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Effect of n-3 fatty acids on patients with advanced lung cancer: a double-blind placebo-controlled study

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Abstract

Background: PUFAs from fish oil appear to have anti-inflammatory and anti-oxidative effects and improve nutritional status in cancer patients.

Objective: the aim of the study was to investigate the effect of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), on inflammatory condition, oxidative and nutritional status in patients with lung cancer.

Design: in our multicentre, randomised, double blind trial, 33 patients with a diagnosis of advanced inoperable non small cell lung cancer and undergoing chemotherapy were divided into two groups, receiving 4 capsules/day containing 510 mg of EPA and 340 mg of DHA, or 850 mg of placebo, for 66 days. At the start of chemotherapy (T_0), after 8 days (T_1), 22 days (T_2), and 66 days (T_3), biochemical (inflammatory and oxidative status parameters) and anthropometric parameters were measured in both groups.

Results: a significant increase of body weight in the n-3 group at T_3 versus T_0 was observed. Concerning inflammation, C-reactive protein and IL-6 levels differed significantly between the n-3 and placebo groups at T_3, and progressively decreased during chemotherapy in the n-3 group, evidencing n-3 PUFAs' anti-inflammatory action. Concerning oxidative status, plasma reactive oxygen species levels increased in the placebo group versus the n-3 group at the later treatment times. Hydroxynonenal levels increased in the placebo group during the study, while they stabilized in the n-3 group.

Conclusions: our data confirm that the continual assumption of EPA plus DHA determined an anti-inflammatory and anti-oxidative action which could be considered a preliminary goal in anti-cachectic therapy.

Introduction

The role of fish-oil supplementation in numerous diseases has been emphasised; these include coronary disease, rheumatoid arthritis, inflammatory diseases, and cancer (1, 2, 3, 4, 5, 6).

Fish-oil supplementation has also been proposed for the treatment of cancer cachexia syndrome, an altered metabolic state characterized by anorexia, weight loss, asthenia, anaemia and alterations in carbohydrate, lipid and protein metabolism (7, 8). This syndrome is the major cause of morbidity and mortality in patients with advanced cancer (9).

Previous studies using conventional nutrition have shown that it is impossible to increase the lean tissue in cachexia patients (10); therefore it is important to use natural substances possessing both nutritional and anti-cachectic properties (11). In particular, eicosapentaenoic acid (EPA), an n-3 PUFA present in large amounts in fish oil, can be considered as a potential natural support: it has been shown to have anti-inflammatory properties, down-regulating both pro-inflammatory cytokine production and the acute-phase protein response in cancer patients (12, 13, 14). Pro-inflammatory cytokines, IL-1, IL-6, and TNF-α, are recognized to play a central role in the pathogenesis of cancer-related cachexia (15, 16). Furthermore, EPA has also been shown to inhibit activation of the ubiquitin proteasome pathway, by the proteolysis-including factor, a cachectic factor produced by cancer tissue, which induces atrophy of skeletal muscle in animal models (9).

In 2006 (17) the administration of a dose of 2 g or 4 g of EPA was compared to placebo in 518 cancer patients (gastrointestinal and lung), over an 8-weeks period. The results indicated that there was no benefit with the 4 g dose, but a potentially clinically relevant treatment effect with 2 g EPA per day.

In recent years, many studies have addressed this subject, but without reaching any conclusions concerning survival improvement and weight increase. This failure might be attributed to sample heterogeneity, reduction of patient number at the end of studies, lack of patient compliance, or subclinical toxicity of the higher dose of EPA (18).

The Cochrane analysis published in 2009 (19) concluded that there was insufficient evidence to draw any conclusions about EPA supplementation in cancer patients with cachexia. This systematic review also suggested there is little evidence of harm from using EPA, especially when combined with Megestrol Acetate.

The anti-inflammatory properties of EPA might also be involved in reducing oxidative stress. The association between reactive oxygen species (ROS), carcinogenesis, and progression of lung cancer has been widely demonstrated. The high percentage of oxidants in cigarettes smoke contributes to smoking-associated carcinogenesis (20). Asbestos fibres alter DNA of lung cells, with increased cell proliferation (21), by changing the redox state of the cells. Several mechanisms are responsible for the development of oxidative stress in cancer patients: the altered energy metabolism caused by the impossibility of normal nutrition in patients with anorexia, nausea and vomiting results in a reduced availability of glucose, proteins and vitamins, leading to increased free radicals (22, 23, 24). Furthermore, chronic non-specific activation of the immune system, with excessive production of inflammatory cytokines, is responsible for increased production of ROS (25). An additional mechanism leading to oxidative stress derives from the use of anti-neoplastic therapy: many chemotherapics, and in particular alkylating agents and cisplatin, determine an increasing of ROS (26).

The aim of the present study was to investigate the effect of fish-oil components, namely EPA plus docosahexaenoic acid (DHA), versus placebo, on inflammatory condition, oxidative and nutritional status, in patients with lung cancer

Experimental Methods and Participants

Study design

The study was a multicentre, randomised, double-blind trial conducted between May 2007 and May 2008. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the Scientific Ethics Committee of the City of Turin, Italy (research protocol n° Eudra-CT 2006-002978-21). Written informed consent was obtained from all patients. Patients were randomised at enrolment using a sequential series of numbered sealed envelopes containing computer-generated random assignments. A copy of the randomisation sequence was kept in a locked cabinet apart from the study personnel. Study products were packaged identically and were not distinguishable from one another.

