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ORIGINAL ARTICLE

Marine phospholipids—a promising new dietary approach to tumor-associated weight loss

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Abstract
Goals of work Advanced tumor disease very often evokes excessive loss of body weight. Among others, fish oil or marine fatty acid ethyl esters were investigated for treatment of cancer cachexia with controversial results. In this study, a new formulation of marine fatty acids was investigated, the marine phospholipids, with more than 50% of phospholipid-bound fatty acids being eicosapentaenoic and docosahexaenoic acid.
Materials and methods Thirty-one tumor patients with various tumor entities suffering from weight loss were asked to take marine phospholipids (1.5 g/day) as softgel capsules for a period of 6 weeks. Compliance, body weight, appetite, and quality of life as well as the fatty acid profile in plasma and blood cells were monitored; 17 patients could be analyzed.
Main results Marine phospholipids were very well accepted; low-dose supplementation resulted in a significant increase of eicosapentaenoic and docosahexaenoic acid in plasma phospholipids; therefore, significantly reducing the $n-6$ to $n-3$ fatty acid ratio. A stabilization of body weight was achieved (median weight change of +0.6% after 6 weeks), while appetite and quality of life improved.
Conclusions These promising first results encourage further investigation of marine phospholipids in cancer care.

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Introduction

The excessive loss of body weight, mostly combined with loss of appetite, is a problem that often occurs with advanced metastatic cancer disease. More than 80% of patients with advanced cancer suffer from the cachexia syndrome [1] which not only impairs quality of life [2] but also increases therapeutic complications [3] and implies a poor prognosis [4]. Although many underlying causes of this syndrome have already been revealed and many approaches [5] have been investigated accordingly, its treatment still remains unsatisfying in a high percentage of cases.

Inflammatory processes have been identified as being partly responsible for weight loss/anorexia [6]. Proinflammatory cytokines like tumor necrosis factor alpha (TNFα, formerly known as cachectin), interleukin 1 and 6 (IL-1/-6), and interferon gamma (INFγ) have been suggested to mediate cancer cachexia [7–11]. The initiation of synthesis and up-regulation of proinflammatory messengers are much dependent on the synthesis of eicosanoids (prostaglandins, leucotrienes, and thromboxanes). The "class 2&4" eicosanoids (for example, PGE_2 and LTB_4), which have proinflammatory effects, all derive from arachidonic acid (AA), an omega-6-polyunsaturated fatty acid (20:4$n-6$) [12]. Upon stimulatory signal, AA is cleaved from the PL in the cellular membrane and is used for eicosanoid synthesis. AA bound in the cellular membrane therefore plays a key role in inflammatory and malignant processes and is an interesting target for therapeutic intervention.

Membrane-forming phospholipids in tumor cells are constantly broken down by PLA_2 at a much higher rate

than in normal cells, rendering a high supply of free AA and, furthermore, the potentially membrane lytic lyso-PC as a breakdown product. As a consequence, the re-synthesis of membrane phospholipids must also be increased in tumor cells to keep up cellular membrane homeostasis. This is in accordance with the results of Baburina et al. [13] who observed an increased membrane turnover in tumor cells.

As AA plays a key role in cancer and associated weight loss, the potential of $n-3$ polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) of marine origin, as a counteracting agent seems promising. EPA is processed in a similar manner as AA without rendering highly active proinflammatory second messengers. Therefore, EPA may act as a competitive substrate of AA at enzymatic binding sites. Anti-inflammatory actions of $n-3$ PUFAs, especially EPA, meanwhile have been established [14]; the pathways appear numerous. Down-regulation of IL-1 and -6 as well as TNFα have been shown by Endres et al. [15] and Wigmore et al. [16]. Ramos et al. [17] showed EPA to prevent cachexia in tumor-bearing rats. Several trials have been conducted with either fish oil or marine fatty acid ethyl esters in order to assess the potentially positive effect of EPA on cancer progression and weight loss, with controversial results. While smaller trials resulted in significant weight gain in 20 cachectic pancreatic cancer patients after 3 to 7 weeks of fish oil-enriched dietary supplementation as shown by Barber et al. [18] and improved survival rate in 60 tumor patients after dietary supplementation of fish oil as shown by Gogos et al. [19], the outcomes of major comparative studies do not clearly support these positive results. Bruera et al. [20] investigated fish oil versus placebo in 60 patients with advanced tumor disease and weight loss and found no significant effect on weight loss or appetite. It must be taken into consideration that appetite was the primary endpoint, not weight loss, and duration of the trial with a 2-week intervention period was very short. Fearon et al. [21] randomized 200 cachectic patients with pancreatic cancer to pure protein and energy dense oral supplement versus the supplement enriched with $n-3$ fatty acids over a period of 8 weeks and found both supplements equally effective at arresting weight loss. Still, the authors conclude that post hoc dose–response analysis suggests only the $n-3$ fatty acid-enriched supplement results in gain of weight and improved quality of life if taken in sufficient quantities. Another study by Fearon et al. [22] resulted in no significant benefit of EPA diethyl ester versus placebo in 518 weight-losing patients with advanced tumor disease. But, a significant improvement of physical function was nonetheless observed after 8 weeks of EPA supplementation. Furthermore, high dosage of fish oil supplementation causes marked compliance problems, especially in anorectic/cachectic cancer patients. As evaluated by Burns et al. in their phase I and II clinical trials with marine fatty acid ethyl ester softgel formulation, relatively high dosage (7.5 g/day EPA plus DHA) was needed to positively affect tumor-associated weight loss, causing marked compliance problems like gastrointestinal side effects and belching with fishy taste or smell [23, 24]. Even lower dose of 1.2 g/day EPA plus 1.2 g/day DHA, supplied as fish oil capsules in the study by Bruera et al. [20] caused significant compliance problems. As critically reviewed by Jatoi [25], the devastating nature of the cancer anorexia/weight loss syndrome and the still persisting lack of its successful clinical management merits further studies and novel approaches to $n-3$ fatty acid supplementation in cancer cachexia.

