Improved body weight and performance status and reduced serum PGE$_2$ levels after nutritional intervention with a specific medical food in newly diagnosed patients with esophageal cancer or adenocarcinoma of the gastro-esophageal junction

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Abstract

Background   The majority of cancer patients loses weight and becomes malnourished during the course of their disease. Metabolic alterations and reduced immune competence lead to wasting and an increased risk of infectious complications. In the present study, the effect of a nutritionally complete medical food, which is high in protein and leucine and enriched with fish oil and specific oligosaccharides, was investigated on immune function, nutritional status, and inflammation in patients with esophageal cancer and compared with routine care.

Methods   In this exploratory double-blind study, 64 newly diagnosed esophageal cancer patients were randomized. All patients received dietary counselling and dietary advice. In the Active group, all patients received the specific medical food for 4 weeks before the start of anticancer therapy. In the routine care control arm, patients with <5% weight loss received a non-caloric placebo product, and patients with weight loss ≥5% received an iso-caloric control product to secure blinding of the study. The required study parameters of body weight and performance status were recorded at baseline and after 4 weeks of nutritional intervention, and patients were asked to complete quality of life questionnaires. In addition, blood samples were taken for the measurement of several immune, nutritional, and safety-parameters.

Results   No effect of the specific nutritional intervention could be detected on *ex vivo* stimulations of blood mononuclear cells. By contrast, body weight was significantly increased ($P < 0.05$) and ECOG performance status was improved after intervention with the specific medical food ($P < 0.05$). In addition, serum Prostaglandin E$_2$ (PGE$_2$) levels were significantly decreased in the specific medical food group and increased in the control group ($P = 0.002$).

Conclusions   Nutritional intervention with the specific medical food significantly increased body weight and improved performance status compared with routine care in newly diagnosed esophageal cancer patients. This effect was accompanied by significantly reduced serum PGE$_2$ levels.

Keywords   Clinical; Cancer; Nutrition; Medical food; Body weight; PGE$_2$

Introduction

The majority of cancer patients loses weight and becomes malnourished during the course of their disease. Worldwide, the incidence of malnutrition during cancer ranges from 30 to 90%, being most prevalent in patients with esophageal, pancreatic, lung, prostate, or colon cancer.[1–5] The incidence and severity of malnutrition are affected by the type, location, grade, and stage of the tumor, as well as by anticancer treatments, patient characteristics, and individual susceptibility.[2,4]

Severe and prolonged malnutrition can lead to cancer cachexia, which is a major contributor to morbidity and mortality, especially in advanced disease. Characteristics of this chronic condition of catabolism include progressive, involuntary weight loss (WL), anorexia, asthenia, fatigue, depletion of lipid stores, and severe loss of skeletal muscle proteins.[6,7]

Other important features of the cachexia syndrome include the presence of a chronic inflammatory state and, paradoxically, a state of impaired immune responsiveness.[6,8,9] Several mediators that are either tumor-derived or host-derived (e.g. pro-inflammatory cytokines, chemokines, and prostaglandins) induce a cascade of events leading to a suppressed immune function, thereby reducing the acute response to infectious triggers.[6,10–12] This compromised immune competence may lead to increased complications, delayed, or suboptimal anticancer treatment, and even to accelerated disease progression, resulting ultimately in a decreased quality of life and reduced survival rates in patients.[13–17]

To reduce the risk of (infectious) complications and to support the performance status of cancer patients, a multidisciplinary approach should be applied, in which nutritional intervention is recommended as an integral part of anticancer therapy to prevent involuntary weight loss and delayed treatment schedules and to improve clinical outcomes and quality of life.[3,6,18,19] In malnourished patients, preoperative nutritional support is associated with a 50% reduction of postoperative complications,[18] including decreased gastrointestinal toxicity, improved performance status, and increased immune responses.[3] However, recent findings show that impaired immune responsiveness and muscle protein degradation may already occur before the onset of WL.[20] Consequently, it is of clinical relevance to provide the optimal treatment support as early as possible, preferably starting at diagnosis and running parallel to the pathway of anticancer therapies.[19] However, today, routine care is often still limited to dietary counselling with, depending on the severity of the nutritional status, protein and energy supplementation.

Recently, a specific medical food* has been developed for application in cancer patients. This medical food is high in protein and leucine and is enriched with emulsified fish oil (containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and a specific oligosaccharide mixture and is designed to reduce complications and to provide optimal treatment support by reducing the inflammatory state, supporting immune function and nutritional status, and preserving muscle mass and function. These effects have been demonstrated in previous preclinical studies using an animal model of tumor-induced cachexia.[21,22] The aim of this exploratory study was to investigate the effects of this medical food on immune function, nutritional status, and inflammation in an early phase in a group of newly diagnosed patients with esophageal cancer or adenocarcinoma of the gastroesophageal junction before the start of anticancer therapy and to compare this with routine care.[1]

Materials and methods

An explorative, randomized, controlled, double-blind study with parallel groups was conducted in order to determine the effect of a 4 week nutritional intervention with a specific medical food on immune function in newly diagnosed esophageal cancer patients before the start of anticancer therapy, compared with the effect of routine care (dietary counseling and advise to increase food intake with the addition of an isocaloric or non-caloric placebo product depending on the nutritional state). The secondary objective was to assess the effects of this specific medical food on nutritional status and inflammation. Data on immune function, nutritional state, and inflammation of healthy volunteers were obtained to compare baseline values and allow an adequate interpretation of the data.

Subjects

In the period between August 2007 and February 2009, 64 newly diagnosed patients with histologically confirmed adenocarcinoma or squamous carcinoma located in the esophagus or gastro-esophageal junction (Siewert–Stein classification type I-III)[24] planned for esophageal cancer treatment were recruited from the Erasmus Medical Center, Rotterdam, The Netherlands. Patients had an age of 18 years and above and were included in the study after informed consent was obtained. Exclusion criteria were life expectancy <3 months, planned start of anticancer treatment within 3 weeks, Eastern Cooperative Oncology Group (ECOG) performance status ≥2, esophagus-related surgery after

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[1]A medical food is in the USA defined in 21 U.S.C. Section 360ee(b)(3) as 'a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognizable scientific principles, are established by medical evaluation'.[23] A comparable definition exists in the harmonized legislation of the European Union (cf. Article 1, 2(b) of Commission Directive 1999/?21/EC of 25 March 1999 on dietary foods for special medical purposes).