Participants were randomly divided into two groups: the placebo group and the n-3 group (Figure 1). The first group was provided with a daily dose of four capsules containing 850 mg of placebo (olive oil), the second with a daily dose of four capsules containing 510 mg of EPA and 340 mg of DHA, for 66 days (the entire period of chemotherapy).

Olive oil was selected as placebo because of evidence that ingestion of this oil was unlikely to change the fatty acid composition of either plasma or cellular phospholipids. Self-reported capsule intake was used to determine patient compliance.

Participants visited the research unit four times: at baseline (T_0), after 8 days (T_1), 22 days (T_2), and 66 days (T_3), at which times a blood sample was taken and measurements were made (Figure 1). T_0 coincided with the start of chemotherapy.

Eligibility criteria

Baseline characteristics of patients recruited into this study are shown in Table 1.

Patients were recruited with a clinical diagnosis of advanced inoperable non small cell lung cancer, in the 18 to 70 year age range, and with 10% or less weight loss over the last three months, before the start the study. Patients received 3 courses of chemotherapy with Cisplatin and Gemcitabine to the following schedule: baseline (T_0), after 8 days (T_1), 22 days (T_2), and after 66 days (T_3). Life expectancy was two months or longer; Karnofsky Performance Status was 80 or higher.

Exclusion criteria

Patients were excluded if they had undergone chemotherapy failure, if metastases were present, if they were diabetic, had cardiovascular or infectious diseases, previous cancer (less than 5 years before or with relapse signs) or inflammatory disease. Patients with neurological deficiency or psychiatric diseases were also excluded.

Nutritional status and dietary intake

Patients were weighed on spring balance scales (Tanita Solar Powered Scale) without shoes and wearing light clothing. BMI was calculated as the ratio of body weight to the square of their height (kg/m^2) (27).

In our study we didn't measure lean body mass with bioelectrical impedance analyser.

At the start of the study, pre-illness weight, unintentional weight loss over the last 6 months, and height were recorded.

Both group received the same dietary counselling with the aim to increase their energy and protein intake. A three day dietary recall was performed prior to assessment at baseline (week 0), and each week in the period of 66 days to assess the patients' dietary intakes. Patients completed a dietary diary, and were instructed by a dietician how fill them out correctly. Data on food intake reported by dietary diaries were then translated into energy and protein intakes by means of specific tables validated for Italian foods (28). Patients were also requested to record the number of capsules of n-3 fatty acids or olive oil supplement taken each day.

Blood analysis

Plasma and erythrocytes were obtained by centrifuging venous blood (collected in tubes containing EDTA and kept on ice until separation) at 3000xg for 5 min at 4°C (centrifuge J6M, Beckman, Palo Alto, CA, USA), and stored at -80°C until use. The percentage content of different fatty acids was

154 determined in the total lipids extracted from plasma, and in phospholipids (PLs) extracted from
155 erythrocytes membranes (29).
156 Albumin was analyzed by an autoanalyzer, whereas prealbumin (TBPA) and transferrin were
157 detected quantitatively by immunoturbidity assay. C-reactive protein (CPR), IL-6, TNFα and PGE2
158 production were evaluated in plasma by the ELISA method (R&D systems, Minneapolis, MN,
159 USA). The levels of reactive oxygen species (ROS) were detected in the plasma using the probe
160 2',7'-dichlorofluorescin diacetate (DCFH-DA) and measured fluorimetrically (30).
161 Hydroxynonenal (HNE) concentration was determined on plasma by the method of EsterBauer et
162 al. (31).
163
164 **Statistical Analysis**
165 Results are expressed as means ± S.D. Significant differences between patients in the n-3 and
166 placebo groups were assessed by the unpaired T test, whereas significant differences within groups
167 were assessed by the paired T test, all tests being two-sided. Statistical analyses were performed
168 using the statistical software package SPSS for Windows Version 17.0 (SPSS, Chicago, Illinois,
169 USA) at baseline (T_0), after 8 days (T_1), 22 days (T_2), and 66 days (T_3).
170
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172 *Results*
173
174 The series comprised 33 participants between 46 and 70 years old; they were randomly assigned to
175 the placebo or the n-3 group. Baseline characteristics (T_0) of patients are shown in Table 1.
176 Fourteen participants, aged 50-70 years (mean age 60.57 ± 7.43 years), were allocated to the
177 placebo group; 19 participants, aged 46-69 years (mean age 58.10 ± 6.72 years), were allocated to
178 the n-3 group; 6 patients in the n-3 group (6 of 19: 31%) dropped out during the double-blind phase
179 (2 patients changed department oncology, 2 refused treatment, 2 had diarrhea with capsules) Thus

13 participants in the n-3 group, (age 46-66 years; mean 55.56 ± 7.35 years) completed the study (Figure 1).

