# 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[1–3]

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## 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 4 wk (B = 1.3 and 1.7 kg, respectively; $P < 0.05$), a better FFM maintenance after 3 and 5 wk (B = 1.5 and 1.9 kg, respectively; $P < 0.05$), a reduced REE (B = −16.7% 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 = −27.9; $P = 0.08$) 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 = 25.0 g, $P = 0.01$, 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. J. Nutr. 140: 1774–1780, 2010.

## 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 the 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

[10] Abbreviations used: ALA, α-linolenic acid; C, control group; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFM, fat-free mass; GEE, generalized estimating equation; HLA-DR, human leukocyte antigen-DR; IL-6, interleukin-6; I, intervention group; MUAC, mid-upper arm circumference; NSCLC, non-small cell lung cancer; REE, resting energy expenditure; sTNF-p55, soluble tumor necrosis factor-p55; TEE, total energy expenditure.

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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-$\alpha$ (TNF-$\alpha$) 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 Fig. 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 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 × *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). $CO_2$ production and $O_2$ 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).

**TABLE 1** Nutritional composition of the oral nutritional supplements

|  | I | C |
| --- | --- | --- |
|  | *unit/can* | |
| Volume, *mL* | 240 | 200 |
| Energy, *kJ* | 1234 | 1255 |
| Protein, *g* | 16.0 | 12.5 |
| Fat, *g* | 6.14 | 9.84 |
|   Monounsaturated fatty acids | 1.22 | 5.82 |
|   SFA | 1.61 | 0.90 |
|   PUFA | 2.83 | 2.60 |
|     Linoleic acid | 0.43 | 2.16 |
|     $\alpha$-Linolenic acid | 0.12 | 0.38 |
|     EPA | 1.01 | — |
|     DHA | 0.46 | — |
| (n-6):(n-3) fatty acids | 0.3: 1 | 5.7: 1 |
| Carbohydrates, *g* | 44.0 | 40.4 |

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 $\mu$g/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 $\mu$g/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 ($\pm$ 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 $\geq$ 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 $\pm$ SD, except where stated otherwise. All *P*-values were 2-sided at a significance level of $\alpha = 0.05$ ($P < 0.05$).

## 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).

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Sixteen patients had stage IIIa NSCLC and 24 patients had stage IIIb NSCLC.

At baseline, the patients had lost 0.5 $\pm$ 2.5 kg in the previous month, 0.9 $\pm$ 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 $\leq$ 18.5 and/or unintentional weight loss $\geq$ 5% in the previous month and/or $\geq$ 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.

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 (drop-outs: 60% vs. 14%: patients who reached wk 3; *P* = 0.02). Stage of disease and Karnofsky performance score were comparable between early drop-outs and patients who reached wk 3. Reasons for drop-out before wk 3 were withdrawal of consent (*n* = 3), disease progression (*n* = 1), or the occurrence of an adverse event (Supplemental Fig. 1).

