intensive medical and nutritional support during several months. They experienced steroid myopathy and fatigue, and had a high risk of infections. These patients represent a general population of patients with severe GVHD. The literature study demonstrated the high degree of malnutrition and weight loss in patients with GVHD, in particular in patients with extensive GVHD. Apart from weight loss, specific micronutrient deficiencies occur, in particular deficiencies of zinc, vitamin B₁₂ and vitamin D. Both GVHD and steroid treatment are associated with a decline in fat free mass.
This review showed that little evidence exists on the appropriate nutritional support strategies for patients with GVHD. Interventions such as immunonutrition, probiotics, low-fat or lactase-free diets have not been investigated in patients receiving allogeneic hematopoietic stem cell transplantations, or patients with GVHD. Probably, recommendations applicable to patients with other inflammatory bowel diseases (colitis, Crohn’s disease, celiac disease) could be extrapolated to patients with GVHD, but no studies on this topic have been performed so far. After having reviewed the literature, we concluded our work with evidence-based nutritional recommendations for patients following allo-SCT and patients with GVHD.

Methodological considerations
Strengths and limitations of the research described in this thesis have already been addressed in the relevant chapters. This section summarizes the most important methodological considerations and addresses additional relevant issues.

We conducted a high-quality double blind RCT, which is the preferred design for a clinical trial and is characterised by a high internal validity. With intensive follow-up and having one investigator to organise the measurements and speak to the participants, we experienced relatively low attrition rates, attributed to patient withdrawal, disease progression or adverse events.

Only meta-analyses of RCTs surpass individual RCTs and are generally accepted as the preferred methodology for summarizing literature findings. In the literature studies in this thesis, it was however not possible to perform meta-analyses, due to the heterogeneity in outcome parameters and study designs.

An overall limitation of the studies was the limited sample size of the RCT (n = 40), the retrospective study (n = 51) and the feasibility study (n = 4). Although many patients were treated in this university hospital, not all patients were eligible to participate in an intervention study, for instance because patients participated in another clinical trial, or because they were too ill or did not meet in- and exclusion criteria. It was also hard to screen and include patients in time, before chemotherapy commenced. Consequently, the power of the studies was low, which may have resulted in type II errors, i.e. false-negative outcomes. This could have been the case for the effect parameters in our RCT, as well as for the preoperative risk factors in the retrospective analysis. Multi-centre trials could have solved this problem, but because of specialized measurement and treatment procedures (e.g. indirect calorimetry to measure REE, and laboratory procedures within 2 hours after blood sampling), it was not feasible to extend our trials to other centres in the
Netherlands, because most use different nutritional and laboratory assessments. Another issue concerns selection bias; patients with lung cancer who were willing to participate in the clinical study, and did not drop out during the study may have been the ones with a better overall physical performance and nutritional status. This could have made them less motivated to use two cans of oral nutritional supplements on a daily base. As a result, the adherence to the study supplements was far from optimal in both the intervention and control group, despite the intensive nutritional counselling during the study. Nevertheless, we found significant differences in a number of effect parameters, and these differences were larger when selecting the patients who had a good adherence. The recommended dose of EPA to achieve immune-modulation and clinical benefits is 2 g per day. Possibly (smaller) effects could be reached when supplementing a lower dose of EPA, but this hypothesis needs to be tested in future research.

The effect parameters on nutritional status included widely accepted methods for nutritional assessment, such as body weight, mid upper arm circumference, handgrip strength. To assess FFM, the only affordable and feasible method was bio impedance analysis. Bio impedance analysis in patients with cancer has a limited accuracy, and thus the FFM data should be taken with care. More accurate methodologies such as DXA or CT imaging are preferred to estimate FFM in future studies.

Relevance and implications for clinical practice and future research

The findings of our research can be used to develop and implement a framework for (pre)cachexia. As with nutritional screening, nowadays implemented in hospitals in the Netherlands, early detection of (pre)cachexia will raise attention to catabolic processes in patients with cancer. It is apparent that the development and use of definitions for cachexia or precachexia are emerging. Despite the large number of available frameworks and definitions, progress needs to be made to validate and refine these. Ideally, cancer centres around the world need to collect baseline and follow-up data on cachexia parameters and biomarkers in a uniform way, and agree upon one definition and classification, after analyses of the merged data. The cachexia definition should be validated against outcomes such as response to cancer treatment or anti-cachexia treatment, rather than overall survival.

After screening, patients diagnosed with (pre)cachexia need to be offered appropriate treatment. Although more research is required, the current evidence points to multimodal treatment, consisting of (immune-modulating) nutritional support, pharmacological treatment and symptom control to reduce catabolic processes and maintain muscle mass,
functional status and quality of life (52, 53). A combination intervention of medroxyprogesterone or megestrol acetate (both appetite stimulants), oral supplementation with eicosapentaenoic acid, the amino acid L-carnitine, and thalidomide proved to be more effective than treatment of one of these agents (54).

So far, the evidence for n-3 PUFAs is inconclusive, and effects appear to be small. In order to improve the tolerance and compliance n-3 PUFAs could be prescribed in a form that patients choose themselves (fish oil capsules, oral nutritional supplements, or tube feeding). Probably, inflammation causes a higher turnover of n-3 PUFAs; n-3 PUFAs washed out more rapidly in patients with sepsis than in patients undergoing surgery. This mechanism needs to be confirmed in future research. Furthermore, studies on the optimal dose and duration of n-3 PUFA supplementation in specific circumstances, e.g. during radiotherapy, would be useful.

Patients with precachexia could be easily overlooked and may benefit from early nutritional or pharmacological intervention. Future studies need to investigate the effects of early nutritional and/or pharmacological intervention in patients with precachexia.

Another component of multimodal treatment should be a physical exercise intervention. To reach muscle synthesis, protein supplementation needs to be combined with resistance training (55). Physical exercise also decreases fatigue symptoms (56). A large body of evidence shows the beneficial effects of physical exercise training during or after cancer treatment (56, 57), but it is unknown whether patients with cancer cachexia would benefit from physical exercise training. Studies first need to confirm the benefits of physical exercise training on muscle quality and responsiveness in these patients. Furthermore, initiatives to investigate the feasibility and effects of physical activity training are needed for patients with lung cancer, who may have a reduced lung capacity, pain and dyspnoea due to the local disease, lung resection, and/or pre-existing COPD (56).

Future research on the degree of sarcopenia and cachexia in patients with cancer requires measurements and follow-up of skeletal muscle mass, preferably by DXA or CT-scans, which are more valid and reproducible to quantify skeletal muscle than bio-impedance analyses (58).

**Conclusion**

In conclusion, weight loss, (pre)cachexia and muscle wasting are prevalent in patients with stage III NSCLC as well as in patients with GVHD-DT, and are associated with a reduced quality of life and shorter survival. Supplementation of fish oil during combined modality treatment improves nutritional status, quality of life, performance status and physical activity in patients with stage III NSCLC. Furthermore, intermittent fish oil infusions in
patients with GVHD-DT alter the fatty acid composition of plasma and blood cells, but can be complicated by a reversible increase in serum triglycerides. Chemoradiotherapy and allogeneic HSCT have become a part of the usual cancer care and will be applied more and more. Consequently, the number of patients who experience nutritional issues, severe complications and GVHD will increase. The findings of this thesis can be used to improve the condition and quality of life around chemoradiotherapy and allogeneic HSCT. Future research needs to focus on the refinement and validation of (pre)cachexia definitions, as well as on nutritional support strategies for GVHD and multimodal treatments for (pre)cachexia.
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