Nutritional Status

The patients' nutritional status was not severely compromised, partly because some patients were overweight at the start of chemotherapy. Mean weight of the n-3 group was 75.10 kg (± 16.12) at baseline and 78.50 kg (± 15.94) at T_3: an increase of 3.4 kg occurred, which was statistically significant (Figure 2). In the placebo group there was no increase: mean weight was 68.00 kg (± 12.85) at baseline and 68.92 (± 13.44) at T_3 (Figure 2). There was no statistical significance between the two groups at T_3.

Both groups had a satisfying calorie intake (1.02 g/kg in n-3 group and 0.93 g/kg in placebo group) but they took a different amount of proteins daily. Data for BMI and dietary intake (calories and proteins) revealed no statistically significant differences between the two groups. There was a non-significant increase in daily calorie and protein intake in the n-3 group (Table 1).

Nutritional blood parameters such as albumin, TBPA and transferrin did not differ between the two groups (data not shown).

Percentage content of n-3 fatty acids in plasma and erythrocytes

The percentage content of EPA in total lipids from plasma and in PLs from erythrocyte membranes is reported in Figure 3, which shows that EPA increased significantly in both plasma (panel A) and erythrocyte membranes (panel B) for the n-3 group, compared to the placebo group, at the two later experimental times.

No significant difference was evident in the percentage content of EPA in the placebo group at any of the experimental times, for either plasma or erythrocytes, whereas in the n-3 group there was a significant increase in EPA between the T_1 and T_0, T_2 and T_0, T_3 and T_0, confirming the consumption of supplementary capsules.

The percentage content of DHA, in total lipids from plasma and in PLs from erythrocyte membranes, is reported in Figure 3 (panels C and D), which shows that DHA increased significantly in the plasma (panel C), but not in the erythrocyte membranes (panel D) for the n-3 group, compared to the placebo group. Considering the percentage content of DHA in the placebo group throughout the experimental time, no variation was evident for either plasma or erythrocytes, whereas in the n-3 group, significant variations were detected, only for the plasma, between the T_1 and T_0, T_2 and T_0, T_3 and T_0.

The percentage content of docosapentaenoic acid (DPA), which is an intermediate between the EPA and DHA, was also measured, showing no significant change in the plasma and in the erythrocyte membranes from placebo and n-3 groups at all experimental times.

Inflammatory parameters

Figure 4 shows the trends of CPR, IL-6, TNF-α, and PGE2. In the n-3 group, CPR was not significantly changed between T_0 and T_3 (from 12.89 mg/l to 10.09 mg/l), while in the placebo group the increase, from 11.50 mg/l at T_0 to 27.09 mg/l at T_3, was significant. Comparing the two groups at T_3, the difference was statistically significant (Figure 4, panel A).

IL-6 values decreased at T_3 from their T_0 values in the n-3 group, and increased in the placebo group. Comparing the two groups at T_3, the difference was statistically significant (Figure 4, panel B). TNF-α were higher in the placebo group than in the n-3 group, although not significantly so, possibly due to subject variability (Figure 4, panel C). Since n-3 PUFAs are able to inhibit the production of pro-inflammatory PGE2, this prostaglandin was evaluated in the plasma, using the ELISA test. A significant decrease in the n-3 group occurred during treatment, but no variation in the placebo group (Figure 4, panel D).

Oxidative status

Since n-3 PUFAs could be damaged by ROS with the production of HNE, both HNE (panel A) and ROS (panel B) were evaluated in the plasma (Figure 5). Both these parameters increased at the later experimental times in the placebo group, whereas they decreased in the n-3 group. The HNE decrease in the n-3 group was statistically significant between T_0 and T_3, and the difference between the two groups was statistically significant at T_3. ROS values were significantly less in n-3 group than in the placebo group, at the latter two experimental times.

Discussion

Several studies (32, 33, 34) have emphasized that conventional nutritional support can only partially stop lean mass reduction in cancer patients: it is only possible to increase muscular mass by resolving metabolic alterations (35). Nutritional support is often wasted due to the hyper-metabolic state of the inflammatory pattern, and for this reason, in cancer patients, the first target of nutritional therapy should be to reduce the inflammatory state; n-3 PUFAs could have this effect (36, 37). For this reason, our study looked at the effect of n-3 PUFA administration in patients with advanced lung cancer, in order to evidence the ability of these compounds to improve patients' nutritional status and reduce the inflammatory and oxidative pattern. In this trial, EPA and DHA, two PUFAs present in fish oil, were administered in combination, rather than EPA alone, as in most studies. Administration was in capsules rather than supplements, as occurred in Fearons' trials (9, 17), and adherence to the study protocol was observed more closely. Detailed, consistent and persistent dietetic counselling, with assessment of any disorders connected with capsule assumption throughout the study, helped to obtain good compliance with the therapy and with the nutritional intake. Patients' observance of dietetic recommendations was confirmed by the increase, in the plasma of the n-3 group, of EPA and DHA percentage contents from T_0 to T_3, compared with the placebo group. Changes in DHA content were not due to variation on DPA percentage content,

since the percentage content of this fatty acid did not show significant differences in all experimental times and in both groups.