diagnosis before inclusion, chemotherapy and/or radiotherapy in the past 5 years, altered immune function, dysphagia score of 4, dependency on tube feed or parenteral nutrition during the previous 4 weeks, use of fish oil-containing supplements during the previous 4 weeks, intolerance or allergy to dairy products, fish, or other ingredients of the study products, dependency on fibre-free diet, pregnancy or lactation, dementia or altered mental status that would prohibit the understanding and giving of informed consent, any other medical condition that may interfere with the safety of the patient or the outcome parameters or uncertainty about the willingness or ability of the patient to comply with the protocol requirements, according to the investigator's judgment. In addition to the patients, a reference group of 40 healthy volunteers was recruited for the study from the database of Vaxinostics BV, University Vaccine Center Rotterdam Nijmegen, Rotterdam, The Netherlands. Subjects in the reference group were age-matched and sex-matched with the Dutch esophageal cancer population, had a body mass index (BMI) between 18.5 and 30 kg/m^2, and were included in the study after signing an informed consent. Exclusion criteria for subjects in the reference group were significant involuntary weight loss in the past year, smoking, acute or chronic disease, altered immune function, pregnant or lactating, and any other condition that may interfere with the definition 'healthy volunteer' according to the investigator's judgment.

Study design

The study was conducted in compliance with the principles of the 'Declaration of Helsinki' (52nd WMA General Assembly, Edinburgh, Scotland, October 2000, including the Notes of Clarification as added in 2002, Washington, and in 2004, Tokyo) according to the International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and was approved by the Ethics Committee of the Erasmus MC, Rotterdam, the Netherlands. The trial was registered in the International Standard Randomized Controlled Trials Number (ISRCTN) database with the Trial registration ID: ISRCTN28490479. After initial screening of the patients, subject characteristics, relevant medical history, dysphagia score, and anthropometrics were determined at visit 1 (baseline). Patients were randomized to the Active group receiving the specific medical food or to the Control group receiving routine care (dietary counselling and advice with the addition of an iso-caloric or non-caloric placebo product depending on the nutritional state), using a computerized randomization program after stratification based on their nutritional status. Patients with 0 to <5% weights loss in the past 3 months and a dysphagia score of 0 or 1 (group 0–5% WL) were either assigned to the Active group receiving the medical food or to the Control group receiving a Placebo product. Patients with ≥5% weight loss in

the past 3 months and/or a dysphagia score of 2 or 3 and/or prescribed sip feed in the last 4 weeks (group ≥5% WL) were either assigned to the Active group receiving the medical food or to the Control group receiving an iso-caloric control product.

Patients were asked to complete the quality of life questionnaires and the required study parameters, as body weight and performance status were recorded at baseline (visit 1) and after 4 weeks of nutritional intervention (visit 3). In addition, blood was drawn for the measurement of several immune-, nutritional-, and safety-parameters. Two weeks after the start of the study, patients visited the clinic (visit 2) to monitor the use of concomitant medication, body weight, and product palatability. The amount of study product taken was recorded daily in a diary by the patient. Patients with an intake of <75% of the minimum amount of 2×200 mL Active or Control product per day were considered as noncompliant.

The subjects in the reference group were prescreened, and eligible subjects visited the clinic once. Subject characteristics, relevant medical history, and anthropometrics were determined, and the required study parameters were recorded. In addition, blood was drawn for the measurement of several immune- and nutritional-parameters. Subjects in the reference group did not receive any intervention with a study product.

Nutritional intervention

All patients received dietary counselling in addition to the nutritional intervention. The prescribed product intake during the study was 2 doses (2 × 200 mL sip feed) of either the Active medical food or Control product daily for patients in group 0–5% WL and at least 2 doses for patients in group ≥5% WL. The Active medical food is an energy dense (163 kcal/100 mL), nutritionally complete oral supplement (FortiCare) that is high in protein and leucine (9.9 g protein/100 mL of which 3.2 g whey protein/100 mL, 5.6 g casein/100 mL, and 1.1 g free leucine/100 mL) and is enriched with emulsified fish oil (0.6 g EPA and 0.3 g DHA/100 mL), specific oligosaccharides (1.2 g galactooligosaccharides (GOS) and 0.2 g fructooligosaccharides (FOS)/100 mL) and a balanced mix of vitamins, minerals, and trace elements. The Control product is for group 0–5% WL, a non-caloric Placebo product and for group ≥5% WL, an energy dense (163 kcal/100 mL) iso-caloric standard nutritional product to provide patient with routine care and secure sufficient nutrient intake for a good preparation for their treatment. All products (active, iso-caloric, and placebo) were provided in white 200 mL tetra packs in three different flavors (mocha, banana, and fruitcake). (Nutricia NV, Zoetermeer, The Netherlands, for details, see *Table* S1,). The products were delivered as ready-to-drink with a straw attached to each pack for patient convenience. The study products carried identical

product labels and were packaged in such a way that the double-blind design of the study was effectively maintained throughout the study. Labels on all study products contained information required for regulatory, as well as identification purposes.

Study outcome

The primary outcome parameters of the study were the *ex vivo* Concanavalin (Con)A-stimulated T-lymphocyte proliferation and cytokine (Interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12 and Interferon (IFN)-γ) production by Blood Peripheral Mononuclear Cells (PBMC) as markers for immune function.[25] PBMC were isolated from heparin blood using density-gradient centrifugation and stored in liquid nitrogen. PBMC were thawed and stimulated with 2.5 and 10 µg/mL ConA (Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) in medium with 10% autologous serum for 44 h at 37°C and 5% CO_2. T-lymphocyte proliferation was measured by the addition of tritiated thymidine (^{3}H-TdR) 16 h prior to harvesting the cells. In addition, PBMC were stimulated with 10 ng/mL Lipopolysaccharide (LPS, *Escherichia coli*, B55:O55, Sigma-Aldrich Chemie) in medium with 10% autologous serum for 44 h at 37°C and 5% CO_2 to measure *ex vivo* B-lymphocyte proliferation as described earlier and for 20 h to measure cytokine (IL-1β, IL-6, IL-8, IL-10 and Tumor Necrosis Factor (TNF)-α) and PGE_2 production by PBMC. Serum samples were assayed for levels of inflammatory mediators (IL-1β, IL-6, IL-8, IL-10, TNF-α, PGE_2 and C-reactive protein (CRP)). Cytokine levels in culture supernatants and serum were measured using a Bio-Plex Cytokine bead immunoassay (Bio-Rad, Veenendaal, The Netherlands) according to the manufacturer's protocol, and PGE_2 was measured using a commercial enzyme immunoassay (Biotrak Amersham, Buckinghamshire, UK) according to the manufacturer's protocol.

