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ORIGINAL ARTICLE

Fish Oil Decreases C-Reactive Protein/Albumin Ratio Improving Nutritional Prognosis and Plasma Fatty Acid Profile in Colorectal Cancer Patients

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Abstract Previous studies have shown that n-3 polyunsaturated fatty acids n-3 (n-3 PUFA) have several anticancer effects, especially attributed to their ability to modulate a variety of genomic and immune responses. In this context, this randomized, prospective, controlled clinical trial was conducted in order to check whether supplementation of 2 g/day of fish oil for 9 weeks alters the production of inflammatory markers, the plasma fatty acid profile and the nutritional status in patients with colorectal cancer (CRC). Eleven adults with CRC in chemotherapy were randomized into two groups: (a) supplemented (SG) daily with 2 g/day of encapsulated fish oil [providing 600 mg/day of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)] for 9 weeks ($n = 6$), and (b) control (CG) ($n = 5$). All outcomes were evaluated on the day before the first chemotherapy session and 9 weeks later. Plasma TNF-α, IL-1β, IL-10 and IL-17A, the pro/anti-inflammatory balance (ratio TNF-α/IL-10 and IL-1β/IL10) and serum albumin, showed no significant changes between times and study groups ($p > 0.05$). C-reactive protein (CRP) and the CRP/albumin ratio showed opposite behavior in groups, significantly reducing their values in SG ($p < 0.05$). Plasma proportions of EPA and DHA increased 1.8 and 1.4 times, respectively, while the ARA reduced approximately 0.6 times with the supplementation (9 weeks vs baseline, $p < 0.05$). Patients from SG gained 1.2 kg (median) while the CG lost −0.5 kg (median) during the 9 weeks of chemotherapy ($p = 0.72$). These results demonstrate that 2 g/day of fish oil for 9 weeks of chemotherapy improves CRP values, CRP/albumin status, plasma fatty acid profile and potentially prevents weight loss during treatment.

Keywords Fish oil · n-3 Polyunsaturated fatty acids · Colorectal cancer · Chemotherapy · Cytokines · Fatty acid profile

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Abbreviations

ARA	Arachidonic acid
BMI	Body mass index
CG	Control group
COX	Cyclooxygenase
CRC	Colorectal cancer
CRP	C-Reactive protein
DHA	Docosahexaenoic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
HPLC	High pressure liquid chromatography
IL	Interleukin
NF-κB	Nuclear factor κB
PG	Prostaglandin
PINI	Prognostic inflammatory and nutritional index
PPAR	Peroxisome proliferator-activated receptors

PUFA	Polyunsaturated fatty acid
SC	Santa Catarina
SG	Supplemented group
TNF	Tumor necrosis factor
TNM	Tumor staging
VB	Visual basic

Introduction

Studies have suggested that n-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in fish oil, may have numerous applications in anticancer treatment. Besides inhibiting the progression of various cancers [1, 2] including colorectal [3, 4] improve the sensitivity of tumor cells to various anticancer drugs [5, 6] and radiation therapy [7], n-3 PUFAs have also been associated with weight gain in patients with cancer-related cachexia [8, 9] and responsible for several immunomodulatory effects [10, 11].

The daily intake of n-3 PUFA, independently of the route, results in a selective incorporation of these fatty acids in fatty constituents of blood and in membranes of immune, tumor and tissue cells, rather than the n-6 PUFA or any other fatty acid with smaller number of unsaturations. This involves changes in a variety of biological functions related to the cell membrane, such as fluidity, receptor expression, signal transduction, cell interaction, enzymatic activity associated with membrane and, in particular, the production of eicosanoids [12].

The association between overexpression of cyclooxygenase-2 (COX-2) in tumor cells of colon cancer and the imbalance of the n-6:n-3 ratio intake, particularly in the western diet, have favored the maintenance of a chronic inflammatory state sustained by the production of prostaglandins (PG) of the pair suffix, especially the PGE_2, with pro-inflammatory and pro-tumorigenic characteristics [13]. Correction of n-6:n3 balance, made by ingestion of foods rich in n-3 PUFA, such as fatty fish or by isolated fatty acids supplementation, directs the production of eicosanoids by COX-2 to an odd numbered class, such as PGE_3, with antitumorigenic activities and less inflammatory potential [14, 15]. In addition, n-3 PUFA can interfere with genic transduction of transcription factors, such as nuclear factor κB (NF-κB) and the peroxisome proliferator-activated receptors (PPAR), inhibiting the production of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and, therefore, the inflammatory response coordinated by these, characterized by elevated levels of CRP and reduction of plasma albumin concentrations [16].

Values of pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α), as well as counter-regulatory cytokine (IL-10), are elevated in colorectal cancer (CRC), showing the presence of an active and permanent inflammatory state [17]. High values of CRP, primarily synthesized by hepatocytes in response to inflammation, are associated with malignancy and the stage of the disease, and indicate worse prognosis [18].

The inflammatory process, which remains exacerbated in cancer, coupled with side effects resulting from chemotherapy toxicity, a series of clinical outcomes, particularly those related to nutritional status, may be harmed. Thus, strategies that can reduce inflammation and its consequences and/or that can prevent adverse changes in nutritional status, such as the use of n-3 PUFA, can provide significant benefits to the patient during treatment.

Therefore, the objective of this study was to determine whether supplementation of 2 g/day of fish oil for 9 weeks in patients with colorectal cancer receiving chemotherapy alters inflammation markers, the CRP/albumin ratio, nutritional status and the plasma fatty acid profile. It is hypothesized that the intake of the supplement source of n-3 PUFA promotes positive changes in the parameters analyzed.