The patients did not take other dietetic supplements unless n-3 fatty acids or olive oil capsules.

In regard to the nutritional status, we evidenced a slight increase, although not significant, of daily calorie and protein intakes during the study in the n-3 group, from start to end of chemotherapy, while calorie and protein intakes stabilized in the placebo group.

The difference between two groups are random, in fact already the n-3 fatty acid group at T0 took more calories and this trend continued throughout the time. Moreover, a statistically significant increase in body weight was achieved in the n-3 group at T_3 versus T_0. It is not feasible that the slight increase of daily calorie and protein intakes may influence the variation in body weight.

These data are particularly interesting compared to reports in the literature, which are not always univocal on this point: some studies (9, 10, 38, 39) that have examined the effect of fish oil in cachectic patients expressed the opinion that valid conclusions are difficult to draw, for several reasons (short duration of trial, poor tolerability of supplementation, inability of patients to complete the study). Also the recent Cochrane (19) review about EPA for treatment of cancer cachexia did not confirm or reject the use of EPA in clinical practice; the results of the systematic review suggest that there is little evidence of harm deriving from the use of EPA. On the contrary, other studies have reported that EPA + DHA, as in our research, or EPA alone, reduce weight loss in patients with advanced cancer (33, 40, 41). In the case of other studies (9, 17, 40-42) a comparison with this research is difficult, because of methodological differences (lung, pancreatic or gastrointestinal cancer, treated with pure EPA).

A reduction of inflammatory parameter values found in the n-3 group versus the placebo group, although not always statistically significant, was observed. For example, CPR and IL-6 levels showed a significant difference between n-3 and placebo groups at T_3 ($p<0.05$) and a progressive decrease during chemotherapy in the n-3 group, evidencing an anti-inflammatory action of n-3 PUFAs. On the contrary, variations in TNFα were not significant, and those of PGE2 (expression of

283 pro-inflammatory factors) were statistically significant ($p<0.05$) from T_0 to T_2 and T_3 in the n-3
284 group, but not between the two groups.
285 Some studies (10, 33, 43) have reported that n-3 PUFAs may suppress inflammatory cytokines in
286 patients with advanced cancers; our data confirm this result . Van der Meij et al (43) recently
287 published the results of a randomised, case-control, double-blind trial of 40 patients with stage III
288 NSCLC, who received chemotherapy and radiotherapy, together with either supplements containing
289 2 gr of EPA or isocaloric control supplements. After five weeks of treatment they observed that
290 levels of inflammatory markers had decreased during chemotherapy and that IL-6 production was
291 lower in the intervention group than in the control group.
292 To evaluate the oxidative status throughout the period of the trial reported here, ROS and HNE
293 levels were determined. Plasma ROS levels were higher in the placebo group than in the n-3 group
294 at the later treatment times ($p<0.05$). HNE levels (expression of the injury from cellular oxidation)
295 significantly ($p<0.05$) increased in the placebo group during the study, while they stabilized in the
296 n-3 group; this demonstrates the cellular oxidative effect of chemotherapy drugs, and the probable
297 protective action of EPA + DHA. The difference between the two groups at the end of the study
298 was also statistical significant.
299 Our data are encouraging with regard to the goals achieved, although the number of patients was
300 limited: a statistically significant increase in body weight together with a reduction of inflammatory
301 and oxidative parameters in the n-3 group confirm that the continual assumption of EPA + DHA
302 showed an anti-inflammatory and anti-oxidative action, which might be considered a preliminary
303 goal in anti-cachectic therapy.
304
305
306 *Conclusions*
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Although numerous studies have addressed this subject and there is great interest in scientific research concerning n-3 fatty acids, there is as yet little clinic proof to justify applying the results to cancer patients.

In our randomised, double-blind study, despite the small number of patients, we analysed clinical, inflammatory and oxidative status during a period of 66 days, until the end of chemotherapy.

From this we may conclude that:

- fewer patients dropped-out than did from other studies;
- compliance with the dieticians' recommendations, and with the EPA and DHA assumption, were good;
- body weight increased significantly in the n-3 group;
- a significant reduction in inflammatory indexes and in oxidative status was observed.

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Contribution of:

C. Finocchiaro designed research,

T. Monge, M. Scigliano, M. Tinivella and E. Tiozzo conducted research,

M. Aragno, , M. Maggiora, M. Oraldi and M. Schena provided essential reagents and patients,

M. Pugliese performed TNFα; M.G. Catalano and N. Fortunati analysed data.

M. Fadda and G. Muzio analysed data and performed statistical analysis,

O. Segre wrote paper,

M. Canuto and C. Finocchiaro had primary responsibility for final content.

All author read and approval the final manuscript. None of the authors had conflicts of interest.