Natural Killer (NK)-cell activity was determined using three different assays, that is, classic NK-cell activity against K562 target cells, lymphokine-activated killer (LAK) activity using Daudi cells and antibody dependent cell-mediated cytotoxicity (ADCC) against P815 target cells using a standard 4 h ^{51}Chromium release assay.[26]

During the visits, body weight and BMI were recorded, and blood was collected to determine white blood cell count and differential, the lymphocyte subset count,[27] pre-albumin and albumin; Also, safety parameters for liver function (ALAT and γ-GT), kidney function (creatinine), and prothrombin time were measured at the Clinical Chemistry Laboratory, Erasmus Medical Center, Rotterdam, The Netherlands. Moreover, the phospholipid fatty acid profile of plasma was measured (gas chromatography),[21] ECOG performance status was assessed,[28] quality of life was recorded (QLQ-C30, OES18, EuroQoL-5D), dysphagia was assessed,[29] and study product intake and palatability were scored.

Statistical analysis

The study was considered an exploratory study; the primary parameters have not been reported in newly diagnosed cancer patients before. Therefore, the expected difference between the Active and Control group and its variance was estimated. Based on two studies, it was assumed that a sample size of 40 for each of the two groups was sufficient to detect a statistically significant result between the groups.[30,31] A blinded interim analysis on primary efficacy and safety was performed after 64 patients. The results were reviewed to check whether the calculated sample size was adequate and that no safety concerns had arisen. From this interim analysis, it was concluded that in order to find differences on the primary outcome, the sample size had to be adjusted to an unrealistically high number of patients. Therefore, it was decided to stop the study and perform the final analysis on the available 64 patients. All subjects that received the study products were included in the intention-to-treat (ITT) analysis. For baseline comparisons, the differences between healthy volunteers and total patients, group 0–5% WL and group ≥5% WL and between the Active and Control group were determined. Moreover, the differences between visit 1 and 3 were compared between the Active and Control group and between group 0–5% WL and group ≥5% WL.

The results of the ConA and LPS stimulations were corrected for the unstimulated cultures by subtraction of the latter. ANOVA, with treatment and stratification for group 0–5% WL and group ≥5% WL as covariates, was used to analyse the measurement of the study parameters. When the data were not normally distributed, the Mann–Whitney *U* test adjusted for stratification (group 0–5% WL and group ≥5% WL) was used, and correlations were made using the Spearman's Rank test. NK cell activity was measured at four different E : T ratios, and the Weighted Mean of Specific Lysis was calculated.[26,32] For the ordinal variables performance status (ECOG) and dysphagia score, visits 1 and 3 were compared between the groups using the Mann–Whitney *U* test adjusted for stratification (group 0-5% WL and group ≥5% WL). All adverse events (AEs) were assessed, and medical history and medication use were checked individually for subjects having AEs. The statistical analyses were performed using SPSS for Windows Release 15.0.0.

Results

Study population and compliance

Of the 201 subjects that were screened in the study, 67 subjects were randomized and 64 subjects received the study products (*Figure* 1). Subjects that were considered a screening failure did not fulfill the inclusion criteria or received an
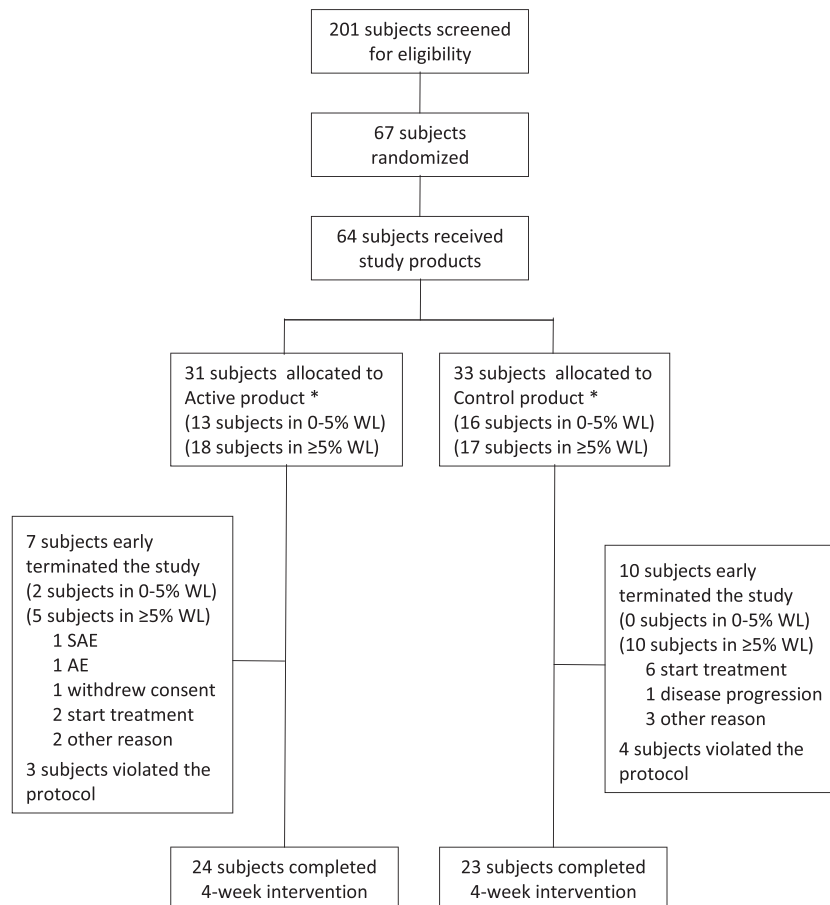
anticancer treatment within 3 weeks. Of the 64 subjects that received the study products, 31 subjects were allocated to the Active product (13 subjects in group 0–5% WL and 18 in group ≥5% WL) and 33 were allocated to the Control product (16 subjects in group 0–5% WL and 17 in group ≥5% WL), and all these subjects were included in the ITT analysis. A total of 17 subjects terminated the study early (7 subjects in the Active group and 10 subjects in the Control group), with most of the patients coming out of the group ≥5% WL. These patients terminated the study due to the start of chemotherapy, an increase of dysphagia score or other reasons. Product compliance was not significantly different between the groups with 89% for the Active product and 87% for the Control products, respectively.