Methods

Subjects

Participating in this randomized, prospective and controlled clinical trial were patients with colorectal cancer treated in the Ambulatory Care Clinic and Oncologic Research Center of Florianópolis, SC, Brazil, from July 2011 to March 2012. The eligibility criteria were: age >19 years; histopathological diagnosis of colorectal carcinoma and chemotherapy treatment indication. Not included were those in palliative care; taking statin or anti-inflammatory drugs; with allergy to fish and derivatives; without oral intake conditions; previously subjected to chemotherapy; patients with autoimmune diseases or whose pathogenesis is related to an inflammatory process and; those who had used supplements containing fish oil or n-3 PUFA for a prolonged period (>120 days) during the 6 months prior to study initiation. A non-probabilistic convenience sample was defined by the time saturation.

Study Design

Eligible patients were randomly allocated into two treatment groups: a supplemented group (SG) and a control group (CG).

The participants of SG were instructed to ingest four capsules/day of fish oil (2 g/day), in addition to their habitual dietary intake, for 9 weeks. They were instructed to ingest the capsules in a dose-fractionated form (two by two capsules), about 20–30 min before two main meals and accompanied by liquid. In addition, they were instructed to record capsule intake in a specific form provided. The supplementation started on the first day of the chemotherapy.

Randomization was performed with a tool designed for Microsoft Office Excel®, which used a VB programming language and designated patients to one of the groups multiplying the number of the patient's records in the institution by a random number between 0 and 1 generated by Excel®, which was modified for each selection. Odd results allocated patients to SG, and pairs to CG. To keep the proportion of participants in the study groups, the second patient from each pair was assigned to the opposing group of the first, regardless of the group assigned by the randomization tool.

The study protocol was approved by the Human Research and Ethics Committee of the institution and was in accordance with the Declaration of Helsinki (1964) [19]. All participants agreed to participate by signing a consent form.

This trial was registered at Platform TrialGov with the number NCT01575340.

Dietary Supplement

The fish oil supplement was offered in the form of gelatinous capsules with 500 mg/capsule (Omega 3, Phytomare®, Governor Celso Ramos, SC, Brazil) extracted from salmon, mackerel and sardines, plus gelatin and glycerin. Each capsule contained 150 mg of n-3 polyunsaturated fatty acids (90 mg of EPA and 60 mg of DHA), 130 mg of monounsaturated fatty acids and 160 mg of saturated fatty acids. The experimental dosage of 2 g/day of fish oil (intake of four capsules/day) provided approximately 600 mg of omega-3 polyunsaturated fatty acids daily.

Blood Collection and Processing

Blood samples (15 ml) were collected after approximately 8 h fasting at two times: on the day prior to the first chemotherapy (baseline) and 9 weeks later (week 9). The venous access was performed in the region of the forearm by a trained professional. The material was collected in vacuum tubes containing anticoagulant (heparin) for the determination of cytokines and plasma fatty acid profile or in separating gel tubes for determination of serum CRP and albumin.

For separation, the sample containing anticoagulant was centrifuged at 400 g for 7 min at a temperature of about 4 °C and plasma aliquots (500 µl) stored at −80 °C. The sample in the separating gel was left at room temperature for about 30 min to complete coagulation, then, centrifuged at 400*g* for 10–15 min to extract the serum, which was investigated for serum albumin and CRP.

Inflammation Markers

At the end of the study. aliquots of plasma were thawed at room temperature, homogenized and immediately subjected to determination of cytokines (IL-1β, IL-10, IL-17A and TNF-α) by solid phase sandwich ELISA (BD Opt-EIA™, BD Biosciences, San Jose, or eBioscience, San Diego, California, USA), according to the protocol described by the manufacturers. The minimum threshold detection of the kits used was: 2 pg/ml for TNF-α and IL-10, and 0.8 pg/ml for IL-1β. Sensitivity test of IL-17A was 4 pg/ml. Concentrations were expressed as pg/ml.

Serum CRP was quantified by the turbidimetric method [20] and albumin by the colorimetric method with bromocresol green [21]. Concentrations were expressed as mg/l and g/dl.

The inflammatory balance was calculated from the ratio of the pro-inflammatory cytokines IL-1 or TNF-α with anti-inflammatory cytokine IL-10, provides important information about the state of inflammation.

CRP/Albumin Ratio

The CRP/albumin index provides a prediction of nutritional and inflammatory prognosis of the patient and was calculated by dividing the serum CRP by the albumin. The classification adopted was: without risk: <0.4, low risk: 0.4–1.2; moderate risk: 1.2–2.0, high risk: >2.0 [22].

Plasma Fatty Acids Profile

Plasmatic fatty acids constituent of phospholipids, triacylglycerols, cholesterol esters, and free fatty acids were extracted using chloroform:methanol (2:1, vol:vol). Lipid extracts were suspended in methanol and pH adjusted to ≥12 with 5 mol/l NaOH. The aqueous solution was acidified with hydrochloric acid (pH ≤ 3) and subjected to a renewed lipid extraction using hexane, followed by evaporation with a stream of N_2 gas at 37 °C. Fatty acids were derivatized with 4-bromomethyl-7-coumarin and acetonitrile and subsequently separated on a pro-liquid chromatograph Varian Star (SpectraLab Scientific Inc., Ontario, Canada) high performance using an octadecyl silica column (25 cm × 4.6 mm id; particle size 5 mm). Fatty acid derivatives were detected by fluorescence (325 nm excitation, 398 nm emission) and the integrated data and analyzed using a LCPro-Star 6.0 workstation.