In this study, a new formulation of $n-3$ fatty acids was investigated, the "marine phospholipids" (MPL). MPL are extracted from salmon roe and contain 30% phospholipids. More than 50% of all fatty acids contained are EPA and docosahexaenoic acid (DHA). The high percentage of phospholipids on one hand should reduce side effects like fishy taste or smell or indigestion because of the natural detergent effect of phospholipid molecules. Furthermore, the uptake and metabolism of the $n-3$ fatty acids bound to phospholipids differs from those bound to triacylglycerol. Triacylglycerols are degraded in the gastrointestinal tract and transported to the liver as chylomikrones, passing most of the fatty acids contained to heart or muscle tissue for energy provision or adipose tissue, while phospholipids are mostly degraded to lyso-PL [26] by cleavage of the fatty acid at the s$n-1$ position of the glycerol backbone by pancreatic lipase or at the s$n-2$ position by PLA_2 and partly reassembled to phospholipids after passage of the enteromucosa. Orally ingested phospholipids and their fatty acids are incorporated preferentially into the high-density lipoprotein (HDL) phospholipid fraction [27, 28]. It appears probable that lyso-PC found in the bloodstream not only results from the lecithin-cholesterin-acyltranferase (LCAT) activity (cleaving at the s$n-2$ position) in HDL particles and the action of the endothelial lipase cleaving phospholipids at the s$n-1$ position [29], but also from lyso-PC passing directly from the gastrointestinal tract through the epithelium into the circulation and binding to albumin. From the blood, cells could access lyso-PC directly for re-acylation to form membrane phospholipids. Incorporation of fatty acids taken up bound to phospholipids into plasma phospholipids [30] and tissues is remarkably higher than fatty acids bound to triacylglycerols [31, 32]. Therefore, this formulation was expected to be more effective in a relatively low dose than fish oil or ethyl ester formulations regarding weight loss in tumor patients. Participating patients were asked to take 1.5 g MPL (three softgel

capsules) daily for 6 weeks; body weight, appetite, pain, and quality of life as well as blood parameters were monitored.

Materials and methods

Subjects

Thirty-one patients (16 women, 15 men) were included in the investigation. All participants gave informed written consent. The study protocol was approved by the Ethics Committee of the University of Freiburg, Germany. Patients were recruited as in-patients from the Department of Medical Oncology at the Tumor Biology Center Freiburg between June 2007 and October 2008. Patients all suffered from various tumors (see Table 1) in metastatic stage and weight loss (≥5% of body weight since first diagnosis; CTC grade I), currently without radio- or chemotherapeutic intervention.

Inclusion criteria: age>18 years, diagnosis of solid tumor, written informed consent, and tumor-associated weight loss (CTC Grade I).

Exclusion criteria: allergy against food from marine sources, current radio- and/or chemotherapy for at least 4 weeks previous to dietary intervention.

Mean age of the patients was 59 years, mean body mass index was 20 kg/m^2, and mean weight loss since first diagnosis (% BW) was −26% (see Table 1).

Marine phospholipid formulation

MPL preparation was obtained from Bio-Sea (Tromsø, Norway). Lipid composition was 67.72 g/100 g neutral lipids and 32.75 g/100 g polar lipids with 87.5% phosphatidylcholine. Fatty acid profile was 18.8 g/100 g eicosapentaenoic acid (EPA) and 22.8 g/100 g docosahexaenoic acid (DHA) bound in neutral lipids and 16.5 g/100 g EPA and 33.7 g/100 g DHA bound in phospholipids. The MPL preparation was encapsulated into softgel capsules, 500 mg per capsule, by pharmagel (Italy).

Treatment protocol

Participating patients were asked to take marine phospholipids as softgel capsule preparation of 500 mg three times a day with their meals for a period of 6 weeks. A patient diary was handed out, capsule intake, weight, appetite, and pain scores were to be documented daily. Patients were examined at three time points: before intervention, after 3 and after 6 weeks. Examination included blood sampling for routine analysis and determination of lyso-PC and fatty acid profiles in plasma phospholipids as well as red blood cells (RBC) and mononuclear lymphocytes (MNL), physical exam, bioelectrical impedance analysis (BIA), and completion of the EORTC QLQ-C30 (version 3.0) quality of life questionnaire.

Examination of nutritional status (BIA)

Participants were weighed and measured, and BMI was calculated as (weight in kg)/(height in m)^2.

Bioelectrical impedance analysis (BIA) estimates body water from whole body impedance against an alternating high-frequency current. For the test, a low voltage is applied to wrist and ankle via self-adhering electrodes (Fresenius Kabi AG, Medical Devices, Bad Homburg, Germany) and an alternating current produced of about

Table 1 Baseline characteristics of patients included in MPL investigation

Baseline characteristics	All patients (n=31)	Analyzed patients (n=17)
Gender (no. of patients)		
Female	16	10
Male	15	7
Age (years; mean ± SD)	59.2±13.4	62.2±8.9
Tumor entity (no. of patients)		
Urogenital[a]	3	3
Gynaecological[b]	8	4
Gastrointestinal[c]	12	5
Other[d]	8	5
BMI (kg/m^2; mean ± SD)	20.1±3.6	20.2±3.7
Body weight (kg; mean ± SD)	58.2±12.1	58.9±12.7
Weight loss since first diagnosis (% BW; mean ± SD)	−26.3±15.5	−26.2±16.1
Appetite (median; EORTC QLQ-C30v3.0)	0	16.7
Global health (median; EORTC QLQ-C30v3.0)	33.3	25

[a] Kidney, ureter, prostate

[b] Breast, ovaries, cervix

[c] Colon, rectum, pancreas, stomach, esophagus, liver, appendix

[d] Lung, thymus, astrocytoma, endocrine gland (not defined), peritoneal mesotheliom, desmoplastic tumor of the abdomen

0.8 mA [11]. The analysis was performed using a calibrated impedance spectrum analyzer (Hydra 4200, Xitron Technologies, San Diego, USA), an alternating current at multiple frequencies from 5 to 1,000 kHz and nonlinear regression analysis using the Cole–Cole method. Further parameters obtained are lean body mass (LBM), total body water (TBW), as well as intracellular (ICF) and extracellular water (ECF) [33].