Baseline characteristics

At baseline, BMI of the total patient group significantly differed from the healthy volunteer (HV) group ($P < 0.01$, *Tables* 1a and 1b). By definition, patients in group ≥5% WL

had lost significantly more weight in the past 3 months than patients in group 0–5% WL ($P \le 0.001$). Furthermore, patients in group ≥5% WL scored lower on the quality of life scales (EQ-VAS and EQ-5D) ($P \le 0.001$), had a higher tumor length ($P \le 0.02$), and a higher dysphagia score than patients in group 0–5% WL ($P \le 0.001$). Control and Active groups matched very well with regard to baseline characteristics, except for smoking history (patients in the Active group had smoked longer than patients in the Control group ($P = 0.001$)). In more than 50% of the patients, the tumor was located in the esophagus, and in the remaining patients, the tumor was located at the gastrointestinal junction. Clinical stage ranged from I to IV and was equally distributed.

Efficacy

At baseline, the primary parameter, ConA-stimulated T-lymphocyte proliferation and cytokine production in PBMC, was not significantly different between the total patient group and HV (*Table* 2), with the exception of IFN-γ

Table 1. (a) General baseline characteristics of the study groups and (b) Disease specific baseline characteristics of the study groups

Variable	Presented as	Healthy volunteers (n = 40)	Total patients (n = 64)	Active (n = 31)	Control (n = 33)
(a) General baseline characteristics of the study groups					
Sex	n (%)				
Female		8 (20.0%)	14 (21.9%)	7 (22.6%)	7 (21.2%)
Male		32 (80.0%)	50 (78.1%)	24 (77.4%)	26 (78.8%)
Age (years)	mean ± SD	63.6 ± 10.2	61.4 ± 9.2	61.1 ± 9.2	61.6 ± 9.4
BMI (kg/m^2)	mean ± SD	27.0 ± 2.1	25.4 ± 4.1[A]	25.5 ± 4.6	25.4 ± 3.6

Variable	Presented as	Patients 0–5% WL (n = 29)	Patients ≥5% WL (n = 35)	Active (n = 31)	Control (n = 33)
(b) Disease specific baseline characteristics of the study groups					
Body weight change in past 3 months (%)	mean ± SD	0.8 ± 2.7	−8.0 ± 4.5[b]	−4.2 ± 6.0	−3.8 ± 5.7
Days since diagnosis*	median (IQR)	0 (−2–19)	4 (−4–14)	0 (−1–18)	0 (−6–14)
Score on EQ-VAS (mm)	median (IQR)	80 (70–90)	60 (50–72.5)[b]	60 (50–70)	77.5 (60–87.5)
Score on EQ-5D index	median (IQR)	0.81 (0.81–1.0)	0.69 (0.37–0.81)[b]	0.81 (0.68–0.84)	0.81 (0.69–1.0)
Tumor length (cm)	median (IQR)	3.8 (2.0–5.0)	5.4 (3.0–7.8)[c]	4.0 (3.0–5.5)	4.0 (3.0–6.0)
Years smoked	mean ± SD	30.0 ± 12.1	37.8 ± 17.3[C]	40.9 ± 11.9[d]	27.9 ± 16.1
Tumor location	n (%)				
Esophagus		20 (69.0%)	18 (54.5%)	20 (64.5%)	18 (58.1%)
Gastro-esophageal junction: I, II, III		9 (31.0%): 7, 2, 2	15 (45.5%): 5, 4, 6	11 (35.5%): 6, 3, 3	13 (41.9%): 6, 3, 5
Histology	n (%)				
Adenocarcinoma		22 (75.9%)	29 (82.9%)	25 (80.6%)	26 (78.8%)
Squamous carcinoma		6 (20.7%)	5 (14.3%)	6 (19.4%)	5 (15.2%)
Other		1 (3.4%)	1 (2.9%)	0 (0%)	2 (6.1%)
TNM stage	n (%)				
I		2 (6.9%)	0 (0%)	2 (6.5%)	0 (0%)
IIA		6 (20.7%)	6 (17.1%)	4 (12.9%)	8 (24.2%)
IIB		5 (17.2%)	1 (2.9%)	3 (9.7%)	3 (9.1%)
III		4 (13.8%)	9 (25.7%)	8 (25.8%)	5 (15.2%)
IV		1 (3.4%)	2 (5.7%)	1 (3.2%)	2 (6.1%)
IVA		5 (17.2%)	5 (14.3%)	5 (16.1%)	5 (15.2%)
IVB		0 (0%)	1 (2.9%)	0 (0%)	1 (3.0%)
Unknown		6 (20.7%)	11 (31.4%)	8 (25.8%)	9 (27.3%)
Dysphagia score	n (%)				
Score 0		13 (44.8%)	4 (11.%)[e]	6 (19.4%)	11 (33.3%)
Score 1		13 (44.8%)	13 (37.1%)	14 (45.2%)	12 (36.4%)
Score 2		3 (10.3%)[†]	11 (31.4%)	7 (22.6%)	7 (21.2%)
Score 3		0 (0%)	7 (20.0%)	4 (12.9%)	3 (9.1%)

Data represent the baseline characteristics as the number of subjects (*n*) and percentages or means ± SD of the healthy volunteers group (*n* = 40), the total patient group (*n* = 64), the Active medical food group (*n* = 31) and the Control group (*n* = 33) (*Table* 1a) and the baseline disease specific characteristics as the number of subjects (*n*) and percentages, means ± SD or medians and interquartile ranges (IQR, 25th–75th percentiles) of the patients with 0–5% WL (*n* = 29), the patients with ≥5% WL (*n* = 35), the Active medical food group (*n* = 31) and the Control group (*n* = 33) (*Table* 1b). Patients with 0–5% weight loss in the past 3 months and a dysphagia score of 0 or 1 were assigned to the group 0–5% WL and patients with ≥5% weight loss in the past 3 months and/or dysphagia score of 2 or 3 and/or prescribed sip feed in the last 4 weeks were assigned to the group ≥5% WL.

BMI, body mass index; EQ-VAS, EuroQol-Visual Analogue Scale (a standard vertical 20 cm visual analogue scale for recording an individual's rating for their current health-related quality of life state in which the higher the score, the better); EQ-5D, EuroQol 5-dimension (descriptive system of health-related quality of life states consisting of five dimensions [mobility, self-care, usual activities, pain/discomfort, and anxiety/depression]), each of which can take one of three responses. The responses record three levels of severity (no problems/some or moderate problems/extreme problems) in which the higher the score, the better; TNM, tumor, node, metastasis; WL, weight loss.

