A randomized study of the effect of fish oil on n-3 fatty acid incorporation and nutritional status in lung cancer patients

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A Randomized Study of the Effect of Fish Oil on n-3 Fatty Acid Incorporation and Nutritional Status in Lung Cancer Patients

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Abstract

Long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) have been proposed to have beneficial effect on cancer cachexia. The aims of the present study were to a) determine the incorporation of n-3 LCPUFA in erythrocytes (RBC) as a measure of compliance to fish oil (FO)-supplement in lung cancer patients undergoing anti-neoplastic therapy; and b) evaluate the effect of the FO-supplement on weight-loss, mid arm muscle circumference, energy and protein intake, hand grip strength and quality of life. Forty-two patients with advanced lung cancer were randomized immediately after referral to ingest either 20 ml of FO or 20 ml of rapeseed oil (RO) daily. Patients were evaluated every three weeks. Twenty-five patients participated in the study for more than 21 days. The RBC content in FO-group increased with 35%, 137% and 44%, respectively (p < 0.001, p < 0.001 and p < 0.001), but did not change in the RO-group. Neither intention-to-treat analysis nor per-protocol-analysis revealed any statistically significant differences between the groups with respect to clinical outcomes.

Keywords: Lung cancer; Cachexia; Fish oil; n-3 Fatty acids

Introduction

Progressive nutritional deterioration with insufficient intake of macro- and micro-nutrients is common in cancer patients and is associated with changes in carbohydrate, lipid and protein metabolism [1,2]. Malnutrition occurs in 60% of all patients with small cell- and non-small cell lung cancer [2], and this is partly a consequence of cachexia, which is a multi-factorial syndrome including anorexia, severe weight loss, muscular atrophy and weakness [1,3,4,5]. Previous investigations have shown that cachexia deteriorates the patients quality of life (QoL) [6], shortens survival time, and reduces the response to chemotherapy [1,7,8,9]. Supplementation with energy and protein has not been proven efficient in inhibiting the deterioration [6,10,11], and the potential weight gain, if any, consists primarily of fat and water, and not lean body mass [6,11,12,13,14].

Cachexia is partly due to an increase in inflammatory cytokines, and as long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) have been shown to have anti-inflammatory and cytokine reducing effects [15], they may be beneficial in cancer patients with a high inflammatory response. n-3 LCPUFA has been shown to normalize some of the metabolic abnormalities in cachexia such as hypermetabolism and insulin-resistance in patients with pancreatic cancer [16,17,18]. Furthermore, good clinical results primarily on QoL have been achieved in studies with palliative pancreatic cancer patients, if patients are compliant to the treatment with fish-oil (FO) containing supplements [19].

For these reasons we wanted to test if supplements with n-3 LCPUFA lead to incorporation of these fatty acids into the cell membranes during cytotoxic treatment and if that has beneficial effects on weight loss in patients with lung cancer.

Materials and Methods

The investigation was approved by the regional ethical committee.

Patients with small cell- or non-small cell lung cancer referred to the Clinic of Oncology, Rigshospitalet, Copenhagen, were eligible for the study. All patients received platinum-based chemotherapy (Carboplatin or Cisplatin) in combination with Vinorelbine or Etoposid. Patients were consecutively included disregarding previous weight loss. Patients were excluded, if they had ingested daily supplements of n-3 LCPUFA within 60 days prior to the time of randomization, suffered from spontaneous bleeding tendency or were in anti-coagulant therapy. Overall 44 patients were randomized to the two treatment groups (RBC) as a measurement of compliance to fish oil (FO)-supplement in lung cancer patients undergoing anti-neoplastic therapy; and b) evaluate the effect of the FO-supplement on weight-loss, mid arm muscle circumference, energy and protein intake, hand grip strength and quality of life. Forty-two patients with advanced lung cancer were randomized immediately after referral to ingest either 20 ml of FO or 20 ml of rapeseed oil (RO) daily. Patients were evaluated every three weeks. Twenty-five patients participated in the study for more than 21 days. The RBC content in FO-group increased with 35%, 137% and 44%, respectively (p < 0.001, p < 0.001 and p < 0.001), but did not change in the RO-group. Neither intention-to-treat analysis nor per-protocol-analysis revealed any statistically significant differences between the groups with respect to clinical outcomes.