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445

TABLE 1 - Nutritional status and calorie and protein intakes at baseline and at study end (T_0 and T_3)

		n-3 group (n= 13) (M/F 8/5)	SD	Placebo group (n= 14) (M/F 11/3)	SD
Weight loss %	T_0	3.0	3.5	2.0	3.3
Karnofsky Performance Status	T_0	80	10	80	10
BMI (kg/m2)	T_0	26.19	6.98	25.25	3.92
	T_3	27.65	7.02	25.58	4.43
Calorie intake/day (Kjoule/Kg)(Kcal/day)	T_0	8072.47 1990.56	1596.3 393,44	7371.46 1685.57	1421.95 169.32
	T_3	8840.08 2160	1147.7 330.8	7131.32 1730.29	1016.10 242.69
Protein intake/day (g/day)	T_0	68.56	15.03	60.00	8.5
	T_3	74.88	17.33	61.43	8.52
Calorie intake/Kg (Kjoule /Kg) (K/Kg)	T_0	119.31 29.22	26.85 6.38	110.63 26.42	23.64 5.65
	T_3	120.48 29.63	26.69 7	109.32 26.11	17.08 4.08
Protein intake/Kg (g/kg)	T_0	1.02	0.29	0.94	0.24
	T_3	1.04	0.32	0.94	0.16

The weight loss percentage is related to the last three months, before the start of the study.

Figure legends

FIGURE 1 – Study design

FIGURE 2 – Comparison of weight changes (Kg) in the placebo and n-3 groups during the experimental time

Data are expressed as means ± S.D. * "paired t test" $p<0.05$ T_3 versus T_0.

(–▲–) placebo group, (–□–) n-3 group

FIGURE 3 – Comparison of changes in EPA and DHA in plasma and in erythrocyte membrane content in the placebo and n-3 groups during the experimental time

Data are expressed as means ± S.D. and are the percentage content of EPA in plasma (panel A) and erythrocyte membranes (panel B), and the percentage content of DHA in plasma (panel C) and erythrocyte membranes (panel D).

*"paired t test" $p<0.05$ T_1, T_2, T_3 versus T_0.

§ "unpaired t test" $p<0.05$ n-3 group versus placebo group.

(–▲–) placebo group, (–□–) n-3 group

FIGURE 4 – Comparison of changes in CPR, IL-6, PGE2 and TNF-α content in plasma in the placebo and n-3 groups during the experimental time

Data are expressed as means ± S.D. and are the plasma content of CPR (panel A), IL-6 (panel B), and PGE2 (panel C).

* "paired t test" $p<0.05$ T_2, T_3 versus T_0.

§ "unpaired t test" $p<0.05$ n-3 group versus placebo group.

(–▲–) placebo group, (–□–) n-3 group

476 FIGURE 5 – Comparison of changes in HNE and ROS content in plasma in the placebo and n-3
477 groups during the experimental time
478 Data are expressed as means ± S.D. and are the plasma content of HNE (panel A) and ROS (panel
479 B).
480 * "paired t test" $p<0.05$ T_3 versus T_0.
481 § "unpaired t test" $p<0.05$ n-3 group versus placebo group.
482 (–▲–) placebo group, (–□–) n-3 group