Blood sampling and routine analysis

Samples for lyso-phosphatidylcholine and fatty acid profile analyses were collected as EDTA blood (3× S-Monovette® 9 ml with 1.6 mg EDTA/ml blood, Sarstedt, Nümbrecht, Germany) and centrifuged for 10 min at 2,000 rpm (805×*g*) at room temperature; the resulting plasma was stored in aliquots of 500 µl at −80°C until analysis.

All routine blood parameters (CRP, albumin, leukocytes, thrombocytes, GOT, GPT, CHE, triglycerides, total cholesterol, VLDL, LDL, HDL) were determined from serum in the clinical chemistry routine laboratory according to standard procedures.

Lyso-phosphatidylcholine analysis

Lyso-phosphatidylcholine (Lyso-PC) analysis was performed by high-performance thin layer chromatography (HPTLC) after lipid extraction (modified Bligh and Dyer [34]) from blood plasma according to our description published in 2007 [35].

Seventy-five microliters of the plasma sample and 500 µl of five calibration standards, solutions of lyso-phosphatidylcholine (Sigma, Steinheim, Germany) in 0.9% NaCl aqueous solution, ranging from 80 to 400 µM in concentration were extracted with $CHCl_3$/MeOH (2:1 *v*/*v*). Dry extracts were dissolved in 75 µl, calibration standards in 500 µl of Hexan/Isopropanol/H_2O (40/50/8 *v*/*v*/*v*), 5 µl of each calibration standard and of each sample were applied to a preconditioned HPTLC plate (Merck, Darmstadt, Germany) with the Camag Automatic Sampler TLC III. After development, plates were dried and stained with a copper sulfate/phosphoric acid solution (14.7% *w*/*v*; 10% *v*/*v*). Quantification was performed with a Camag TLC-Scanner II equipped with a tungsten bulb at 530 nm.

Analysis of cytokines

The cytokines interleukin 1 (IL-1) and interleukin 6 (IL-6) were determined by Quantikine® Human IL-1α/IL-1F1 and Quantikine® Human IL-6 ELISA assays, TNFα by Quantikine® HS Human TNF-α/TNFSF1A high sensitivity ELISA assay in EDTA plasma according to the manufacturer's instructions (R&D Systems, Wiesbaden, Germany).

Fatty acid profiles in plasma phospholipids, RBC, and MNL

Fatty acid analysis of plasma phospholipids as well as RBC and MNL cell pellet lysates was performed by GC in combination with liquid extraction and SPE (LiChrolut® glass columns from Merck, Darmstadt, Germany and Chromabond aminopropyl silicagel from Macherey&Nagel, Düren, Germany) according to the method established and validated in our group [36].

Lipid extraction was performed according to the modified method of Bligh and Dyer [37]. Five hundred microliters of the plasma sample and whole MNL and RBC cell pellets resulting from gradient centrifugation (performed with Ficoll-Paque™ Plus, GE Healthcare, München, Germany) were extracted. Prior to first extraction, a quality standard (heptadecanoyl-lyso-PC (Avanti Polar Lipids, Alabaster, USA), 1.3 ml of a methanolic solution 0.25 mg/ml) was added to each plasma sample. The resulting dry lipid extracts were either kept in a refrigerator at +4°C until direct methylation of the whole lipid extract (MNL and RBC) or subjected to SPE (plasma samples).

SPE was performed by shaking the dry samples with acetone and passing the solution over aminopropyl columns, followed by elution with chloroform/methanol/water (5:5:1 vol/vol/vol). The resulting phospholipid fraction was taken to dryness at 40°C under a stream of nitrogen.

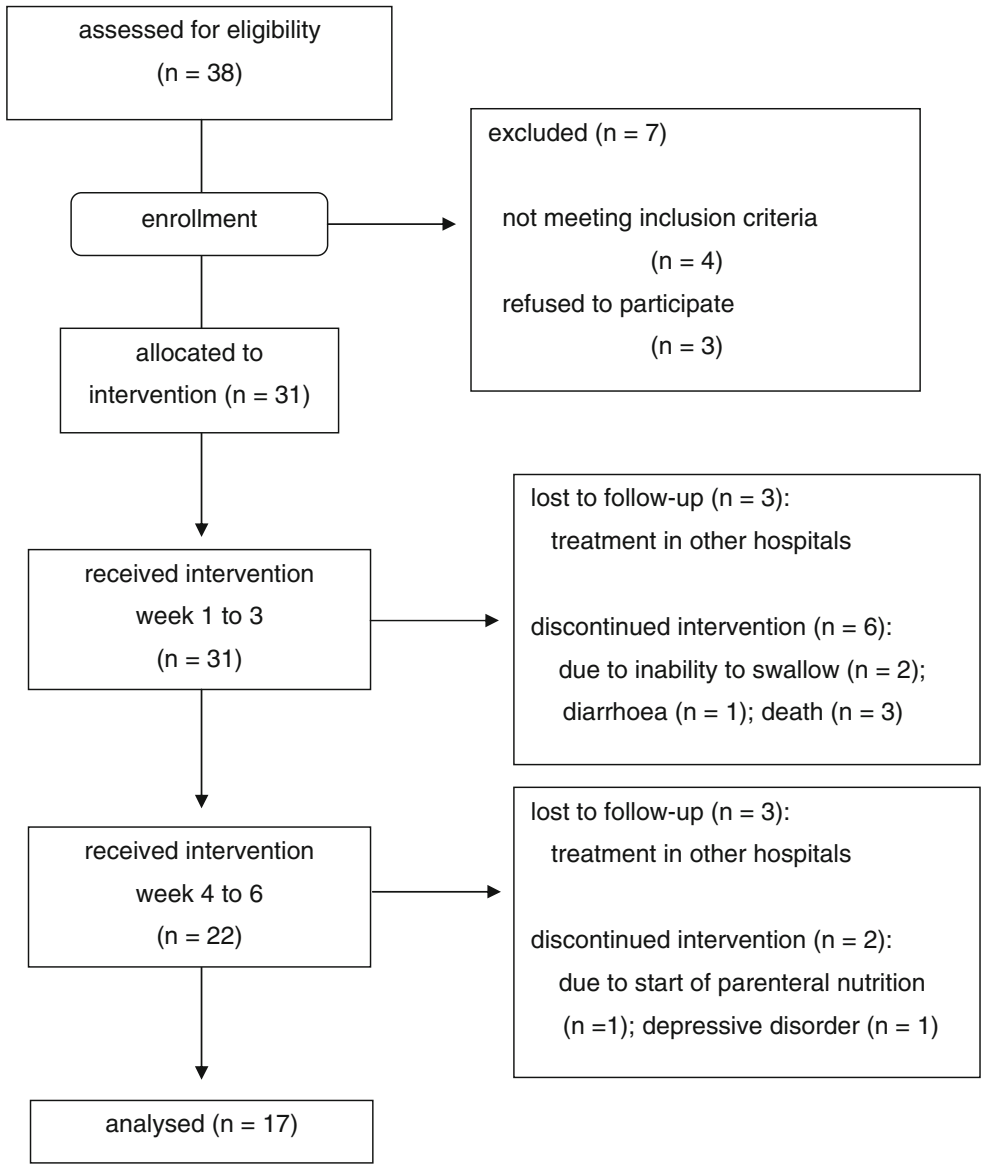
GC analysis required derivatization; all fractions were methylated with TMSH (Macherey&Nagel, Düren, Germany) before analysis of 5 µl with a HP 5890 Series II Plus, equipped with an Agilent Technologies (Böblingen, Germany) DB-23 column (30 m, 0.25 mm ID, 0.25 µm) with helium at 1 ml/min, oven temperature programming starting with 150°C for 3 min, up to 220°C with a rate of 5°C/min, 220°C for 3.5 min, split injection (1:100), injector temperature 260°C and FID at 280°C.