**TABLE 2** General and baseline characteristics of 40 patients with stage III NSCLC[1,2]

| | I | C | P |
|---|---|---|---|
| *n* | 20 | 20 | |
| Female, *n (%)* | 4 (20) | 15 (75) | <0.001 |
| Age, *y* | 58.4 ± 12.0 | 57.2 ± 8.1 | 0.70 |
| Karnofsky performance score | 84.0 ± 11.4 | 80.5 ± 10.0 | 0.31 |
| Stage of disease, *n (%)* | | | 0.75 |
| IIIa | 9 (45) | 7 (35) | |
| IIIb | 11 (55) | 13 (65) | |
| Chemotherapy, *n (%)* | | | 0.62 |
| Cisplatin and docetaxel | 11 (55) | 8 (40) | |
| Cisplatin | 8 (40) | 11 (55) | |
| Cisplatin and bevacizumab | 1 (5) | 1 (5) | |
| Nutritional status | | | |
| BMI, *kg/m²* | 24.8 ± 4.1 | 23.0 ± 2.4 | 0.42* |
| Weight loss previous month, *%* | −0.3 ± 2.4 | −1.5 ± 4.7 | 0.32 |
| Malnutrition, *n (%)* | 3 (15) | 5 (25) | 0.70 |
| Body weight, *kg* | 77.1 ± 14.6 | 64.7 ± 7.4 | 0.12* |
| FFM, *kg* | 58.0 ± 8.7 | 48.0 ± 7.3 | 0.26* |
| FFM index, *kg/m²* | 18.5 ± 2.0 | 16.6 ± 1.4 | 0.47* |
| MUAC, *mm* | 289.4 ± 36.2 | 269.6 ± 23.1 | 0.43* |
| REE, *% of expected* | 113.6 ± 15.1 | 110.5 ± 13.7 | 0.51 |
| REE, *kJ/kg body weight* | 102.5 ± 17,6 | 99.6 ± 15.5 | 0.57 |
| REE, *kJ/kg FFM* | 136.8 ± 4.7 | 141.0 ± 22.2 | 0.53 |
| Inflammatory markers | | | |
| Serum CRP, *mg/L* | 39.1 ± 42.9 | 50.4 ± 66.8 | 0.54 |
| Serum albumin, *g/L* | 35.9 ± 5.2 | 35.8 ± 6.0 | 0.96 |
| Leukocytes, *x10⁹/L* | 8.7 ± 4.5 | 9.3 ± 7.4 | 0.76 |
| Serum IL-6, *mg/L* | 7.0 ± 6.5 | 4.9 ± 7.8 | 0.38 |
| Serum sTNF-p55, *μg/L* | 3.0 ± 1.1 | 2.8 ± 1.0 | 0.65 |
| HLA-DR expression on monocytes, *kMESF* | 95.2 ± 43.1 | 88.1 ± 37.8 | 0.59 |

[1] Results are mean ± SD or *n* (%).* *P*-value of difference between groups after adjustment for sex (linear regression analysis with sex as covariate).
[2] *P*-value of difference between groups (independent samples *t* test).

## 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 (**Fig. 1**). In both groups (I: $n = 1$ vs. C: $n = 3$), there were patients with baseline plasma phospholipid EPA $\geq 1.6\%$, 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%; $P = 0.65$).

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
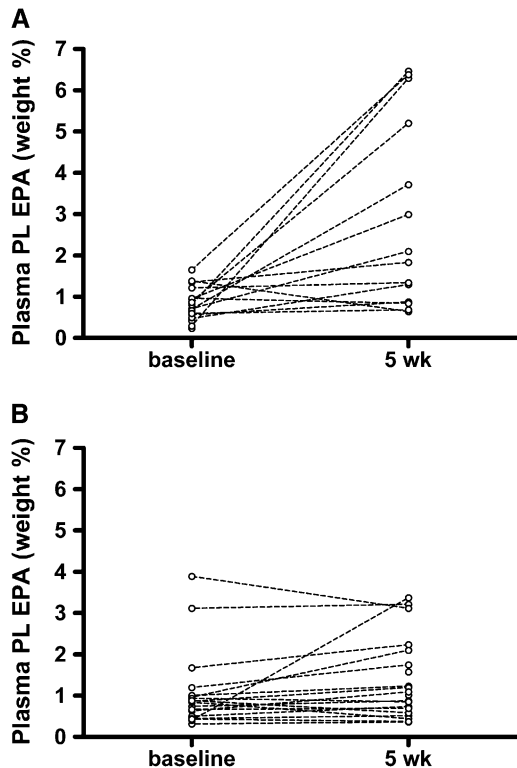
I group decreased more after 3 wk (B = −16.7% of predicted, $P = 0.01$ and B = −4 kJ/kg body weight, $P = 0.07$) (**Table 4**).

After 4 wk, the I group had a greater energy intake than the C group (B = 2456 kJ/d; $P = 0.03$). After 1 and 4 wk, the I group had a greater absolute protein intake than the C group (B = 12.4 g/d, $P = 0.08$ and B = 25.0 g/d, $P = 0.01$, respectively). After 2, 3, and 5 wk, protein intake did not differ between the groups. After correcting for individual energy requirements and for intake per kilogram body weight, energy balance and protein intakes did not differ between the groups (Table 3).