Anthropometric Data

Anthropometric data were measured professionally with standardized techniques at the baseline and at week 9. Weight and height were measured with an electronic platform scale with a coupled vertical stadiometer, brand Toledo® (Toledo Company of Brazil, São Bernardo do Campo, SP, Brazil). Their normal weight was taken as that reported by the patients. Triceps, biceps, subscapular and suprailiac skinfold were measured with compass Lange Skinfold Caliper® (Beta Technology Incorporated, Santa Cruz, California, USA). All anthropometric measurements were measured following standard techniques [23].

Nutritional Status and Estimated Body Composition

Body mass index (BMI) was obtained by the ratio of weight (kg) to the square of the height (m). The cutoff points for classification adopted were proposed by the World Health Organization [24]. The weight loss percentage was calculated by dividing the difference in weight in the last 6 months at baseline and baseline weight of the patient [25]. Body fat percentage was estimated from the sum of four skinfolds measured [26] and lean mass, by subtracting the weight corresponding to body fat.

Lifestyle and Other Data

Smoking habits, surgical resection and frequency of fish consumption were collected from direct interview. Data regarding the date of birth, location of tumor and clinical stage were obtained from the patient's records.

Statistical Analysis

For analyzes, the intake of fish oil was considered as the exposure variable. Concentrations of IL-1β, IL-10, IL-17A, TNF-α, CRP and albumin, CRP/albumin ratio and plasma fatty acid profile were the primary outcomes evaluated.

The symmetry of the data was tested by applying the Shapiro–Wilk test. The Student's t test or Mann–Whitney test were used to test for differences between groups at the two time points of the study. Paired t test or Wilcoxon test for paired data were used to test the differences between the different time points in study groups. Fisher's exact test was used for to test the differences between dichotomous variables of characterization.

External validity was tested using age, sex, clinical stage and tumor location.

All analyses were performed with STATA 11.0, version for Windows, considering $p < 0.05$ for statistical significance.

Results

Fifty-seven new cases of CRC were identified at the Oncologic Research Center of Florianópolis between July 2011 and March 2012. Of this total, eight patients were not eligible according to the inclusion criteria. Twelve potentially eligible patients were not identified before the start of chemotherapy. Thus, 37 patients were invited to take part in this clinical trial. The percentage of rejection was 62 %. Of the 14 patients who agreed to participate, three were hospitalized to start the first chemotherapy, and therefore could not collect the study's first blood sample. Finally, 11 patients (22 % of eligible population) were randomly assigned into two study groups, as shown in Fig. 1. None of the randomized patients dropped out or were withdrawn from the study.

The characteristics of age, gender, location and tumor stage of the eligible patients lost ($n = 38$) were not significantly different from the group assessed ($n = 11$) (Table 1).

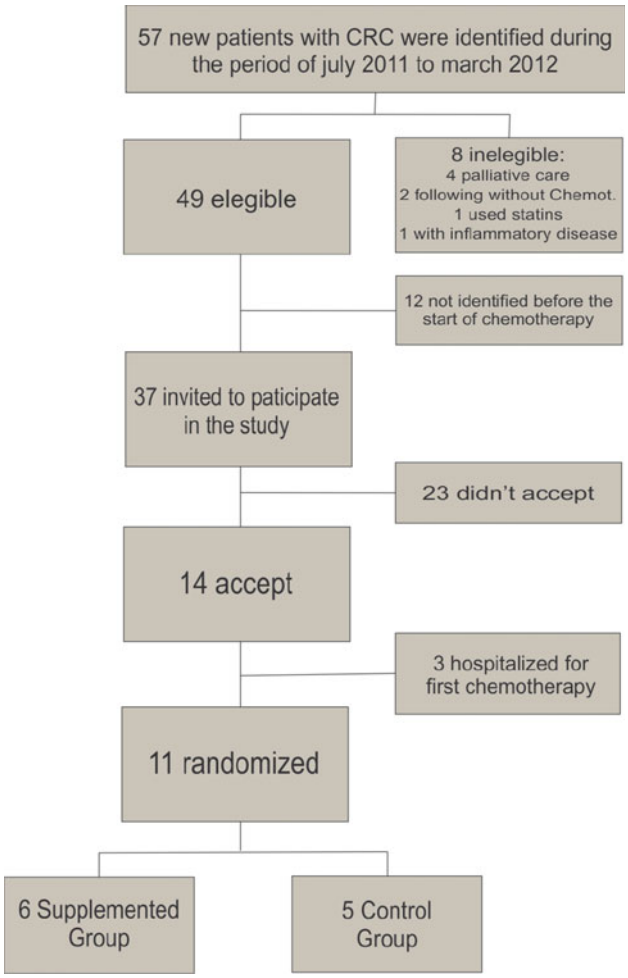

Fig. 1 Flowchart of recruitment of study patients

Table 1 Characteristics of eligible patients ($n = 49$) recruited on the Oncological Research Center of Florianópolis in the period from July 2011 to March 2012

Characteristics	Groups		p value
	Lost group ($n = 38$)	Randomized group ($n = 11$)	
Age (years)	58.5 (10.2)	54.5 (9.8)	0.25*
Gender (n)			
Male	20	6	0.74†
Female	18	5	
Tumor localization (n)			
Colon	24	6	0.73†
Rectum	14	5	
TNM classification (n)			
II	7	0	0.17†
III	19	9	
IV	10	2	

Mean values (SD) or median [IQR]

* Test t

† Chi^2 test

Table 2 Baseline characteristics of randomized patients ($n = 11$) according to study groups

Characteristics	Study groups		p value*
	Control group ($n = 5$)	Supplemented group ($n = 6$)	
Age (years)	53.6 (12.9)	55.2 (7.7)	0.80
Male (n)	3	3	1.00†
Smokers (n)			1.00†
Ex-smoker	2	3	
Never smoked	3	3	
Tumor localization (n)			0.58†
Colon	2	4	
Rectum	3	2	
TNM classification (n)			1.00†
III	4	5	
IV	1	1	
Tumor resection in the last 4 months (n)	4	5	1.00†
Fish consumption (n)			0.57†
≥1 time/week	2	4	
<1 time/week	3	2	
Weight loss in the last 6 months (%)	1.8 [−1.0; 10.5]	7.6 [1.4; 9.2]	0.72‡

Mean (SD) or median [IQR]

* Data refer to the t test, unless when specified

† Chi^2 Fisher's exact

‡ Mann–Whitney test

Characteristics of the Study Participants

Baseline characteristics of the eleven randomized patients show that the study groups were comparable. No statistical difference was observed between groups for characterization variables (Table 2).