Statistics

The statistical analysis (*t* tests, Mann–Whitney and correlation as well as quartile analyses) was performed with SigmaStat 3.1 (Systat Software, USA, 2004) and "XLStatistics—Excel Workbooks for Statistical Data Analysis" (© Rodney Carr 1997–2008).

Results

Phosfood dietary intervention—flow chart



Compliance of MPL formulation

The compliance of the MPL formulation was very satisfactory, the preparation was highly accepted, very few side effects were recorded. One patient disliked the smell, one patient suffering from chronic diarrhea perceived this condition as worsening during MPL intake; only in these cases, noncompliance of MPL was the reason for dropout. On average, 94%±2% of prescribed MPL capsules were taken.

Tumor progression during MPL intake

Nine out of 17 patients had no measurable tumor progression (imaging performed) during MPL intake; only one patient suffered progression who had been classified as

stable disease in the period of time before intervention. All other patients experiencing tumor progression during MPL intake were experiencing progressive disease in the period of time before start of the dietary intervention. Therefore, no measurable effect of MPL on tumor growth or disease progression was seen in this investigation.

Laboratory parameters

The results of routine blood analysis show no changes in all routine blood parameters documented. Median CRP was 1.7 mg/dl at time point zero and 1.6 mg/dl after 6 weeks.

The results of the additional laboratory analyses (lipid electrophoresis, lyso-PC determination by HPTLC and cytokine (IL-1, IL-6, and TNFα) determination by ELISA assays) are listed in Table 2.

The results from lipid electrophoresis revealed no changes in average triglyceride and total cholesterol levels; VLDL and LDL levels decreased but did not change significantly. Only the high-density lipoprotein (HDL) fraction significantly increased 30% on average after 6 weeks of MPL intake (p=0.029).

Average lyso-PC levels were low (187±103 μM) and did not increase after 6 weeks MPL intake.

IL-1 was not detectable in any of the patient samples (lowest calibrator 3.9 pg/ml). Average IL-6 plasma levels increased after 6 weeks MPL as well as average TNFα plasma levels.

Bioelectrical impedance analysis

The results from the bioelectrical impedance analysis (BIA) showed no changes in all determined parameters (see Table 3).

Table 2 Laboratory results in plasma of analyzed patients (n=17) at weeks 0 and 6 (mean ± SD)

Parameter	Week 0	Week 6
Triglycerides (mg/dl)	139±72	136±68
Total cholesterol (mg/dl)	177±59	170±42
VLDL (mg/dl)	13±9	10±6
LDL (mg/dl)	133±58	118±45
HDL (mg/dl) *	43±13	56±18
Lyso-PC (μmol)	187±103	182±131
CRP (mg/dl)	1.7 (median)	1.6 (median)
IL-1 (pg/ml)	n.d. (< 3.9)	n.d. (< 3.9)
IL-6 (pg/ml)	27.9±37.2	38.9±53.0
TNFα (pg/ml)	2.3±3.1	2.9±4.1

*p=0.029

Table 3 Bioelectrical impedance analysis (BIA) results of analyzed patients (n=17) at weeks 0 and 6 (mean ± SD)

Determined parameter	Week 0	Week 6
BMI (kg/m^2)	20.2±3.7	20.2±3.4
Phase angle (at 50 kHz)	4.9±0.9	4.7±1.1
Lean body mass (kg)	39.0±8.6	39.6±7.7
Fat mass (kg)	21.2±7.2	20.1±7.0
Intracellular water (l)	15.2±3.3	14.4±3.0
Extracellular water (l)	15.7±3.4	16.3±4.4

Influence of MPL intake on fatty acid profiles of blood cells and plasma

The intake of MPL capsules over a period of 6 weeks induced a significant change in the fatty acid profiles of blood cells and plasma PL regarding the PUFA (AA, EPA, DHA) content. While the percentage of AA decreased only slightly in plasma PL and MNL, a significant decrease (p=0.037) could be observed in the RBC. The increase of the $n-3$ fatty acids EPA and DHA in plasma PL and MNL is impressively high, while the increase is less in RBC. Average EPA percentage of total FA increased significantly in plasma PL (p=0.002) and MNL (p=0.044), only slightly in RBC. Average DHA percentage increased significantly in plasma PL (p= 0.0007), but only slightly in MNL and decreased slightly in RBC (see Fig. 1a). The calculation of the changes of these PUFAs relative to the first measurement show that EPA increases between 27% and 70 % (median) of its value at week 0 in all three blood compartments, the greatest relative increase is found in plasma PL. Median relative DHA change is between −8% and 35%; AA decreases slightly by −2% to −17% (median; see Fig. 1b).