[a] Significantly different from healthy volunteers group, $P < 0.01$ (Mann–Whitney),
[b] Significantly different from group 0–5% WL, $P \leq 0.001$ (Mann–Whitney),
[c] Significantly different from group 0–5% WL, $P \leq 0.02$ (Mann–Whitney),
[d] Significantly different from the Control group, $P = 0.001$ (Mann–Whitney),
[e] The distribution over the different dysphagia scores is significantly different from group 0–5% WL, $P \leq 0.001$ (Mann–Whitney), the lower the score, the better.
*Negative numbers indicate patients that were already included in the study before the official pathological diagnosis and confirmation of the disease. These subjects already had their screening visit based on macroscopic diagnosis or based on referral from other hospitals.
[†]These patients should have been included in the other group with ≥ 5% WL. In the per protocol analysis, these patients were omitted because they have not been treated according to the protocol.

Table 2. Proliferation response and cytokine and PGE2 production in ConA- and LPS-stimulated PBMC

	Baseline (visit 1)			Δ(visit 3-visit 1)	
	Healthy volunteers	Total patients	Patients 0–5% WL Patients ≥5% WL	Active	Control
ConA-stimulated PBMC					
Proliferation (cpm)	3229 (2199–8507)	4478 (2947–6369)	3684 (2398–6603) 5170 (4038–6316)	−546 (−2232–291)	−464 (−1281–235)
IL-2 (pg/ml)	164 (91.2–289)	162 (96.6–230)	164 (96.6–305) 162 (84.1–217)	−10.0 (−39.5–39.6)	−15.2 (−66.5–27.4)
IFN-γ (pg/ml)	398 (261–775)	214 (119–446)	233 (89.9–662) 198 (131–420)	−52.0 (−250–64.3)	5.5 (−157–104)
LPS-stimulated PBMC					
Proliferation (cpm)	276 (98.2–706)	182 (78.9–486)	222 (82.9–774) 149 (59.9–224)	−43.1 (−97.8–25.2)	−6.1 (−97.9–90.0)
IL-6 (pg/ml)	3084 (2185–4664)	3102 (1906–5392)	2706 (1749–3407) 3682 (2215–7402)	−141 (−1037–945)	−24.3 (−956–676)
TNF-α (pg/ml)	239 (162–380)	227 (166–509)	224 (175–447) 336 (143–728)	−65.2 (−257–9.8)	11.5 (−73.0–54.0)
PGE$_2$ (pg/ml)	4744 (2583–11808)	6245 (2903–9286)	6693 (2903–9921) 5202 (2696–8779)	−840 (−3243–656)	79.3 (−3932–4774)

Data represent medians and interquartile ranges (25th–75th percentiles) of the proliferation response and cytokine and PGE$_2$ production in ConA-stimulated (10 μg/ml) and LPS-stimulated (10 ng/ml) PBMC of the healthy volunteers group (n=40), the total patients group (n=46) and the patient groups 0–5% WL (n=26) and ≥5% WL (n=20) at baseline. For the comparisons of the Active medical food group (n=24) and the Control group (n=22), the deltas between visit 1 and visit 3 are presented.

[a]A Significantly different from healthy volunteers group, p = 0.005 (Mann-Whitney). Abbreviations: ConA, Concanavalin A; cpm, counts per minute; IL, interleukin; IFN-γ, interferon-gamma; LPS, lipopolysaccharide; TNF-a, tumor necrosis factor-alpha; PGE$_2$, prostaglandin E$_2$; WL, weight loss.

Figure 2 Body weight change (kg) in the Active medical food group (*n* = 24) and the Control group (*n* = 23) in the total patient group (A), in the Active medical food group (*n* = 11) and the Placebo group (*n* = 16) in group 0–5% WL (B) and in the Active medical food group (*n* = 13) and the Iso-caloric control group (*n* = 7) in group ≥5% WL (C) after a 4 week nutritional intervention period. Data are presented as the delta between visit 1 (baseline) and 3 in means ± SEM. * Significantly different from visit 1 (baseline), *P* < 0.05 (ANOVA).
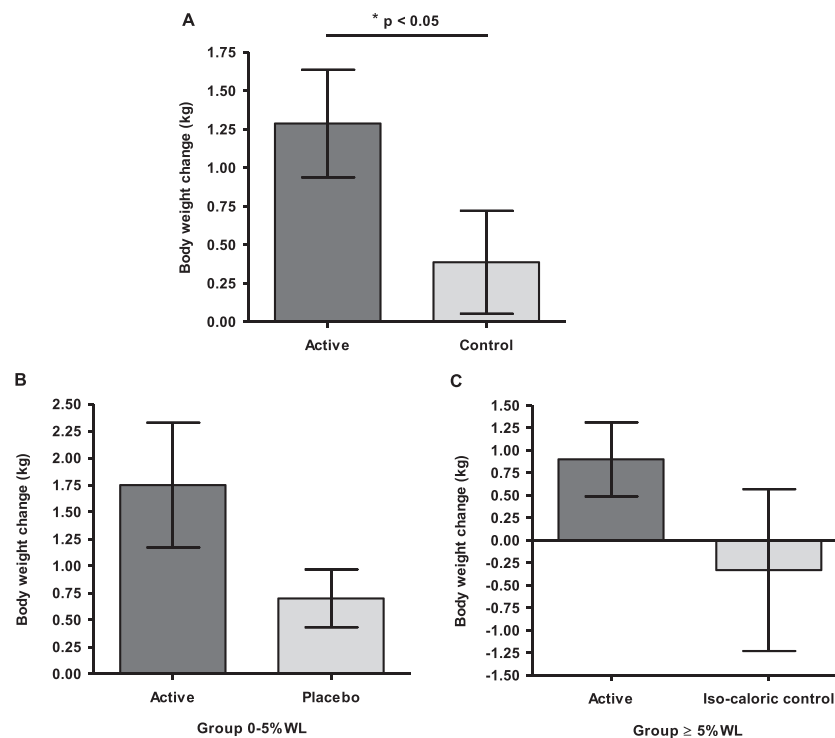
Figure 3 Change in ECOG score (% of patients) in the Active medical food group ($n = 24$) and the Control group ($n = 23$) after a 4 week nutritional intervention period. Improved means ECOG score improved with 1 score, stable means ECOG score did not change, worsened means ECOG score worsened with 1 score. Data are presented as the delta between visit 1 (baseline) and 3 as the percentage of patients. * Significantly different from visit 1 (baseline), $P < 0.05$ (Mann–Whitney U).
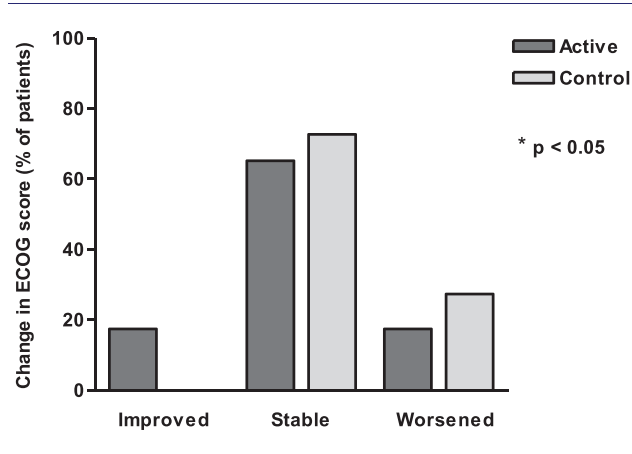
production, which was significantly lower in the total patient group ($P = 0.005$). Also, no baseline differences between group 0–5% WL and ≥5% WL were observed. Furthermore, after 4 weeks of nutritional intervention, no differences between the Active and Control groups were observed on the change from baseline (as the delta of visit 3–visit 1) regarding the primary parameter. Similarly, no differences on LPS-stimulated B-lymphocyte proliferation or on cytokine production by monocytes in PBMC were observed between the total patient group and HV at baseline or after the nutritional intervention.