Nine patients (82 %) underwent tumor resection in the previous 4 months preceding their inclusion in the study, three (27 %) had tumor invasion to other organs or structures (T4), seven (64 %) had metastases in one to three regional lymph nodes (N1) and, two (18 %), distant metastasis (M1). No patients were classified as malnourished, despite three presented weight loss greater than 10 % after tumor resection. Seven (64 %) were overweight, three obese (BMI ≥30 kg/m^2).

The chemotherapeutic drugs used alone or combined for treatment of these patients were xeloda ($n = 5$), oxaliplatin ($n = 6$), 5-fluorouracil ($n = 6$) and leucovorin ($n = 6$). The infusional chemotherapy sessions were performed every 15–21 days. Moreover, oral chemotherapy, characterized by chemotherapy drug administration in the form of tablets/pills, was performed daily.

Inflammatory Markers and CRP/Albumin Ratio

Plasma concentrations of TNF-α, IL-1β, IL-10 and IL-17A, as well as the balance pro/anti-inflammatory (ratio IL-1β and TNF-α/IL-10) were similar at the stipulated times and between study groups, with no statistical difference observed between them (Table 3).

Serum albumin also showed no significant difference at the two time points between groups, despite their values decreasing in CG at the end of the study (week 9—baseline = −0.32 ± 0.26 g/l, $p = 0.051$). In contrast, serum levels of CRP were significantly reduced in SG (week 9—baseline = −8.8 ± 13.5 mg/l, $p = 0.028$) while increased in the CG (week 9—baseline = 27.4 ± 30.2 mg/l, $p = 0.043$), with significant difference between groups at week 9 ($p = 0.045$) (Table 3).

The CPR/albumin index shown in Fig. 2, presented the opposite behavior in study groups. While patients of CG changed their classification categories from low to medium risk to high risk of complications, in the group that ingested fish oil for 9 weeks (SG), all patients remained in the low risk category (week 9—baseline: CG 3.04 (2.72;7.15); SG −0.30 (−4.13; −0.09); $p = 0.006$).

Plasma Fatty Acid Profile

With daily supplementation of 2 g of fish oil (600 mg of n-3 PUFA), the plasma levels of EPA and DHA increased on an average by 1.8 and 1.4 times, respectively, showing a

Table 3 Inflammation markers and inflammatory balance at the two timepoints and among the study groups

Inflammatory markers	Study groups		*p* value*
	Control group ($n = 5$)	Supplemented group ($n = 6$)	
TNF-α (pg/ml)			
Baseline	2.48 (0.00)	2.49 (0.01)	0.21
Week 9	2.48 (0.01)	2.48 (0.01)	0.41
p-value	0.85§	0.10‡	
IL-1β (pg/ml)			
Baseline	1.56 (0.00)	1.57 (0.01)	0.28
Week 9	1.57 (0.01)	1.57 (0.01)	0.93
p-value	0.31§	0.69§	
IL-10 (pg/ml)			
Baseline	2.32 (0.08)	2.29 (0.08)	0.54
Week 9	2.30 (0.07)	2.35 (0.06)	0.25
p-value	0.31‡	0.13‡	
IL-17A (pg/ml)			
Baseline	2.34 (0.00)	2.35 (0.01)	0.28
Week 9	2.348 (0.01)	2.35 (0.01)	0.82
p-value	0.44‡	0.86‡	
Ratio IL-1β/IL-10			
Baseline	0.67 (0.02)	0.69 (0.02)	0.41
Week 9	0.68 (0.02)	0.67 (0.02)	0.25
p-value	0.20‡	0.09‡	
Ratio TNF-α/IL-10			
Baseline	1.07 (0.04)	1.08 (0.04)	0.57
Week 9	1.08 (0.03)	1.06 (0.03)	0.32
p-value	0.30‡	0.19‡	
C-reactive protein (mg/l)			
Baseline	4.85 [1.01; 6.33]	3.39 [1.49; 18.24]	0.71†
Week 9	18.14 [12.16; 35.51]	1.46 [1.20; 2.71]	0.04†
p-value	0.04§	0.03§	
Albumin (g/l)			
Baseline	4.34 (0.29)	4.23 (0.25)	0.53
Week 9	4.02 (0.24)	4.27 (0.24)	0.13
p-value	0.05‡	0.77‡	

Mean (SD) or median [IQR]

C-reactive protein analysis power: 70.2 %

* Unpaired *t* test, unless if specified

† Mann–Whitney test

‡ Paired *t* test

§ Wilcoxon test for paired data

significant difference (at baseline vs week 9: $p < 0.05$). Arachidonic acid also changed with supplementation of fish oil, reducing significantly (from 29.4 to 17.41 %) its concentration (at baseline vs week 9: $p = 0.028$). Significant differences between study groups was observed for