The great increases of the $n-3$ FA also induce a significant decrease of the $n-6/n-3$ ratio in plasma PL (p=0.0002) and MNL (p=0.002), but not in the RBC (see Fig. 1c).

Influence of MPL intake on body weight

Body weight was measured every day by the patients and documented in the patient diary. Baseline weight was the median body weight in the first week of MPL intake and was taken as 100% value. The BW median was calculated for each following week, and the relative changes of BW to baseline value were calculated. The individual relative weight changes for all 17 patients analyzed are depicted as interrupted lines in Fig. 2. The continuous line in the figure represents the median relative weight change of all patients. Compared to baseline, median BW is stable throughout the 6 weeks of MPL intake (+0.6% BW).

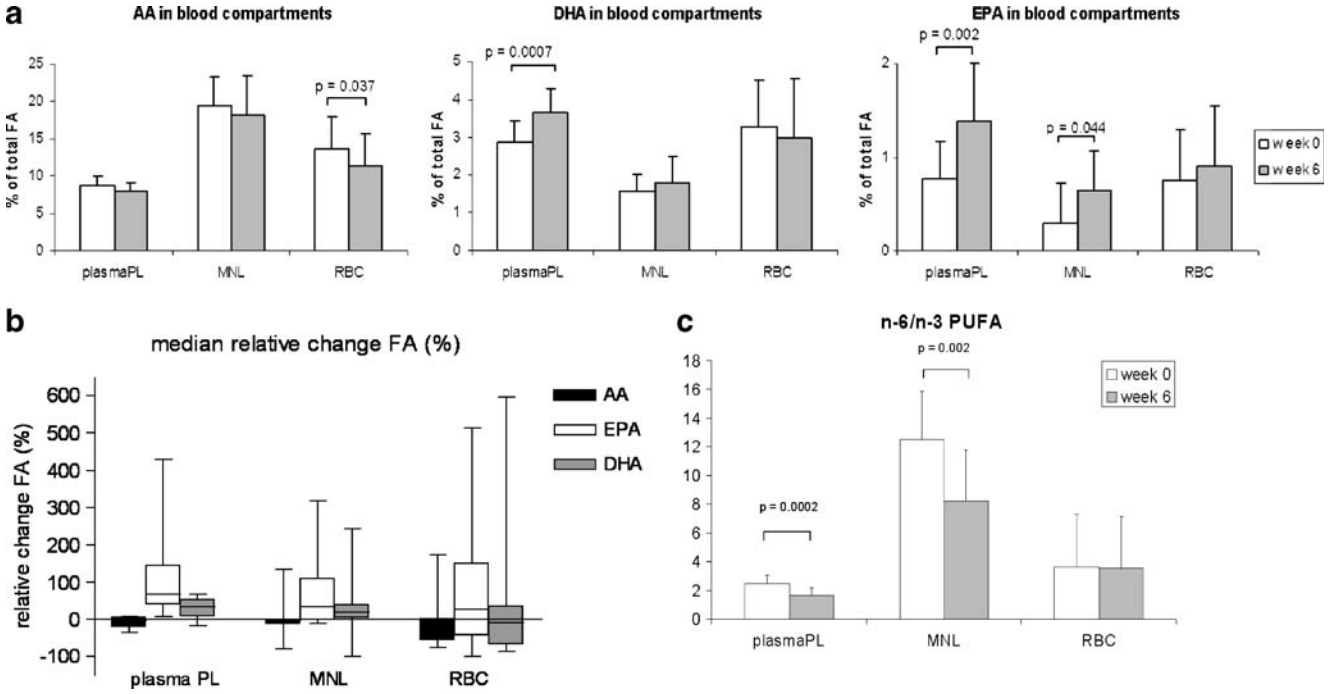


Fig. 1 **a** Changes of AA, DHA, and EPA in plasmaPL, MNL, and RBC in % of total FA, compared at weeks 0 and 6. **b** Median relative changes of AA, DHA, and EPA in plasmaPL, MNL, and RBC in %, after 6 weeks relative to baseline (=100%) at week 0. **c** Changes of $n-6/n-3$ FA ratios in plasmaPL, MNL, and RBC compared at weeks 0 and 6, $n-6$=AA, $n-3$=EPA+DHA

Patients were asked their BW at the time point 6 weeks before start of the dietary intervention. These single values were related as percent to the baseline BW. Median weight loss in the group of analyzed patients during the 6 weeks before MPL intake was −2.0% BW (see Fig. 3). Median change of BW before MPL intake is clearly of negative value, while the median change during MPL intake is slightly positive. The difference would be significant (p= 0.037), but it has to be mentioned that a mathematical comparison is problematic here, as the BW values 6 weeks before intervention were only single values which were furthermore not documented in the same fashion as the BW values during MPL intake. Five out of the 17 patients (29%) already experienced some weight gain during the 6 weeks previous to MPL intake while 10 out of 17 (59%) gained weight during MPL intervention.

Correlation between body weight change and fatty acids

The observed effects of MPL intake on the fatty acid profiles in the plasma PL and the patients' BW correlate. The higher the percentage of EPA in total plasma PL FA

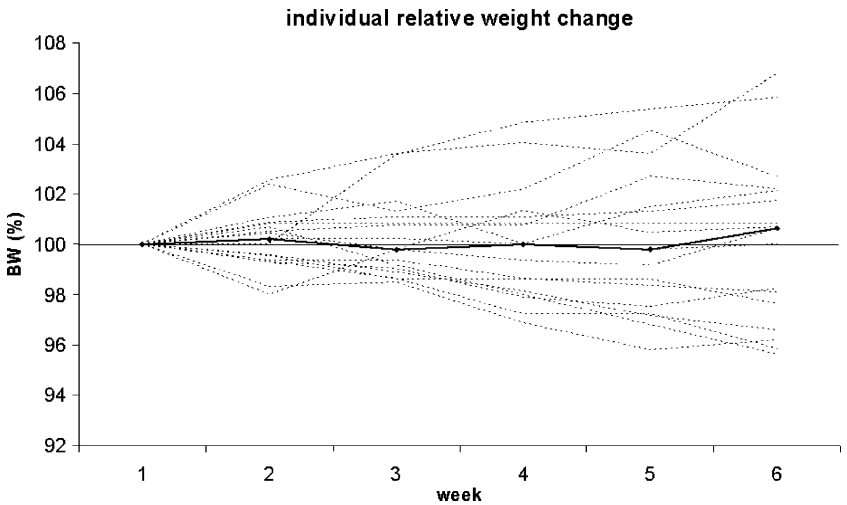


Fig. 2 Individual relative body weight changes of 17 analyzed patients baseline (=100%) is median BW in week 1, the median relative BW change is calculated for each following week to week 6, the *continuous line* represents the median relative BW change of all analyzed patients (n=17)