Figure 1

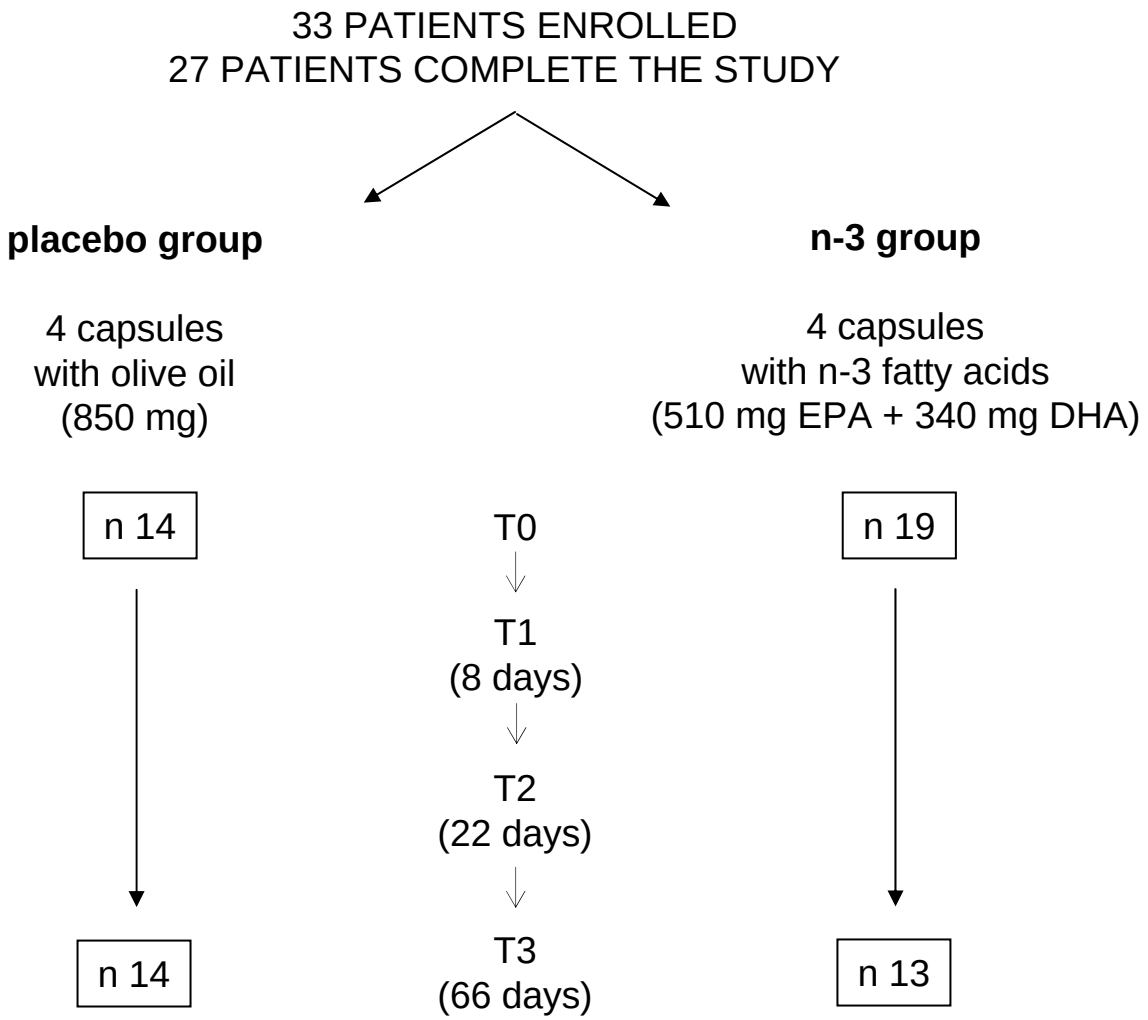

33 PATIENTS ENROLLED
27 PATIENTS COMPLETE THE STUDY
placebo group
4 capsules
with olive oil
(850 mg)
n-3 group
4 capsules
with n-3 fatty acids
(510 mg EPA + 340 mg DHA)
n 14
T0
T1
(8 days)
T2
(22 days)
T3
(66 days)
n 19
n 14
n 13