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deliveried in dark bottles packed by the producer to contain either FO or RO. Patients were instructed to ingest a total of 20 ml of the given oil every day with their meals. Both oils supplied 681 kJ pr. 20 ml. The FO was produced by FF-Denmark and contained 0.235 g of n-3 LCPUFA pr. ml (0.1 g/ml of eicosapentaenoic acid (EPA) and 0.12 g/ml of docosahexaenoic acid (DHA)). The RO was supplied by Aarhus Karlshamn A/S and contained 0.078 g of ALA pr. ml and no 0.12 g/ml of docosahexaenoic acid (DHA)). The RO was supplied by Aarhus Karlshamn A/S and contained 0.078 g of ALA pr. ml and no 0.12 g/ml of DHA.

The known side effects of FO are regurgitation, nausea and transient diarrhoea [20-25]. In case of suspected side effects of the oils, these were discontinued for three days to evaluate if they were responsible for the inconvenience. If a subject experienced serious side effects that could be potentially attributed to the oils, the code was broken by the doctor in charge of the patient. Ten patients withdrew from the study because of side effects; diarrhea (2), vomiting (3), increased illness (5). Furthermore, seven subjects dropped out of the study before the minimum period of 21 days due to death (2), reason not specified (4) and loss of contact (1). The reasons for drop-out were evenly distributed among the two oil groups (Figure 1).

All patients were blindly evaluated and interviewed by the same person at three-week intervals. Outcome measures at these clinical control visits were:

**Weight:** Muscular strength was measured by a dynamometer (Jamar, Sammons Prestons Inc). Mid-arm circumference was measured on the right arm. Energy and protein intake was estimated by two different techniques: initially by a three days dietary registration at home (two weekdays and one week-end-day), and after 12 days by 24-hour dietary recall interview as patients were not able to perform any more registrations. A special designed photomap of portion sizes was used for quantification during the interviews.

Patients completed the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-C30 and LC13, which is specific for lung cancer patients. The scores related to fatigue and overall health state was used to reflect the patient’s quality of life (QoL).

Five ml of blood was collected in ice-cold EDTA-conditioned tubes. The blood samples were centrifuged (2300 g for 5 min at 4 °C) to isolate erythrocytes (RBC), which was washed three times in isotonic NaCl solution. Isolated RBC were reconstituted 1:1 in physiological saline with 1mM EDTA and 0.005% BHT and kept at -80°C until they were analyzed. Lipids were extracted from RBC by the Folch procedure [26], Trans esterified by BF₃ and the resulting fatty acid methyl esters were separated using a gas chromatography (Hewlett-Packard - HP6890 with a Supelco SP2380 capillary column (30 m, i.d. 0.25 mm, and film thickness of 0.2 mm)). The levels of the individual fatty acids are presented as percent of the total gas chromatogram area (area %, equivalent to percent of all fatty acids

<table>
<thead>
<tr>
<th>Table 1: Baseline characteristics of the patients with lung cancer referred to anti-neoplastic treatment in the two intervention groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (male/female)</strong></td>
</tr>
<tr>
<td>13 / 7</td>
</tr>
<tr>
<td><strong>Ages (y)</strong></td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m2)</strong></td>
</tr>
<tr>
<td><strong>Weight change during 3 month prior to inclusion (%).</strong></td>
</tr>
<tr>
<td><strong>Energy intake (MJ/d)</strong></td>
</tr>
<tr>
<td><strong>Protein intake (g/d)</strong></td>
</tr>
<tr>
<td><strong>QoL: Fatigue score</strong></td>
</tr>
<tr>
<td><strong>QoL: Global health score</strong></td>
</tr>
<tr>
<td><strong>MS (kg)</strong></td>
</tr>
<tr>
<td><strong>MaC (cm)</strong></td>
</tr>
</tbody>
</table>

Data are given as mean ± SD-value for normal distributed data (student t-test) and median (1. quartile - 3. quartile) for non-parametric data (Mann-Whitney).

Abbreviations: BMI: Body Mass Index; QoL: Quality of Life measured by EORTC-C30 and LC13. Scores are sums in the main sections of the questionaires, MS: Muscular Strength, MaC: Mid-arm Circumference.
The duration of the intervention was (mean ± SD) 37.0 ± 23.3 in the fish oil group, and 39.8 ± 19.7 in the rapeseed group (p=0.700).

Data are expressed as % of fatty acids and given as mean ± SD. Group comparisons were performed by Student t-test. Abbrevations: SFA: Saturated Fatty Acids, MUFA: Mono Unsaturated Fatty Acids, PUFA: Poly Unsaturated Fatty Acids, ALA: α-Linolenic Acid, EPA: Eicosapentaenoic Acid, DHA: Docosahexaenoic Acid.