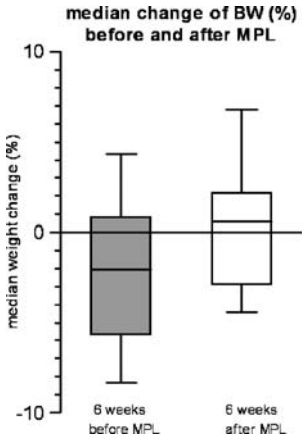


Fig. 3 Median relative body weight changes of 17 analyzed patients in the period of 6 weeks before intake of MPL and after 6 weeks of MPL intake, relative to baseline (=100 %) median BW in week 1

after 6 weeks, the more positive is the median BW change (r=0.64; p=0.006; see Fig. 4). A similar relationship can be seen between percentage of EPA in total RBC FA and median BW change (r=0.64; p=0.005), but not in MNL or with DHA percentages.

The comparison of the upper and lower quartiles regarding the change of the $n-6/n-3$ ratio in plasma phospholipids showed a lower BMI and lower fat mass in the quartile of patients responding with the greatest decrease regarding the $n-6/n-3$ ratio (upper quartile = UQ). The four patients with the least change of the $n-6/n-3$ ratio in plasma phospholipids (lower quartile = LQ) displayed mean BMI and FM in the normal range (see Fig. 5).

Quality of life (EORTC QLQ questionnaire)

The EORTC QLQ-C30 (version 3.0) questionnaire [37] was filled out by patients at weeks 0, 3, and 6. Scores were calculated according to the scoring manual delivered by the EORTC (European Organisation for Research and Treatment of Cancer). Comparison of individual scores before and after MPL intake resulted in positive changes. Regarding the relevant scores in the context of this dietary intervention (functional scores: physical, social, role, and

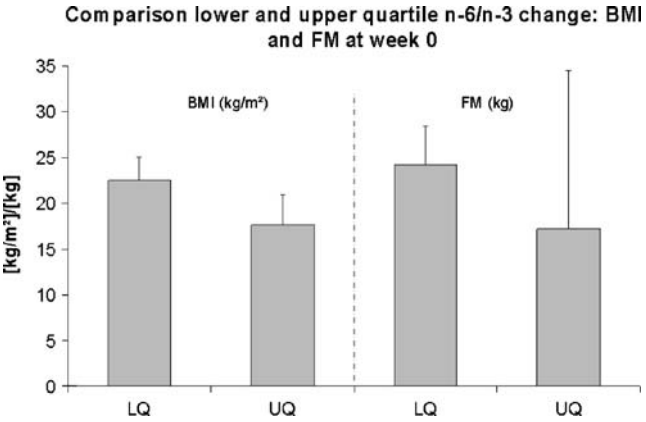


Fig. 5 Comparison of upper and lower quartile of $n-6/n-3$ FA ratio change of 17 analyzed patients; BMI (kg/m^2) and FM (kg) are compared in the LQ and UQ at week 0

symptom scores: pain and appetite as well as global health), all median scores increased after 6 weeks, save for the median pain score which decreased (see Fig. 6). According to the Subjective Significance Questionnaire (SSQ) developed by Osoba et al. [38], changes in the QLQ-C30 scores of 5 to 10 were perceived by patients as "a little," changes of 10 to 20 were perceived as "moderate," and changes greater than 20 were perceived as "very much." Translating the changes of median scores in our investigation, improvement of functional scores was moderate (social score) to very much (physical and role score), improvement of appetite was moderate, while pain reduction and improvement of global health was perceived as very much.

Discussion

The underlying cause of cancer cachexia is an inflammatory process, pro-inflammatory cytokines as IL-1, IL-6, and TNFα are recognized as mainly involved [7–11]. These cytokines can induce phospholipase A_2 (PLA_2) [10, 39–41]

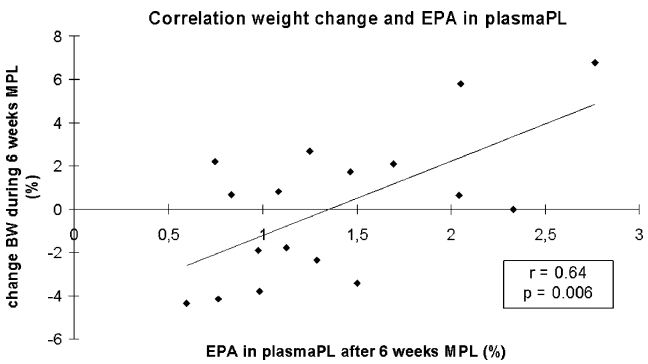


Fig. 4 Correlation between median relative body weight changes and EPA content (% of total FA) of 17 analyzed patients after 6 weeks MPL intake

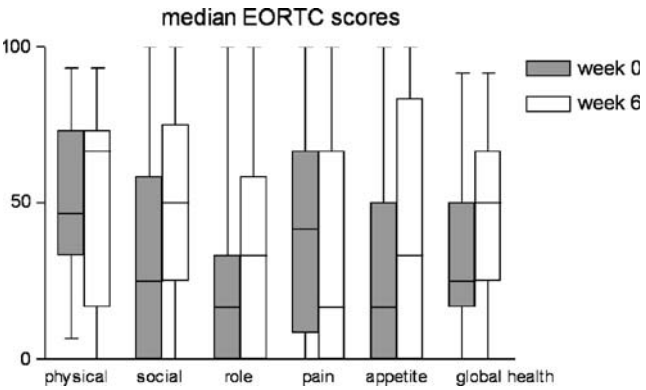


Fig. 6 Comparison of selected median EORTC quality of life questionnaire scores of 17 analyzed patients at weeks 0 and 6

which cleaves arachidonic acid (AA) out of membrane phospholipids (PL). Free AA is the precursor of proinflammatory eicosanoids (e.g., PGE_2) [10, 42], lipid second messengers that promote tumor progression and metastasis [10, 43] along with occurring symptoms of pain as well as weight and appetite loss. To provide the required high amounts of AA for eicosanoid synthesis membrane PL must be broken down by PLA_2, resulting in equimolar amounts of free AA and lyso-PC. Concluding from these facts, one might expect the levels of lyso-PC in the blood of tumor patients to be elevated.