Figure 2

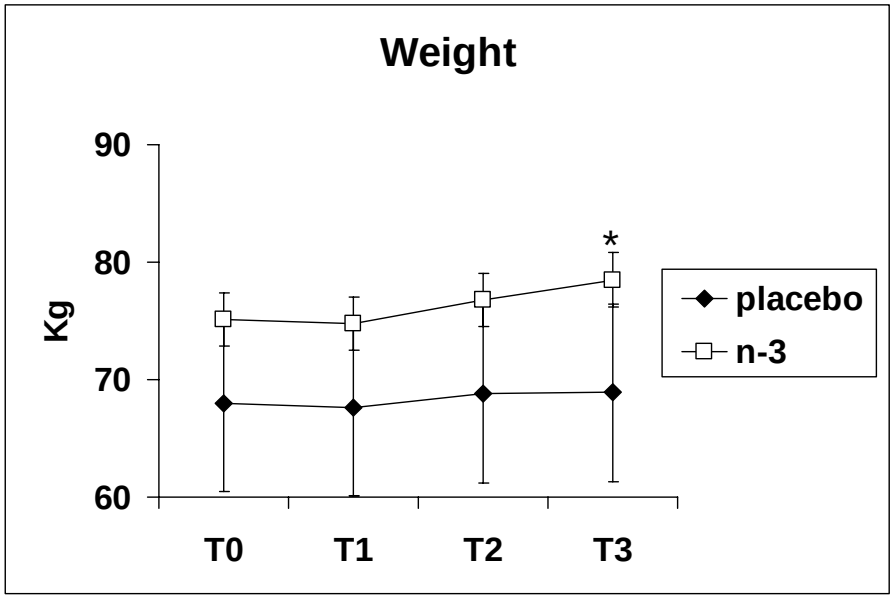


Figure 3

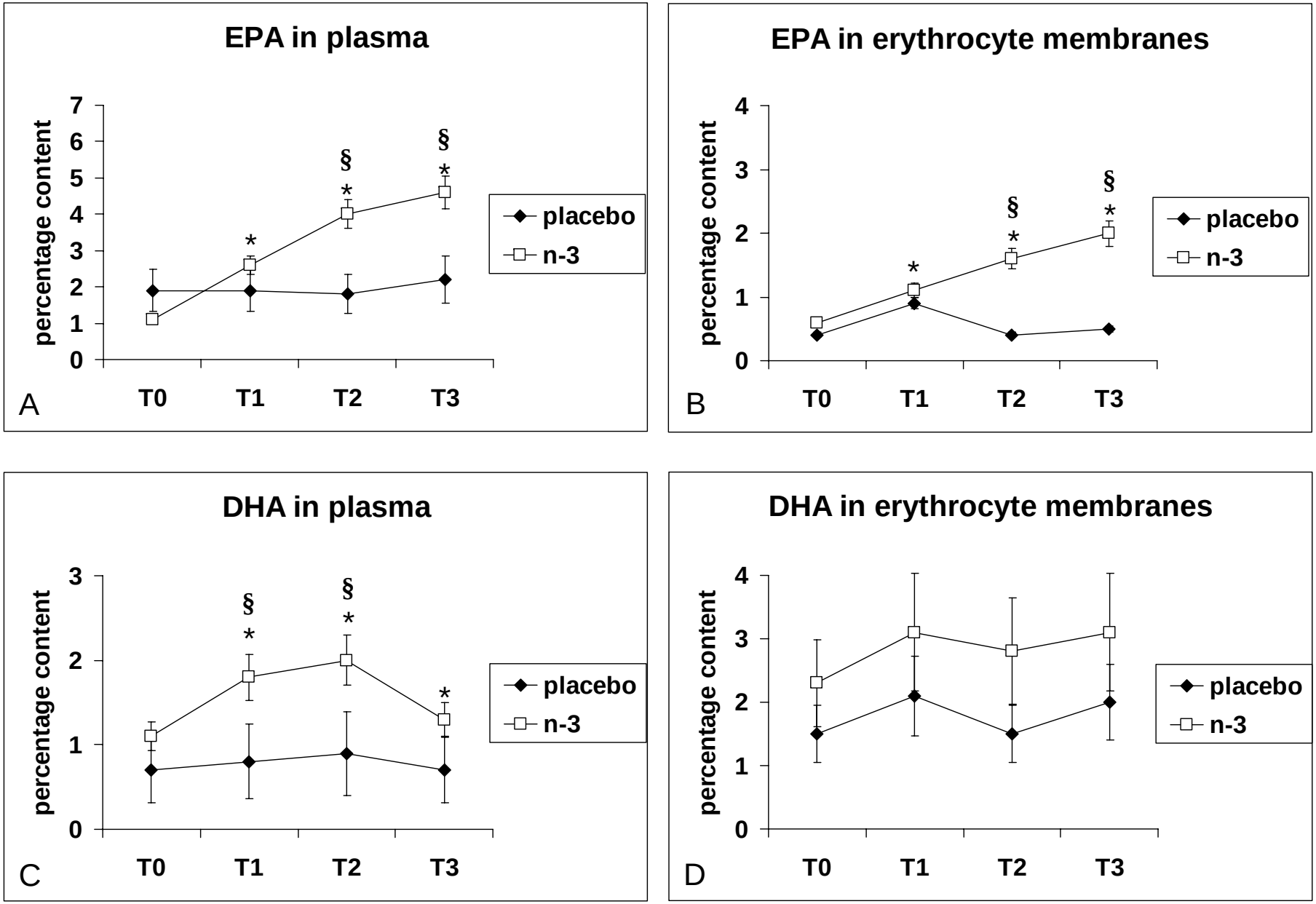

EPA in plasma
percentage content
0
1
2
3
4
5
6
7
T0
T1
T2
T3
placebo
n-3
A
EPA in erythrocyte membranes
percentage content
0
1
2
3
4
T0
T1
T2
T3
placebo
n-3
B
DHA in plasma
percentage content
0
1
2
3
T0
T1
T2
T3
placebo
n-3
C
DHA in erythrocyte membranes
percentage content
0
1
2
3
4
T0
T1
T2
T3
placebo
n-3
D