As already mentioned, at baseline, the BMI of the total patient group differed significantly from the HV ($P < 0.01$), which is partly because of a trend in lower body weights in the total patient group (79.1 ± 15.6, mean ± SD) compared with HV (84.4 ± 8.9, mean ± SD, $P = 0.05$). After the 4 week nutritional intervention period, a significant higher weight gain was observed in the Active group compared with the Control group ($P < 0.05$, Figure 2a). Analysing group 0–5% WL and group ≥5% WL separately, the weight gain in Active group 0–5% WL was more pronounced compared with the total group (Figure 2b). In contrast to the Active groups, patients

Figure 4 Change in serum Prostaglandin E_2 levels (pg/ml) in the Active medical food group ($n = 24$) and the Control group ($n = 23$) in the total patient group (A), in the Active medical food group ($n = 11$) and the Placebo group ($n = 16$) in group 0–5% WL (B) and in the Active medical food group ($n = 13$) and the Iso-caloric control group ($n = 7$) in group ≥5% WL (C) after a 4 week nutritional intervention period. Data are presented as the delta between visit 1 (baseline) and 3 in means ± SEM. * Significantly different from visit 1 (baseline), $P = 0.01$ (ANOVA).
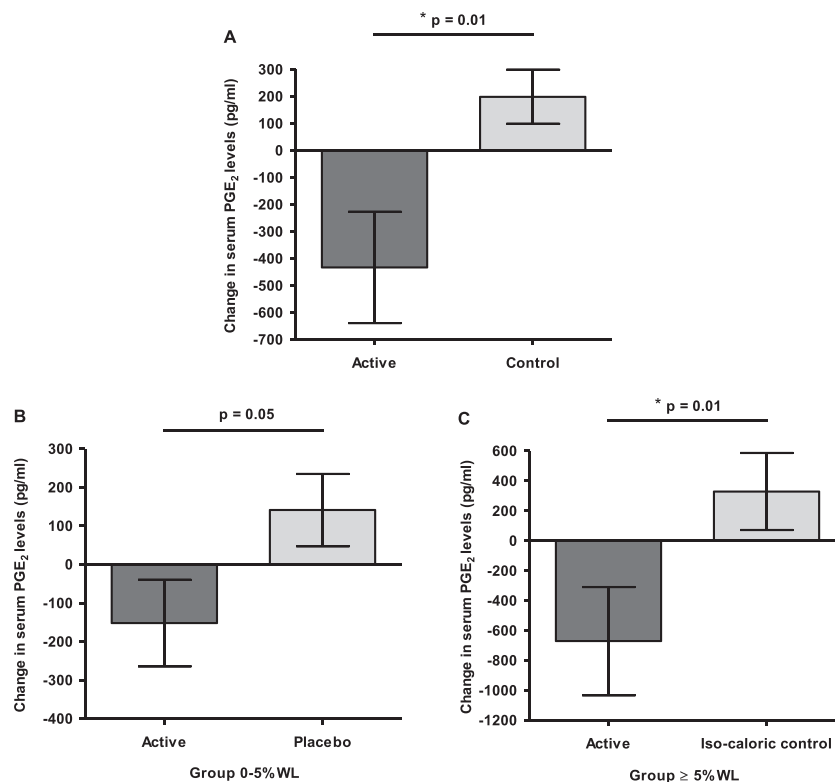
in Control group ≥5% WL appeared to lose body weight, de-spite receiving an iso-caloric control product (*Figure* 2c).

The performance status of the patients was assessed by ECOG score. At baseline, no differences were observed between the Active and Control group, whereas patients in group ≥5% WL had a significant worse performance status compared with patients in group 0–5% WL ($P < 0.01$, data not shown). After the 4 week nutritional intervention, the performance status was significantly different between the Active and Control group ($P < 0.05$). ECOG improved with a score of 1 in 17.4% of the patients in the Active group compared with 0% in the Control group, was stable in 65.2% of the patients in the Active group compared with 72.7% in the Control group, and was worsened in 17.4% of the patients in the Active group compared with 27.3% in the Control group (*Figure* 3).

At baseline, absolute levels of leucocytes were significantly higher in the total patient group compared with HV ($P \leq 0.001$), which is mainly caused by the higher number of neutrophils ($P \leq 0.001$); also, monocytes were significantly higher in the total patient group compared with HV ($P \leq 0.001$) (*Table* S2). By contrast, NK-lymphocytes were significantly lower in the total patient group compared with HV ($P = 0.002$), but nevertheless, levels of all mentioned cell types were within the normal range. Moreover, no differences in cell types were observed between the Active and Control group after the 4 week intervention period. In addition to the number of NK-lymphocytes, NK-cell activity was measured as a parameter of innate immune function (*Table* S2). After the 4 week nutritional intervention period, no differences between the Active and Control group were observed.