Surprisingly, our own earlier investigations resulted in the contrary observation; it was shown that lysophosphatidylcholine (lyso-PC) levels in serum of tumor patients are decreased in comparison to healthy individuals. This is in accordance with the findings of other groups [44–47]. Even more astonishing was our finding that especially those patients suffering from tumor-associated weight loss displayed low lyso-PC serum levels [35].

In vitro experiments performed in our group with tumor cells exposed to exogenous lyso-PC in physiological concentration may offer some explanation for these seemingly contradictory in vivo findings. All investigated cell lines derived from solid tumors displayed a rapid and extensive elimination of lyso-PC from the supernatant. The extent of elimination of lyso-PC was impressive; lyso-PC corresponding up to 125 times of the whole PC content of the cells was eliminated in only 24 h (Jantscheff et al. 2009, submitted). Possibly a similar process takes place in tumor patients, accounting for the lowered lyso-PC levels.

However, our in vitro studies did not only show that tumor cells eliminate massive amounts of lyso-PC, but also revealed that all cell lines derived from solid tumors showed a remarkable change of the fatty acid (FA) pattern in their cellular membrane. When exogenous lyso-PC containing 90% palmitic acid (C16:0, Pal) was offered, the content of Pal in the cellular membrane was 1.5- to 3-fold increased after 48 h of incubation (Jantscheff et al. 2009, submitted). This observed incorporation of lyso-PC into the cellular membrane can be explained by the increased membrane turnover of tumor cells [13]. The phospholipid (PL) content of cellular membranes is at a homeostasis. The increased degradation of membrane PL, providing AA for eicosanoid synthesis, must therefore be balanced by augmented re-synthesis of membrane PL. This takes place quicker and more effectively by re-acylation of lyso-PC than by de novo synthesis. The presence of lyso-PC has been shown to downregulate the de novo synthesis of membrane PC and to act as negative regulator in mouse macrophage cell lines [48] and in prostate cancer cells [49]. Provisioning of lyso-PC with a certain FA can therefore induce a change in membrane FA pattern, probably especially extensive in cells with a high membrane turnover, as tumor cells. The augmented content of this certain FA in the cellular membrane must then result in a decrease of all other FA due to PL homeostasis of cellular membranes which we also have observed in the in vitro experiments.

The in vivo supply of lyso-PC carrying FA that may counteract the pro-inflammatory AA, therefore appears a promising therapeutic strategy against tumor-associated weight loss and systemic inflammation. Incorporation of lyso-PC with $n-3$ FA into cellular membranes would not only decrease the AA content of the membrane but also provide a higher amount of $n-3$ FA in the membrane PL. Due to its structural similarity to AA, especially the marine $n-3$ FA, eicosapentaenoic acid (EPA) may act as a competitive substrate at enzymatic binding sites. In order to achieve this, an oral formulation of PL with bound $n-3$ FA—the marine phospholipids (MPL)—seems most appropriate as the uptake and metabolism of the $n-3$ FA bound to PL differs from those bound to triacylglycerols (TG), as described in the "Introduction" section. Providing MPL orally should result in a high amount of $n-3$ FA carrying lyso-PC in the blood.

Therefore, in this investigation, patients suffering from tumor-associated weight loss were given marine phospholipids (MPL). In accordance with our earlier findings, average plasma lyso-PC levels were low (187±103 μM) in this patient group, compared to the levels found in healthy individuals (300 to 400 μM) (own unpublished data) [45, 47]. Oral supplementation of PL in the form of MPL for 6 weeks did not change the average lyso-PC levels. Concluding from our in vitro experiments, the lyso-PC elimination might be too extensive to influence plasma lyso-PC levels by low dose oral supplementation of PL. Still, a rapid exchange and incorporation of the n-3 FA bound to MPL was to be expected. Indeed, the FA patterns in blood plasma and cells—used as surrogate parameters—of the 17 analyzed patients changed remarkably. EPA and DHA increased significantly in blood plasma PL, EPA increased significantly in MNL, and AA decreased significantly in RBC. AA does not appear to be remarkably displaced in the examined blood components by $n-3$ fatty acids, even though we could observe a tendency of less AA in plasma and MNL, only the RBC displayed significantly lowered AA percentages.

MNL are metabolically active cells and display a high membrane turnover when immune reactions are activated. The content of AA in their cellular membrane may be highly regulated [50] in order to maintain the proper immunological functionality. RBC are not metabolically active cells; one could therefore regard them as long-term marker membranes. Here, we find a significant reduction of AA, possibly indicating that the effect of MPL on the whole FA profile is rather slow. Very likely, a change of

diet (less intake of meat, eggs, or other animal fats except fish) combined with MPL intake would induce a greater decrease of AA in all compartments.