Figure 4

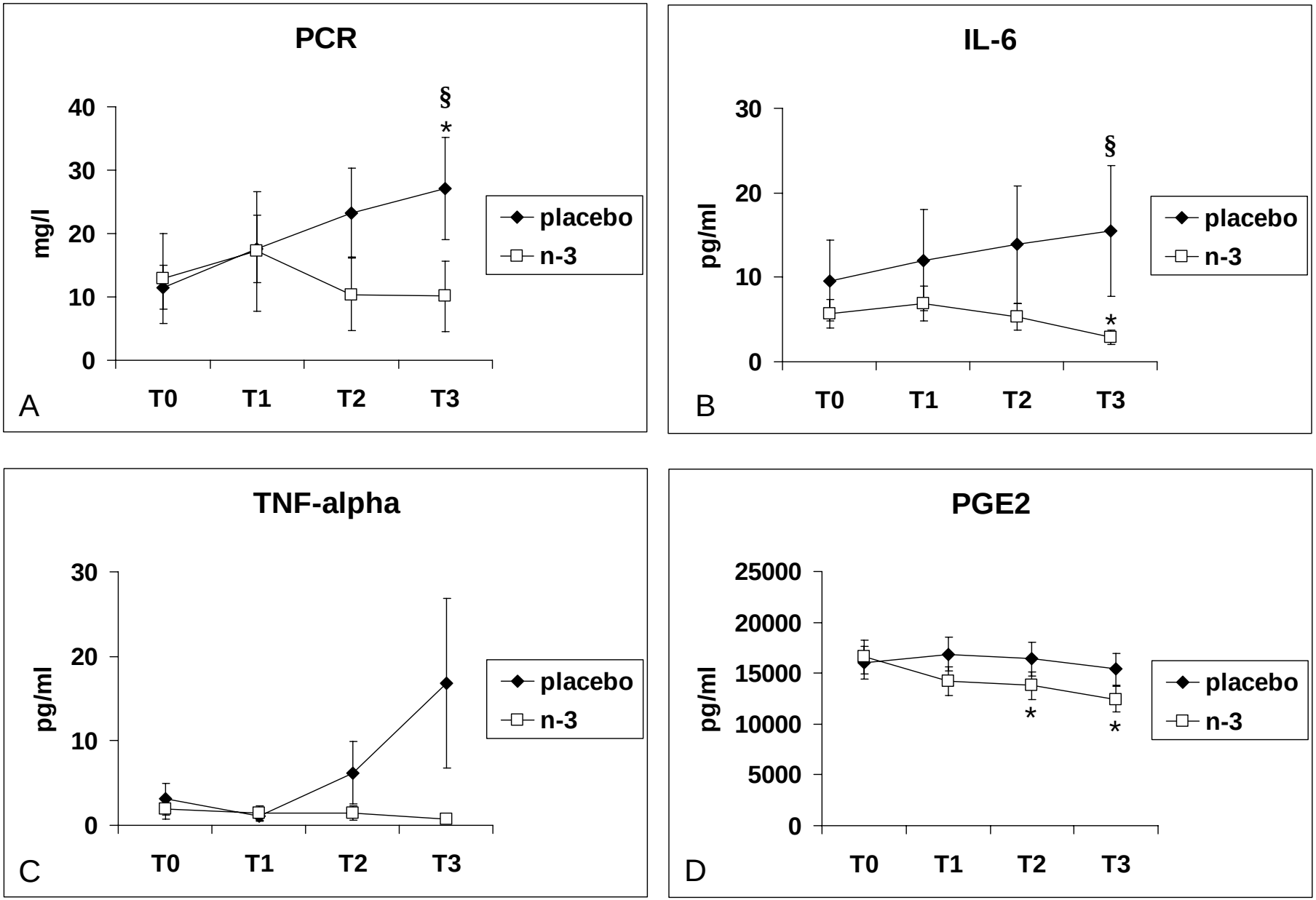
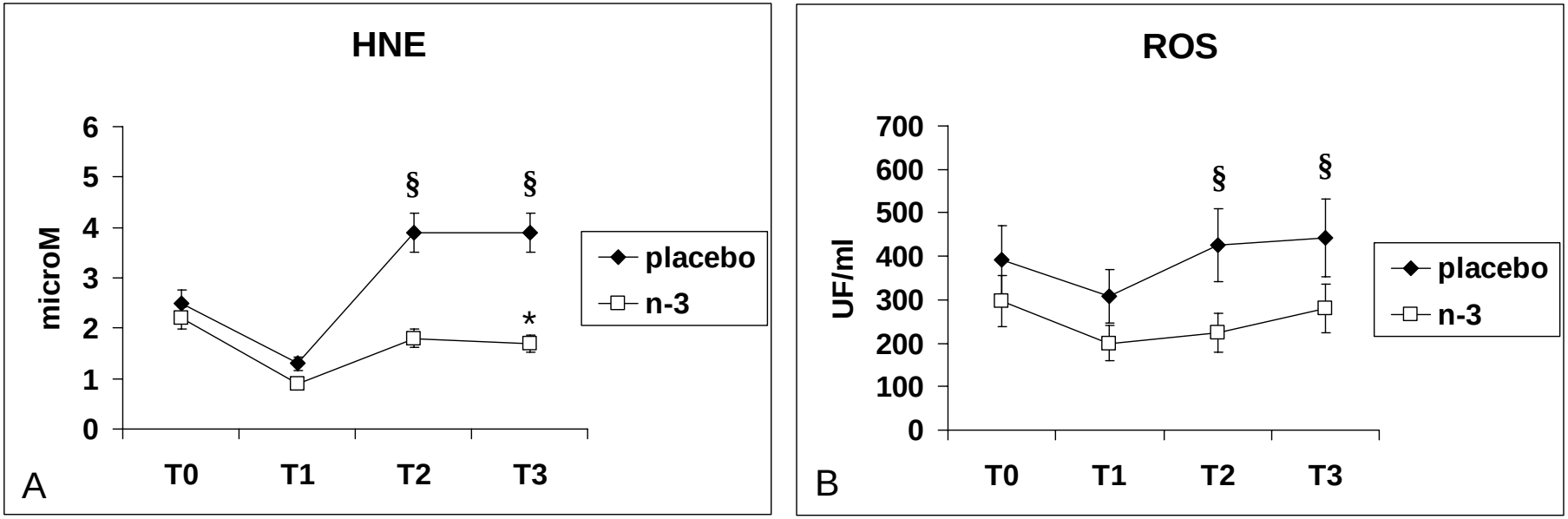

PCR
mg/l
40
30
20
10
0
§
*
T0
T1
T2
T3
placebo
n-3
A
IL-6
pg/ml
30
20
10
0
§
*
T0
T1
T2
T3
placebo
n-3
B
TNF-alpha
pg/ml
30
20
10
0
T0
T1
T2
T3
placebo
n-3
C
PGE2
pg/ml
25000
20000
15000
10000
5000
0
*
*
T0
T1
T2
T3
placebo
n-3
D

Figure 5


HNE
microM
6
5
4
3
2
1
0
§
§
*
T0
T1
T2
T3
placebo
n-3
A
ROS
UF/ml
700
600
500
400
300
200
100
0
§
§
T0
T1
T2
T3
placebo
n-3
B