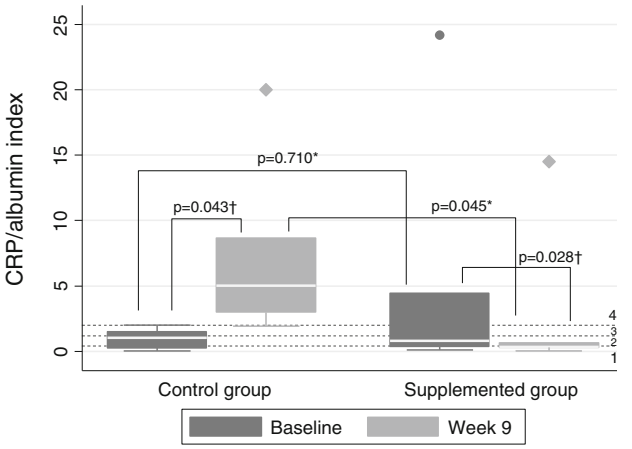

Fig. 2 CRP/albumin ratio on different moments (baseline and week 9) and study groups (control group, $n = 5$, and supplemented group, $n = 6$). Categories: *1* without risk; *2* low risk; *3* moderate risk; *4* high risk. *Wilcoxon test for paired data; †Mann–Whitney test. Analysis power: 73.3 %

plasmatic proportion of EPA and DHA on week 9 ($p < 0.05$). Oleic (monounsaturated fatty acid) and palmitic (saturated fatty acid) fatty acids showed opposite behavior in the treatment groups, with no significant difference (Fig. 3).

The plasmatic ratio of n-6:n-3 increased from 8.1:1 to 8.7:1 in the control group ($p > 0.05$), and decreased from 10.2:1 to 4.6:1 in supplemented group ($p = 0.116$). Comparing n-6:n-3 ratio among study groups, significant difference was observed only at week 9 ($p = 0.010$).

Anthropometric Parameters and Nutritional Status

Considering the timepoints and the study groups, there were no significant changes in weight, BMI, body fat percentage and lean mass (Table 4). However, when performing an analysis of individual behavior of these indicators, is observed that three (60 %) patients of the CG lost weight during treatment, while on the SG, only one lost weight (17 %) (Fisher's exact, $p = 0.24$). Considering the difference in individual weight (week 9—baseline) observed that patients of the CG reduced −0.5 kg (−1.1; 1.4 kg) whereas patients of the SG increased by 1.2 kg (0.4; 1.3 kg), with clinical importance, but no statistical difference ($p = 0.72$).

Discussion

Interest in the modulation of immune and inflammatory responses in colorectal cancer with the use of n-3 PUFA has grown in recent years with the publication of several studies [27–32]. However, they all have different study