Due to the great increase of $n-3$ FA, the ratio $n-6$ to $n-3$ FA was significantly lowered in plasma PL and MNL. The $n-6$ to $n-3$ ratio of FA in cellular membranes plays an important role in cellular behavior as shown by Xie et al. [51] who observed a reduced invasive potential of lung cancer cells in vitro by inducing a decreased $n-6/n-3$ ratio in cellular membranes. In the group examined here, all patients experienced a decrease of the $n-6/n-3$ ratio in plasma PL, some to a greater extent than others. From the data resulting from this investigation, we cannot yet explain why the extent of decrease varies as much as observed. A genetical variation in enzymes and regulatory pathways involved in the metabolism and synthesis of lipids and FA due to single nucleotide polymorphisms (SNPs) may play a role here. Furthermore, the comparison of upper and lower quartiles of $n-6/n-3$ ratio responders hints at the fact that low BMI and low fat mass (FM) of patients may predict a better response regarding the FA pattern change. The quartile of patients responding with the greatest decrease regarding the $n-6/n-3$ ratio change have a lower BMI and lower FM than the quartile of patients with the least change of the $n-6/n-3$ ratio in plasma PL. Possibly a higher FM to a certain extent inhibits the exchange of FA in plasma PL due to release of FFA out of adipose tissue (for mean FM (kg) see Table 3) into the blood stream. This implies that dosage of MPL should be calculated adjusted to FM or at least indicates that the beneficial effect of MPL seems greatest when adipose tissue is no longer present as FFA source.

Meanwhile, the weight loss of the participating patients receiving 1.5 g MPL per day was stabilized during the 6-week intervention, median weight change (in % BW) was +0.6% compared to baseline median BW at week 1. This is significantly better than the median BW change during the 6 weeks previous to intervention of −2.0%, calculated from one single value for each patient, taken out of their medical history. Ten out of 17 patients experienced a stabilization or gain of BW during MPL intake, while only 5 out of 17 did not lose weight in the 6 weeks previous to intervention. Patients were not undergoing any chemo- or radiotherapy for at least 4 weeks before intervention, and patient history was known. Weight loss occurring in that period of time therefore most likely cannot be directly attributed to any acute events in patient history.

Furthermore, the percentage of EPA in blood plasma PL after 6 weeks MPL intake correlated with median BW change. But no correlation was found between the relative change of EPA between weeks 0 and 6 and the median BW change. This indicates that a certain minimum amount of EPA in the blood may be more effective regarding a stabilization of BW than a high relative increase. DHA does not show the same correlation as EPA, and we observed that even though DHA is delivered in greater amounts by the MPL formulation than EPA, the absolute increase (in % of total FA) is comparable for both marine FA. It appears that EPA has a greater positive effect than DHA on tumor-associated weight loss and inflammation. This might be explained by the higher structural similarity of EPA to AA and therefore greater competition at enzymatic binding sites, although other pathways of action are also probable [42, 43]. The suppression of cytokine production as shown by Endres et al. [15] in MNL and Wigmore et al. [16] in patients suffering from pancreatic cancer could not be found in our investigation. IL-1 was not detectable in any patient sample, while average IL-6 and TNFα increased after 6 weeks of MPL supplementation. This finding is corroborated by the results of Jatoi et al. [52] who found no correlation between the three serum cytokines and changes in weight or appetite in patients with advanced cancer disease suffering from anorexia and/or weight loss, as well as by the results of Persson et al. [53] who observed a weight-stabilizing effect of fish oil combined with melatonin in weight-losing cancer patients without finding any major changes in serum cytokine levels.

The MPL formulation used in this dietary intervention trial with cancer patients suffering from cachexia is highly accepted by the participating patients, the regular intake is confirmed by the measured changes of the FA profiles in plasma and blood cells. Of 31 patients included, only one patient reported a side effect of worsening chronic diarrhea, and one patient objected to the smell of the capsules, these were the only cases were noncompliance was the reason for dropout. The high dropout must be attributed mainly to the consequences of the narrow inclusion criteria which allowed only patients without current chemo- or radiotherapy to participate. This restriction was chosen to minimize impairment of appetite and quality of life related to tumour therapy and to therefore allow clearer judgment of the effects of MPL on these parameters. In clinical reality, this lead to a selection of patients who had been treated with various therapy regimes and either no other therapeutic option was recommendable or the patients wished to abandon further chemo-or radiotherapy. In most cases, the tumor disease was very advanced, and multiple disease-related problems other than weight and appetite loss occurred. In cachexia studies, high dropout is inevitable, patients are frail and reasons for drop out were numerous (see flow chart) and in many cases not directly linked to missing benefit. Of the five patients participating for only 3 weeks in this study, only two experienced further weight loss, while one patient was stable and two experienced weight gain. For these reasons a per-protocol analysis was chosen, even though an intention-to-treat analysis would

better reflect the fact that drop outs were in part likely due to missing benefit.

The results regarding quality of life, assessed by a questionnaire filled out by the participating patients, do not show any significant changes after 6 weeks of MPL. But all the functional (physical, social, role) and symptom (pain and appetite loss) median scores considered relevant in this investigation improve, as does the global health score. Evaluating the changes of median scores with the Subjective Significance Questionnaire of Osoba et al. [38], improvement of physical and social scores as well as pain reduction and improvement of quality of life was subjectively perceived as "very much change" by patients. Therefore, the overall results show a consistent trend toward improved quality of life after 6 weeks of MPL intake. $n-3$ FA supplementation improving physical strength in cachectic patients without concomitant increase of mean lean body mass (see Table 3) as observed here is in accordance with the results of Fearon et al. [22] who found a significant improvement of physical function (also assessed with the EORTC QLQ-C30v3.0) after 8 weeks supplementation of EPA as diethyl ester versus placebo in 518 weight-losing cancer patients.

In conclusion, dosage of 1.5 g/day MPL is highly accepted and a compliant formulation of marine fatty acids. Low-dose supplementation induces a significant change in FA profiles in plasma PL, RBC, and MNL in tumor patients suffering from excessive weight loss and advanced disease. Furthermore, it leads to BW stabilization compared to baseline throughout a period of 6 weeks, while quality of life is improved. Further investigation of this new $n-3$ FA formulation in patients at earlier stage of cancer disease and with dosage adjusted to BMI or FM is expected to confirm these results.

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Conflict of interest The Tumor Biology Center Freiburg has applied for a patent pertaining to the use of marine phospholipids for palliative cancer treatment. The patent is intended to be commercialized. The presented investigation of marine phospholipids was funded in its entirety, including the purchase of the marine phospholipid formulation, by research sponsorship as stated in the acknowledgements section.

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