Serum concentrations of inflammatory cytokines were relatively low in both patients and HV, for example most levels were just above the detection limit of the assay (*Table* S3). However at baseline, serum IL-6, IL-1β and CRP levels were significantly higher in the total patient group compared with HV (all $P \leq 0.001$). Additionally, patients in group ≥5% WL had significantly higher CRP levels than patients in group 0–5% WL ($P = 0.005$). No differences were detected on the change from baseline between the Active and Control group after 4 weeks of nutritional intervention. Serum concentrations of PGE$_2$, were not different at baseline between the total patient group and the HV. However, patients in group ≥5% WL showed higher PGE$_2$ levels (953 ± 1229 pg/ml, means ± SD) compared with patients in group 0–5% WL (414 ± 474 pg/ml, means ± SD, $P = 0.05$). After the nutritional intervention, serum PGE$_2$ levels (as the delta of visit 3–visit 1) in the Active group were decreased significantly compared with increased levels observed in the Control group ($P = 0.01$, *Figure* 4a). Analysing group 0–5% WL and group ≥5% WL separately, the differences between Active and Control were more pronounced in group ≥5% WL ($P = 0.01$, *Figure* 4c) compared with group 0–5% WL ($P = 0.05$, *Figure* 4b).

To determine the uptake of fatty acids from the product, the percentage phospholipid fatty acids were measured in plasma (*Table* S4). The only differences at baseline were the lower percentage total n-3 polyunsaturated fatty acids (PUFAs) ($P = 0.006$) and EPA ($P = 0.001$) in the total patients group compared with HV. After the 4 week nutritional intervention period, a significant higher increase was observed in the Active group for total n-3 PUFAs, EPA, DPA and DHA ($P \leq 0.001$) compared with the Control group and a significant higher decrease for total n-6 PUFAs, AA and the ratio n-6/n-3 PUFAs ($P \leq 0.001$) compared with the Control group.

After the nutritional intervention, no significant differences between the Active and Control group were observed on the nutritional parameters pre-albumin and albumin, on quality of life (QoL), or on dysphagia score (data not shown).

Safety and tolerability

A total of 79 AEs were reported: 44 in the Active group (occurring in 37 patients) and 35 in the Control group (occurring in 24 patients), including two product-unrelated serious AEs, but no statistical differences between the Active and Control group were found. Most AEs were gastrointestinal-related with full feeling, nausea, and constipation most frequently observed in the Active group and diarrhea and constipation most frequently observed in the Control group. Blood safety parameter means were all within reference ranges, and no clinically relevant changes on liver and kidney function and prothrombin time were observed.

Discussion

In this double-blind, randomized, placebo-controlled study, a significant increase in body weight and an improved ECOG performance status were observed in newly-diagnosed esophageal cancer patients after a 4 week nutritional intervention with the Active medical food compared with the Control group with standardized routine care. This effect was accompanied by a significant reduction of serum PGE$_2$ levels in the patients. However, no effects of the nutritional intervention were observed on proliferation responses or cytokine production in PBMC.

Patients in group ≥5% WL had lost significantly more weight in the past 3 months than patients in group 0–5% WL, which might contribute to the induction of a (pre) cachectic state and consequently into more inflammation. In the present study, this was confirmed by higher baseline levels of PGE$_2$ in group ≥5% WL compared with group 0–5% WL. After the nutritional intervention with the Active medical food, body weight change was significantly improved in the total group when compared with routine care. Calorie intake in these patients was not controlled, but all patients received dietary counselling and dietary advice to increase food intake, and products were taken in addition to their normal diet. PGE$_2$ levels were also reduced after the nutritional intervention with the Active medical food, demonstrating most

pronounced effects in group ≥5% WL. Accordingly, a relation between the improved body weight and reduced serum PGE$_2$ levels may exist via the reduction of (pre-) cachexia and inflammation by the anti-inflammatory nutrients in the Active product, because body weight shows a significant inverse correlation with serum PGE$_2$ levels in group ≥5% WL ($P = 0.02$). However, the presence of high protein and leucine in the Active medical food may have contributed to the preservation of body weight. Leucine has been added to the Active product to provide an anabolic trigger for muscle protein synthesis, and high levels of protein were added in order to provide sufficient amounts of protein-building blocks. The acute effect of this composition on muscle protein synthesis has recently been shown in a clinical study in catabolic cancer patients with involuntary weight loss,[33] and the effects on muscle mass and function have been demonstrated in previous preclinical studies using an animal model of tumor-induced cachexia.[22] In the present study, the effects of the medical food on muscle function are reflected by a significantly improved ECOG performance status after the intervention with the Active medical food compared with the Control group. Cachectic cancer patients often suffer from a reduced activity and decreased performance status, which is related to the increase of weight loss in these patients affecting the muscle compartments as well.[5,18] Moreover, weight loss and a decreased performance status may negatively affect immune competence, leading to an increased risk of (infectious) complications.[5] In relation with the observed weight loss, McMillan described the link between weight loss, poor performance status, poor response to treatment, and poor prognosis, which is probably due to loss of skeletal muscle. Although the loss of adipose tissue accounts for the majority of the weight loss, the loss of muscle accounts for most of the morbidity and mortality.[34] PGE$_2$ might also be involved, because a rise in PGE$_2$ is suggested to be associated with muscle protein degradation in cancer cachexia.[35] Consequently, reduced PGE$_2$ levels might diminish the loss of muscle function and thereby improve ECOG performance status.

Prostaglandin E$_2$ is one of the best studied eicosanoids that contributes to the inflammatory state and immune suppression during the course of cancer.[13,36] It is involved in several human malignancies including colon, lung, breast, and head and neck cancer and is produced during the course of inflammation in response to growth factors, hormones, and inflammatory cytokines.[36–38] PGE$_2$ is produced by various types of cancer cells and their surrounding cells, leading to a range of oncogenic effects including stimulation of cell proliferation, protection against apoptosis, and induction of migration and invasion.[12,39] In addition, it can induce epithelial cells to secrete growth factors, pro-inflammatory mediators, and angiogenic factors, switching a normal microenvironment to a tumor-supporting environment.[36,37,40] PGE$_2$ contributes to the shift of the tumor microenvironment from an antitumor Th1 response to an immunosuppressive Th2 response by down-regulating Th1 cytokines (IFN-γ, TNF-α, and IL-2) and up-regulating Th2 cytokines (IL-4, IL-6, and IL-10) and has a clear role in the regulation of immune suppression.[37,13]