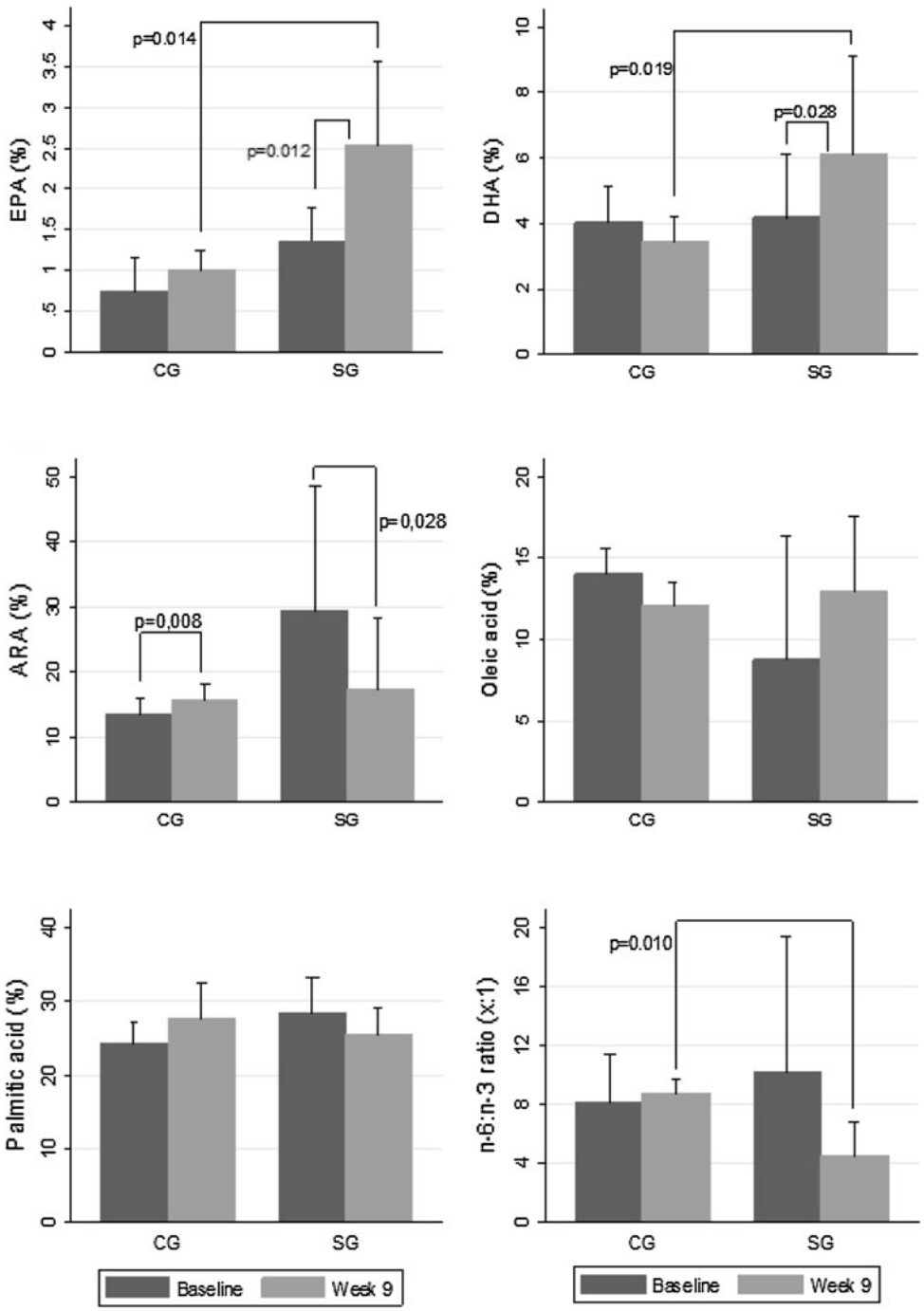

Fig. 3 Average (SD) of plasma fatty acids proportions at the different time points (baseline and week 9) and study groups (SG, $n = 6$; CG, $n = 5$). *CG* control group, *SG* group supplemented, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ARA* arachidonic acid. Tests used: EPA, ARA, palmitic and oleic acids: paired *t* test and unpaired *t* test. DHA and ratio n-6: n-3: Wilcoxon test for paired data and the Mann–Whitney test. Differences were tested between the timepoints of the same group and from different groups for all variables. $p > 0.05$, not shown. EPA analysis power: 80.7 %; DHA analysis power: 70.7 %; ARA analysis power: 64.7 %; n-6:n-3 ratio analysis power: 72.4 %

designs, making it difficult to compare them and consequently reach a conclusion on the evidence presented. Dosages of isolated n-3 PUFA or in combination with other nutrients ranged from 0.6 g [31, 33] to 3.3 g/day [27], and the supplementation time, of 5 days [28] to 6 months [33]. Most of these studies evaluated the immunomodulatory effect of n-3 PUFA on surgical procedures [27, 28, 30, 32, 33], while on chemotherapy, only two studies were found [29, 31].

Modulating effects of n-3 PUFA evidenced by changes in cytokine production were observed in four controlled studies [27, 28, 32, 33]. In these studies, the cytokines production (IL-1β, IL-2, IL-4, IL-6, TNF-α and IFN-γ) were lower in the group that received supplementation with n-3 PUFA compared to their respective control groups.

All findings of this study are consistent with those found in a prior study of our search group [31]. In this study [31], no difference was found for IL-1β, IL-6 and TNF-α

Table 4 Anthropometric parameters and indicators of nutritional status at the two timepoints and within the study groups

Indicators	Study groups		*p* value*
	Control group ($n = 5$)	Supplemented group ($n = 6$)	
Weight (kg)			
Baseline	68.1 (12.1)	72.3 (12.3)	0.58
Week 9	68.0 (±12.1)	72.7 (10.0)	0.50
p-value	0.97‡	0.77‡	
BMI (kg/m^2)			
Baseline	26.4 (3.7)	28.6 (6.3)	0.54
Week 9	26.5 (3.4)	28.7 (5.2)	0.44
p-value	0.93‡	0.86‡	
Body fat (%)			
Baseline	27.0 [16.7; 34.1]	32.8 [16.8; 39.5]	0.41†
Week 9	25.9 [15.7; 38.3]	34.8 [16.4; 38.8]	0.46†
p-value	0.69§	0.35§	
Lean mass (kg)			
Baseline	50.4 (12.5)	50.5 (9.4)	0.99
Week 9	50.2 (13.8)	50.5 (9.0)	0.96
p-value	0.79‡	0.99‡	
Weight difference (kg)	−0.5 [−1.1; 1.4]	1.2 [0.4;1.3]	0.72†
Lean mass difference (kg)	−0.4 [−1.5; −0.4]	0.2 [−0.7; 1.3]	0.58†

Mean (SD) or median [IQR]

Weight and lean mass difference: week 9—baseline

* Unpaired *t* test, unless that specified

† Mann–Whitney test

‡ Paired *t* test

§ Wilcoxon test for paired data

between CRC groups with or without supplementation of 2 g/day of fish oil for 9 weeks of chemotherapy. Likewise, Read et al. [29] found no significant differences in levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and IL-12 after supplementing 480 ml/day of a liquid emulsion containing 2.18 g of EPA and 0.92 g of DHA to patients with advanced CRC in chemotherapy. In contrast, we found important differences in the behavior of serum CRP, with a decrease in concentration of this acute phase protein in the supplemented group and an increase in the control group. Similarly, we observed a significant change in CRP/albumin ratio, with clinical relevance, indicating an increased risk of nutritional and inflammatory complication during treatment in the group without supplementation, and a significant reduction of the risk in those who received 2 g of fish oil/day (600 mg/day of n-3 PUFA).

Elevated levels of CRP have been associated with CRC [34–36]. Its pattern of hepatic production changes according to the presence of an active inflammatory process. Increased levels of serum CRP represent an independent predictor of a poor clinical prognosis and a low survival rate of these patients [18, 37]. The CRP/albumin ratio [22] is a simple indicator of lower cost, with the same power and sensitivity of prognostic inflammatory and nutritional index (PINI) [38] to provide risk of complications degrees. Silva et al. [31] observed that 2 g/day of encapsulated fish oil during 9 weeks significantly decreased the complication risk provided by CRP/albumin ratio of the ten CRC patients in chemotherapy.

The role of IL-17A in cancer-related inflammation is not well understood. Recent studies with animal models [39, 40] have shown that IL-17A may promote development of intestinal cancer, exerting potential pro-inflammatory activity [41]. However, no studies were found that evaluated the behavior of this cytokine with n-3 PUFA supplementation in the CRC, which makes this the first study to provide profile data of IL-17A in CRC.