## 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) (**Fig. 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).

*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 × $10^9$, 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 = −27.9; $P = 0.08$). 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



**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[1–3]

| | I | | C | |
| --- | --- | --- | --- | --- |
| | Baseline | wk 5 | Baseline | wk 5 |
| *n* | 20 | 14 | 20 | 19 |
| Energy, *kJ/24 h* | 6668 ± 2684 | 7646 ± 4132 | 6836 ± 2220 | 6650 ± 3110 |
| Macronutrients, *% of energy* | | | | |
| Protein | 16.5 ± 4.08 | 16.9 ± 3.81 | 14.5 ± 3.34 | 18.2 ± 8.74 |
| Carbohydrates | 51.9 ± 12.0 | 52.4 ± 7.51 | 48.9 ± 7.92 | 46.5 ± 13.7 |
| Fat | 28.6 ± 8.98 | 30.2 ± 8.50 | 34.3 ± 5.26 | 35.4 ± 8.06 |
| PUFA | 4.65 ± 1.82 | 6.11 ± 3.91 | 4.34 ± 2.09 | 6.01 ± 3.88 |
| (n-3) PUFA, *g* | 0.10 ± 0.24 | 2.89 ± 3.37 | 0.10 ± 0.17 | 0.22 ± 0.83 |
| EPA | 0.02 ± 0.08 | 0.87 ± 1.03[3] | 0.03 ± 0.06 | 0.08 ± 0.32 |
| DHA | 0.04 ± 0.10 | 0.43 ± 0.46 | 0.07 ± 0.12 | 0.13 ± 0.48 |
| ALA | 0.04 ± 0.17 | 1.58 ± 1.90[3] | 0.00 ± 0.00 | 0.01 ± 0.03 |

[1] Results are mean ± SD.
[2] 1 kJ = 0.239 kcal, 1 g protein = 17 kJ, 37 g fat = 37 kJ, 1 g carbohydrate = 17 kJ (44).
[3] $P < 0.05$ (difference between groups, analyzed by GEE with baseline value and sex as covariates).

**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[1]

| | wk 3 | | wk 5 | |
|---|---|---|---|---|
| | B | P | B | P |
| REE, *% of predicted* | −16.7 | 0.01 | 4.9 | 0.56 |
| REE, *kJ/kg body weight* | −10.0 | 0.07 | 3.8 | 0.66 |
| REE, *kJ/kg FFM* | −10.9 | 0.10 | 3.6 | 0.66 |
| Energy intake, *kJ/24 h* | 1046 | 0.25 | 256 | 0.81 |
| Energy balance, *% of TEE* | 15.4 | 0.20 | −3.8 | 0.78 |
| MUAC, *mm* | 6.6 | 0.37 | 9.1 | 0.06 |

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

plasma phospholipid EPA levels (Pearson $r$: −0.8, $P = 0.041$ and −0.8, $P = 0.048$, 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
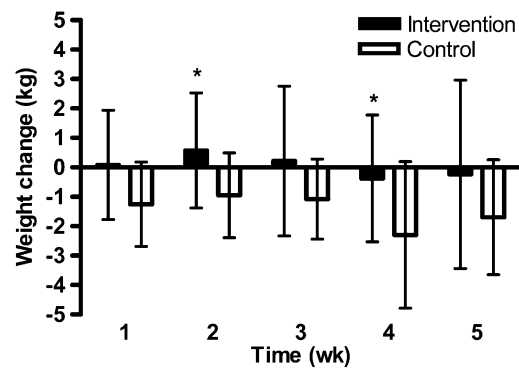
**FIGURE 2** Weight change from baseline in I and C patients with stage III NSCLC. Values are means ± SD, $n = 20$ (baseline), 14 (wk 5, I), or 19 (wk 5, C). *Different from C, $P < 0.05$ (analyzed by GEE, with baseline value and sex as covariates).

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 pre-cachexia, 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 drop-out, 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, pre-cachectic 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

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