A reduction of PGE$_2$ might be important to reduce the inflammatory state and to improve immune responsiveness in cancer patients. Clinically, this may lead to an improved acute response to infectious triggers and may beneficially affect tumor immunity. For that reason, the reduction of serum PGE$_2$ levels in the present study, observed after the nutritional intervention of the medical food, could be beneficial for these cancer patients. Each of the product features, being fish oil, specific oligosaccharides, high protein, and leucine, might play a specific role in this process, but overlapping biological activities and synergistic interactions between them eventually lead to the overall effect.[21,22]

The cancer patients in the present study showed significant lower percentages total n-3 PUFAs ($P = 0.006$) and EPA ($P = 0.001$) in plasma compared with HV. This is previously described by Zuijdgeest et al.,[41] showing reduced n-3 PUFA levels in pancreatic cancer patients and lung cancer patients with weight loss, but not in esophageal cancer patients. This can be related to the higher rate of metabolism in these patients and to the inflammatory state leading to an increased need of PUFAs.[42] Moreover, Murphy et al. even showed a relation to the loss of skeletal muscle mass (sarcopenia) in lung cancer patients, because patients with sarcopenia had significantly lower plasma levels of n-3 PUFA and EPA than non-sarcopenic patients.[43] Providing nutritional support containing n-3 PUFA to these patients can restore these low levels and might even contribute to improved muscle metabolism.

Fish oil contains high amounts of the n-3 PUFAs, EPA, and DHA, playing a major role in the regulation of immune responses and inflammation.[44,45] After intervention with the Active medical food, both the percentages EPA and DHA, as well as the total percentage n-3 PUFAs of plasma phospholipids, were significantly increased (Table S4). This is partly because of the high compliance to the study product, inducing comparable effects as observed in a previous study in healthy volunteers.[46] The increase in n-3 PUFAs was partly at the expense of the n-6 PUFA AA, but the total percentage n-6 PUFAs was decreased significantly. Because AA can be used as a substrate for the COX-enzyme to produce PGE$_2$, a reduction in AA may explain the decrease in PGE$_2$, but other factors were involved. The specific oligosaccharides (GOS/FOS) may affect the process of PGE$_2$ production as well. These non-digestible oligosaccharides are fermentable fibers that have been associated with a reduced production of PGE$_2$ and pro-inflammatory cytokines in different parts of the gut.[47] In addition, immune modulatory effects and other health benefits as an improved gut barrier function have been described, which may be related to their prebiotic properties.[48] Besides the direct effect of fish oil and the

oligosaccharides on PGE$_2$ metabolism, these ingredients were also described to reduce the systemic inflammatory state by decreasing the production of several inflammatory mediators.[44]

In contrast to the effects on PGE$_2$, no effects were observed on other inflammatory mediators as IL-6, TNF-α, IL-8, IL-1β and CRP (*Table* S3), but levels of these markers were very low. Nevertheless, baseline levels of IL-6, IL-1β and CRP were significantly higher in the total patient group compared with healthy volunteers, even though a more severe inflammatory state of these patients was expected before the start of the study. In the patient group, baseline CRP levels were significantly higher in group ≥5% WL, compared with group 0–5% WL, indicating a more severe inflammatory state of the patients in group ≥5% WL.

In addition, no effects of the nutritional intervention were observed on proliferation responses or cytokine production in PBMC. This could partly be explained by the fact that the total patient group did not differ from the healthy volunteers in respect to their immune status at baseline. In contrast to these findings, esophageal cancer patients are frequently described to be at high risk for malnutrition and reduced immune responsiveness, especially around major surgery.[49,50] However, these studies were primarily performed in Japan, where the incidence of squamous cell carcinoma of the esophagus is higher compared with adenocarcinoma,[51] whereas in the present Dutch study, adenocarcinoma of the esophagus is mostly observed.

It appears that the patients included in this study had a better immune function than expected, possibly due to the type of cancer or the early phase of patient inclusion, just after diagnosis. Goto demonstrated a reduced production of IL-2, IFN-γ and IL-10 in PHA-stimulated PBMC and a reduction of IL-12 and TNF-α in LPS-stimulated PBMC of cancer patients compared with healthy controls.[52] However, these patients suffered from different types of advanced cancer, which were irresectable with multiple metastases. Other explanations for the lack of effects on these immunological parameters were the high number of differences between Active and Control already at baseline and the higher drop-out of patients in group ≥5% WL in the Control group, leading to a skewed distribution of patients with milder and more advanced disease between Active and Control groups, possibly obscuring treatment effects. The high variance on the various immune parameters in combination with a relatively small group size was an important discussion point as well. In addition to that, another limitation of the study was the absence of a calorie measurement tool that could have made the conclusion on body weight ascribed to the specific nutritional intervention more specific.

In conclusion, the present exploratory study demonstrates a significant increase in body weight and an improved ECOG performance status in newly diagnosed esophageal cancer patients after a 4 week nutritional intervention with medical food, which is high in protein and leucine and enriched with emulsified fish oil (containing EPA and DHA) and a specific oligosaccharide mixture compared with routine care (control group with an iso-caloric or placebo product). This effect is accompanied by a significant reduction of serum PGE$_2$ levels of the patients and an efficient incorporation of n-3 PUFAs. Moreover, the medical food is well-appreciated with a high compliance rate of study product intake. No clinically relevant safety concerns were reported, and no changes in blood safety parameters were measured. Consequently, these results show that nutritional intervention with the specific medical food may represent a new opportunity for applications in cancer patients being an integral part of disease management to provide optimal treatment support. However, additional research is recommended to elucidate the potential immunological effects in different types and stages of cancer.

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Supporting information

Supporting information may be found in the online version of the article.
Table S1. Nutritional composition of the Active medical food (FortiCare), Placebo product and Iso-caloric control product in g/100 ml.
Table S2. White blood cell counts, differential and NK cell activity.
Table S3. Serum levels of pro-inflammatory cytokines and CRP.
Table S4. Percentages phospholipid fatty acids in plasma.

Conflict of interest

As indicated in the affiliations, J. Faber, A.P. Vos, M. Berkhout and A. van Helvoort are employed within Nutricia Research. This aside, no conflicts of interest are present. M.J. Uitdehaag, M.C.W. Spaander, S.C.L. van Steenbergen, C.H.J. Lamers, H.C. Rümpke, H.W. Tilanus, P.D. Siersema and A. van der Gaast declare that they have no conflict of interest, neither financial or personal relationship, with a third party whose interests could be positively or negatively influenced by this article's content. All authors comply with the Ethical guidelines for authorship and publishing in the Journal of

Cachexia, Sarcopenia and Muscle. (von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle. J Cachexia Sarcopenia Muscle, 2010; 1:7–8).

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