Excess weight at the start of chemotherapy has shown to be a fact observed in other recent studies with cancer patients [29, 31, 42]. Considering the possible chemotherapy toxicity, represented mostly by anorexia, nausea, vomiting and diarrhea, and the metabolic implications of actively exacerbated inflammation, overweight at an early phase, could be seen as a protective factor against the weight loss related to cancer. In the study of Sánches et al. [42] 63 % of 191 patients receiving chemotherapy showed weight loss during treatment. In our study, this percentage was only 36 %. We also observed a gain of 1.2 kg in the group that received daily supplementation of fish oil and a reduction of −0.5 kg in the group without supplementation, although there was no statistically significant difference. These clinical findings are in line with other studies [29, 31], suggesting a possible protective effect of fish oil against chemotherapy-induced weight loss.

The HPLC data showed that 2 g/day of fish oil (360 mg/day of EPA and 240 mg/day of DHA) for 9 weeks was sufficient to alter the fatty acid composition of the plasma lipid constituents. The 1.8 times increase in the proportion of EPA, 1.4 of DHA and a 0.6 decrease of ARA, showed a preference for the incorporation of fatty acids with more unsaturations, as it was also observed in other studies [10, 29] and perhaps these are part of the key events under the action mechanisms of n-3 PUFA on inflammation and immune system.

The strengths of this study are: comparability of study groups; external validity, which validates the extrapolation of the data found for the eligible CRC population; showing that using a formula of n-3 polyunsaturated fatty acids is a potentially effective, easily accessible and inexpensive treatment; and the originality of the proposal to evaluate the behavior of interleukin 17A.

The refusal rate of participation was high probably due to patients having a recent cancer diagnosis, which in some way caused them to be worried and frightened by the uncertainties of treatment and the severity of the illness. Thus, an invitation to participate in a clinical trial was received with great care and in most cases, refused.

Considering the characteristics of the offered fish oil formula it was not possible to blind the study sample, since an oral placebo formula with the same sensory characteristics of fish oil was not available. Placebo formulas containing soy bean oil to replace fish oil were used in double-blind studies [33, 35]. However, these formulas were offered in the form of liquid emulsion administered parenterally. Another important point lies in the fatty acid composition of the placebo. It is believed that placebo formulations having the content of n-3 PUFA wholly or partially replaced by n-6 PUFA are not suitable for studies to evaluate outcomes of inflammation and thus should not be considered a placebo. This statement is justified in study of Furukawa et al. [43], where seven patients undergoing esophagectomy received one fat-free parenteral formula (control), another seven patients received an only formula containing soybean oil and the other nine patients were supplemented with 1.8 g of EPA plus soybean oil for 21 perioperative days (seven pre and 14 postoperative days). The results showed that the concentration of IL-6 and CRP increased in the group that received only soybean oil and decreased in the EPA plus soybean oil group when compared to the control group. Was checked significantly difference between the soybean group alone and EPA plus soybean group, but this groups not differ the control group. This is because n-6 PUFA, especially ARA, can lead to a pro-inflammation status by the production of pro-inflammatory eicosanoids. For these reasons, the development of a placebo formula suitable for fish oil is a challenge that should be encouraged and implemented in the future.

In conclusion, the supplementation of 600 mg of EPA + DHA from dietary intake of 2 g/day of fish oil for 9 weeks for individuals with CRC during chemotherapy was not capable of promoting change in the plasmatic concentrations of pro-inflammatory and/or anti-inflammatory cytokines, and therefore did not alter the inflammatory balance when assessed by these markers. However, there was a significant change in the composition of plasma fatty acids, which might be related to a significant reduction in the production of CRP/Alb ratio in the supplemented group, pointing to an important decrease in the risk of inflammatory and nutritional complications during treatment, and preventing weight loss associated with disease progression and chemotherapy. For the latter reason, this supplementation protocol represents a potential adjuvant in the treatment of colorectal cancer.

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References

1. Connolly JM, Coleman M, Rose DP (1997) Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice. Nutr Cancer 29:114–119
2. Connolly JM, Gilhooly EM, Rose DP (1999) Effects of reduced dietary linoleic acid intake, alone or combined with an algae source of docosahexaenoic acid on MDA-MB-231 breast cancer cell growth and apoptosis in nude mice. Nutr Cancer 35:44–49
3. Calder PC, Davis J, Yaqoob P et al (1998) Dietary fish oil suppresses human colon tumor growth in athymic mice. Clin Sci 94:303–311
4. Chen ZY, Istfan NW (2000) Docohexaenoic acid is a potent inducer of apoptosis in HT-29 colon cancer cells. Prostaglandins Leukot Essent Fatty Acids 63:301–308
5. Jordan A, Stein J (2003) Effect of an omega-3 fatty acid containing lipid emulsion alone and in combination with 5-fluorouracil (5-FU) on growth of the colon cancer cell line Caco-2. Eur J Nutr 42:324–331
6. DeGraffenried LA, Friedrichs WE, Fulcher L et al (2003) Eicosapentaenoic acid restores tamoxifen sensitivity in breast cancer cells with high Akt activity. Ann Oncol 14:969–970
7. Feng Z, Hu W, Marnett LJ et al (2006) Malondialdehyde, a major endogenous lipid peroxidation product, sensitizes human cells to UV- and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. Mutat Res 601:125–136
8. Moses AW, Slater C, Preston T et al (2004) Reduced total energy expenditure and physical activity in cachexia patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. Br J Cancer 90:996–1002
9. Colomer R, Moreno-Nogueira JM, García-Luna PP et al (2007) n-3 fatty acids, cancer and cachexia: a systematic review of the literature. Br J Nutr 97:823–831
10. Bonatto SR, Oliveira HP, Nunes EA et al (2012) Fish oil supplementation improves neutrophil function during cancer chemotherapy. Lipids 47:383–389
11. Cury-Boaventura MF, Torrinhas RS, Godoy AB et al (2012) Human leukocyte death after a preoperative infusion of medium/long-chain triglyceride and fish oil parenteral emulsions: a randomized study in gastrointestinal cancer patients. JPEN J Parenter Enteral Nutr 36(6):677–684
12. Cockbain AJ, Toogood GJ, Hull MA (2012) Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. Gut 61:135–149
13. Janakiram NB, Mohammed A, Rao CV (2011) Role of lipoxins, resolvins, and other bioactive lipids in colon and pancreatic cancer. Cancer Metastasis Rev 30:507–523
14. Hull MA (2011) Omega-3 polyunsaturated fatty acids. Best Pract Res Clin Gastroenterol 25:547–554