### Results and Discussion

Patients in RO-group had prior to inclusion lost significantly more weight than patients in FO-group (Table 1). This is a kind of "bad luck randomization", and as no significant weight loss was observed in either group after the intervention, on could speculate if the rape-seed oil did better that the fish oil, as a progressive weight-loss was stopped. However, the information about weight-loss prior to the entrance in the study was given by the patient and not calculated by measurements, and we know that weight is varying due chemotherapy due to edema. As a consequence we do not find it justified to conclude anything about the influence on the oils on the weight changes. According to the supplement consumption records, the patients in the FO-group consumed an average of 17.2 (12.9-18.0) ml oil pr. day for an average period of 48 days, whereas those in the RO-group ingested an average of 15.9 (11.6-18.0) ml/d in an average period of 49 days (p= NS). RBC fatty acid analysis was performed at baseline in 19 patients in the FO- and in 21 patients in RO-group and revealed no significant difference between the two groups (Table 2). Similarly, there was no difference in the intervention periods in the patients showing up for blood samples (Table 2). Twenty-five patients (60%) were available for analysis after the intervention. Drop-out rates were the same in the two groups. The reasons were also similar, and it is doubtful whether the reasons for drop-out from C14:0 to DHA). Successful blood sampling and RBC fatty acid analysis was obtained from 40 patients at baseline and only 25 patients at the end of the intervention. Compliance was evaluated blindly by comparing the patients’ daily supplement consumption records with the measured n-3 LCPUFA levels in RBC.

All data were analyzed with Analyse-it® Standard statistical Software (Analyse-it Software Ltd, Leeds, United Kingdom) and significance was set to p < 0.05. Data for height and duration of participation were tested for normality using the Shapiro-Wilk normality test. Group comparisons were performed using Student t-test for normal distributed data, and results are given as mean ± SD. Nonparametric distributed data were analyzed using Mann-Whitney test for unpaired data and Wilcoxon test for paired data, and results are given as median (1. and 3. quartile). Chi-square test (χ²-test) or Fishers exact test were used for binary data and the Spearman’s rank test for correlation.

### Table 2: Erythrocyte (RBC) fatty acid content before and after intervention with fish oil or rapeseed oil.

<table>
<thead>
<tr>
<th>Fatty acids in RBC</th>
<th>Before n=19</th>
<th>After n=21</th>
<th>p value</th>
<th>Before n=19</th>
<th>After n=21</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>40.1 ±1.3</td>
<td>39.8 ±2.1</td>
<td>0.652 NS</td>
<td>38.7 ±2.0</td>
<td>38.6 ±2.3</td>
<td>0.917 NS</td>
</tr>
<tr>
<td>MUFA</td>
<td>20.0 ±1.5</td>
<td>20.7 ±1.8</td>
<td>0.202 NS</td>
<td>19.1 ±1.9</td>
<td>20.2 ±1.7</td>
<td>0.070 NS</td>
</tr>
<tr>
<td>PUFA</td>
<td>37.6 ±2.4</td>
<td>37.5 ±3.1</td>
<td>0.894 NS</td>
<td>40.6 ±4.3</td>
<td>39.3 ±3.6</td>
<td>0.300 NS</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>27.8 ±2.3</td>
<td>27.4 ±2.0</td>
<td>0.610 NS</td>
<td>27.5 ±3.6</td>
<td>28.3 ±2.4</td>
<td>0.388 NS</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>9.8 ±2.2</td>
<td>10.1 ±2.7</td>
<td>0.771 NS</td>
<td>13.1 ±4.0</td>
<td>11.0 ±2.4</td>
<td>0.044</td>
</tr>
<tr>
<td>ALA</td>
<td>0.1 ±0.1</td>
<td>0.2 ±0.1</td>
<td>0.486 NS</td>
<td>0.1 ±0.1</td>
<td>0.2 ±0.1</td>
<td>0.035 NS</td>
</tr>
<tr>
<td>EPA</td>
<td>0.8 ±0.3</td>
<td>0.9 ±0.4</td>
<td>0.116 NS</td>
<td>1.9 ±1.5</td>
<td>1.0 ±0.4</td>
<td>0.009</td>
</tr>
<tr>
<td>DHA</td>
<td>4.1 ±1.1</td>
<td>4.6 ±1.6</td>
<td>0.286 NS</td>
<td>5.9 ±1.9</td>
<td>5.1 ±1.4</td>
<td>0.156 NS</td>
</tr>
</tbody>
</table>

Data are expressed as % of fatty acids and given as mean ± SD. Group comparisons were performed by Student t-test. Abbrevations: SFA: Saturated Fatty Acids, MUFA: Mono Unsaturated Fatty Acids, PUFA: Poly Unsaturated Fatty Acids, ALA: α-Linolenic Acid, EPA: Eicosapentaenoic Acid, DHA: Docosahexaenoic Acid. The duration of the intervention was (mean ± SD) 37.0 ± 23.3 in the fish oil group, and 39.8 ± 19.7 in the rapeseed group (p=0.700).