15. Yang E, Chan D, Felix E et al (2004) Formation and antiproliferative effect of prostaglandin E_3 from eicosapentaenoic acid in human lung cancer cells. J Lipid Res 45:1030–1039
16. Chapkin RS, Kim W, Lupton JR et al (2009) Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. Prostaglandins Leukot Essent Fatty Acids 81:187–191
17. Szkaradkiewicz A, Marciniak R, Chudzicka-Strugała I et al (2009) Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. Arch Immunol Ther Exp 57:291–294
18. Chung YC, Chang YF (2003) Serum C-reactive protein correlates with survival in colorectal cancer patients but is not an independent prognostic indicator. Eur J Gastroenterol Hepatol 15:369–373
19. Rickham PP (1964) Human experimentation. Code of ethics of the World Medical Association. Declaration of Helsinki. Br Med J 2:0–177
20. Peltola H, Valmari P (1985) Serum C-reactive protein as detector of pretreated childhood bacterial meningitis. Neurology 35:251–253
21. Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 31:87–96
22. Corrêa CR, Angelili AO, Camargo NR et al (2002) Comparação entre a relação PCR/albumina e o índice prognóstico inflamatório nutricional (IPIN). Jornal Brasileiro de Patologia e Medicina Laboratorial 38:183–190
23. Lohman TG, Roche AF, Martorell R (1988) Anthropometric standardization reference manual. Human Kinetics, Champaign
24. World Health Organization (1998) Obesity: preventing and managing the global epidemic. Report of a WHO consultation on obesity. WHO, Geneva
25. Detsky AS, McLaughlin JR, Baker JP et al (1987) What is subjective global assessment of nutritional status? J Parenter Enter Nutr 11:8–13
26. Durnin JV, Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77–97
27. Braga M, Gianotti L, Vignali A et al (2002) Preoperative oral arginine and fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. Surgery 132:805–814
28. Matsuda A, Furukawa K, Takasaki H et al (2006) Preoperative oral immune-enhancing nutritional supplementation corrects Th1/Th2 imbalance in patients undergoing elective surgery for colorectal cancer. Dis Colon Rectum 49:507–516
29. Read JA, Beale PJ, Volker DH et al (2007) Nutrition intervention using an eicosapenatenoic acid (EPA)-containing supplement in patients with advanced colorectal cancer. Effects on nutritional and inflammatory status: a phase II trial. Support Care Cancer 15:301–307
30. Liang B, Wang S, Ye YJ et al (2008) Impact of postoperative omega-3 fatty acid-supplemented parenteral nutrition on clinical outcomes and immunomodulations in colorectal cancer patients. World J Gastroenterol 21:2434–2439
31. Silva JA, Trindade EB, Fabre ME et al (2012) Fish oil supplement alters markers of inflammatory and nutritional status in colorectal cancer patients. Nutr Cancer 64:267–273
32. Zhu MW, Tang DN, Hou J et al (2012) Impact of fish oil enriched total parenteral nutrition on elderly patients after colorectal cancer surgery. Chin Med J 125:178–181
33. Purasiri P, Murray A, Richardson S et al (1994) Modulation of cytokine in vivo by dietary essential fatty acids in patients with colorectal cancer. Clin Sci 87:711–717
34. Erlinger TP, Platz EA, Rifai N et al (2004) C-reactive protein and the risk of incident colorectal cancer. JAMA 291:585–590
35. Gunter MJ, Stolzenberg-Solomon R, Cross AJ et al (2006) A prospective study of serum C-reactive protein and colorectal cancer risk in men. Cancer Res 66:2483–2487
36. Il'yasova D, Colbert LH, Harris TB et al (2005) Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev 14:2413–2418
37. Shiu YC, Lin JK, Huang CJ et al (2008) Is C-reactive protein a prognostic factor of colorectal cancer? Dis Colon Rectum 51:443–449
38. Ingembleek Y, Carpentier Y (1984) A prognostic inflammatory and nutritional index scoring critically ill patients. Internat J Vit Nutr Res 55:91–101
39. Wu S, Rhee KJ, Albesiano E et al (2009) A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 15:1016–1022
40. Chae WJ, Gibson TF, Zelterman D et al (2010) Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. PNAS 107:5540–5544
41. Chae WJ, Bothwell AL (2011) IL-17 and intestinal tumorigenesis. Drugs Discov Today: Dis Mech 8:e79–e83
42. Sánchez LK, Ugalde ME, Motola KD et al (2012) Gastrointestinal symptoms and weight loss in cancer patients receiving chemotherapy. Br J Nutr 12:1–4
43. Furukawa T, Tashiro H, Yamamori K et al (1999) Effects of soybean oil emulsion and eicosapentaenoic acid on stress response and immune function after a severely stressful operation. Ann Surg 229:255–261