FO supplementation had no effect on weight-loss when analyzing the data as intention to treat or per pre-protocol (Table 3). Furthermore, no significant correlations were observed between EPA-RBC-incorporation and changes in any of the clinical outcomes (data not shown).

Contrary to most studies, the FO was administered relatively early in the course of disease with the objective to prevent weight loss. However, because of the high dropout rate, it is difficult to conclude with certainty whether or not the n-3 LCPUFA supplement may have anything to do with the oils. At the same time as the patients started the oil treatment, they also started chemotherapy, so we are reluctant to relate the symptoms to the oil treatment. About 1/3 of the patients dropped out because of rapid worsening of the disease or death. FO supplementation augmented n-3 LCPUFA and EPA in RBC significant more than RO supplement (Table 2). RBC-EPA was most pronouncedly affected by the FO supplement and increased by an average of 137 %. As shown in Figure 2, the relative increase in the EPA content of the RBC was closely associated with duration of the supplementation period. Figure 2: Correlation between duration of intervention in days and total percent increase in EPA content in RBC, in the fish oil group. r=0.77, p=0.001 (n=14 due to missing data).
have had a clinically relevant effect. Within this field there has been a general focus on EPA as the main anti-cachectic agent in fish oil [19]. However, the role of DHA separated from EPA has not been clearly ascertained as most trials have supplies both fatty acids in combination [27]. One should refrain from concluding that DHA possesses no anti-cachectic features, as DHA has been shown to suppress some of the same pro-cachectic agents as EPA [28].

The FO-supplement gave rise to a significant increase in RBC n-3 LCPUFA, most pronouncedly in EPA, which correlated significantly with the duration of the FO-supplementation period. The effects of FO on RBC fatty acid composition in these cancer patients were comparable to the changes that was observed in two previous trials with healthy subjects [29,30].

Based on the self-reported consumption records, the subjects in the FO-group consumed approximately 17 ml of fish oil per day of the intended 20 ml/d and compliance in this study was thus actually superior to some of the previous trials in cancer patients [31,32]. The ingested dose of FO supplied on average 4.0 g/d n-3 LCPUFA (1.7 g EPA and 2.0 g DHA), which is within the range of doses recommended in a recent systematic review [33]. Others have suggested a minimum of 2 g/d of EPA [34,35], as some studies with lower doses have failed to show any beneficial effects [31,32,35].

There was some variation in absolute increase in RBC EPA-content observed within the individual subjects in the FO-group; one patient had an increase of a factor of five in just 27 days, whereas another patient only had an increase of a factor of three during a period 76 days. This could indicate differences in metabolism, interfering differences in diet, or differences in body composition, but is most likely due to differences in compliance. The difference in achieved tissue level emphasizes the importance of a measurement of the immediate effect on fatty acid composition in membranes. Many of the previous cancer trials has assessed compliance only by self-reported consumption records and some have examined the n-3 LCPUFA content of plasma lipids [3,16,18,32,36,37]. The plasma n-3 LCPUFA content can however only be considered as an indicator of fatty acids ingestion during a short period of time, whereas RBC fatty acid content reflects the intake over a period of weeks or months [38]. As RBC has a mean survival of about 120 days in healthy persons, it is expected, that steady state is achieved in the incorporation after four months. Our results indicated that determination of the content of n-3 LCPUFA in RBC would be a good choice to monitor compliance in cancer patients. The present study shows that n-3LCPUFA is incorporated in RBC in a time dependent manner. A minimal period of supplementation must therefore be expected before any beneficial effect can be expected, and this might explain why two large clinical interventions studies found no advantageous outcome on cancer cachexia of n-3 LCPUFA supplementation for two weeks [31,39].

**Conclusion**

A daily intake of 17 ml/d FO for an average of 48 days did increase RBC membranes levels of EPA and DHA in lung cancer patients to the same extent as in healthy subjects and that RBC fatty acid measurement can be used as an indicator of compliance during FO treatment. However, FO supplement did not affect weight loss in these patients. As this study included few patients and had a high dropout rate, it is difficult to conclude whether n-3 LCPUFA supplementation have clinically relevant effects on cachexia in lung cancer patients when administered early in the course of their disease.

**Acknowledgements**

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