PLASMA AND ERYTHROCYTE UPTAKE OF OMEGA-3 FATTY ACIDS FROM AN INTRAVENOUS FISH OIL BASED LIPID EMULSION IN PATIENTS WITH ADVANCED OESOPHAGOGASTRIC CANCER

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ABSTRACT

Background: It has been demonstrated that **s**hort term intravenous (IV) administration of omega-3 polyunsaturated fatty acids (PUFAs) is more effective than oral supplementation at promoting incorporation of the bioactive omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into plasma, blood cells and tissues. The effect of repeated short term IV infusion of omega-3 PUFAs was investigated in patients with advanced oesophagogastric cancer during palliative chemotherapy.

Methods: Patients with advanced oesophagogastric cancer (n = 21) were recruited into a phase II pilot clinical trial. All patients were scheduled for an intravenous infusion of Omegaven® (fish oil supplement containing EPA and DHA) at a rate of 2 ml/kg body weight for 4 hours once a week for up to six months. Blood samples were collected to assess omega-3 PUFA uptake into plasma non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC) and into red blood cell (RBC) membranes. Fatty acid profiles were analysed by gas chromatography.

Results: Twenty patients received at least one Omegaven® treatment and were included in the analysis. Each infusion of omega-3 PUFAs resulted in increased EPA and DHA in plasma NEFAs, but there was little effect on PUFAs within plasma PC during the infusions. However, with repeated weekly infusion of omega-3 PUFAs, the EPA content of plasma PC and of RBC membranes increased.

Conclusion: Repeated weekly omega-3 PUFA infusion is effective in enriching plasma PC and RBC membranes in EPA in patients with advanced oesophagogastric cancer receiving palliative chemotherapy..

Key words: cancer; oesophageal cancer; fish oil; gastric cancer; Omegaven; polyunsaturated fatty acids, parenteral nutrition

INTRODUCTION

As long ago as 1863 Rudolf Virchow, after noting the presence of leukocytes in cancer specimens, proposed a link between inflammation and cancer development [1-4]. Chronic inflammation leads to release of pro-inflammatory eicosanoids which are metabolites of the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA). These metabolites, which include prostaglandin E_2 and leukotriene B_4, play key roles in the initiation and propagation of colorectal, prostate, breast and pancreatic cancer [2,3,5,6]. In contrast, there is now much evidence that the omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory and anti-cancer properties [1,7-10].

Fish is the major dietary source of EPA and DHA and they are also found in fish oil supplements. One of the main mechanisms of their anti-inflammatory action involves opposing the production and effects of the AA-derived eicosanoids [7]. This mechanism of action is linked to the incorporation of EPA and DHA into cell membranes [11]. Because of the opposing actions of omega-6 and omega-3 PUFAs, both the content of omega-3 PUFAs and the ratio of omega-6 to omega-3 PUFAs in cell membranes are important determinants of their anti-inflammatory effects [12,13].

Omega-3 PUFAs may be administered by oral, enteral or parenteral means [14-16]. Carpentier *et al.* reported that intravenous (IV) infusion of a blend of 80% medium-chain triacylglycerol and 20% fish oil into healthy volunteers led to an increase in EPA in platelet and white blood cell phospholipids within 60 minutes and that the observed enrichment remained for 48 hours[17]. Another study demonstrated incorporation of omega-3 PUFAs from IV fish oil into plasma lipids and red blood cell (RBC) membranes in patients with advanced pancreatic cancer [18]. A study in rats showed that short term IV infusion of omega-3 PUFAs is more effective than oral supplementation at promoting incorporation of the bioactive omega-3 PUFAs EPA and DHA into plasma, blood cells and tissues [19]. Hence, we investigated the effect of once-weekly infusions of a fish oil-based lipid emulsion (Omegaven®) for six months in patients with advanced oesophagogastric cancer

receiving palliative chemotherapy. The outcomes were appearance of EPA and DHA in two plasma lipid fractions (i.e., non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC)), and in RBC membranes.

METHODS

Study design

This was a prospective, single arm clinical trial, evaluating the effect of using intravenous (IV) omega-3 PUFAs in patients with advanced oesophagogastric cancer receiving conventional platinum-based palliative chemotherapy.

Participants and setting

The study recruited adult patients referred to the University Hospitals of Leicester NHS Trust Oesophagogastric Cancer Service, Leicester, United Kingdom with confirmed diagnoses of oesophageal or gastric cancer. Inclusion criteria were, amongst others, patients with inoperable oesophageal, junctional or gastric cancer eligible for palliative platinum-based chemotherapy. Treatment intent along palliative lines was determined after discussion at the weekly multi-disciplinary team meeting by the clinical team.

Recruitment

The study received approval from the National Research Ethics Service East Midlands - Nottingham 2 Committee (reference number 11/EM/0412). Eligible participants were offered a participant information sheet at their first oncology clinic visit. A minimum of 24 hours later potential participants were contacted to enquire about trial participation. Participants were recruited between 1 May 2012 and 31 July 2013. Participant follow-up was continued for one year from the date of the last treatment, disease progression, or death. All participants provided written informed consent for trial inclusion.

Sample size

As this was a pilot and feasibility study, the sample size was selected on pragmatic grounds to make an estimate of recruitment, retention and drug toxicity. Using the Simon two stage model [20], the intention was to recruit 21 participants for the first stage of the study, perform interim analysis and proceed to recruitment of a further 24 participants, provided that eleven or more participants achieved a six month

progression free survival. Here the findings for the first 21 trial participants are reported.

Intervention

Participants received palliative chemotherapy with IV epirubicin (50 mg/m^2) and oxaliplatin (130 mg/m^2) every 21 days and oral capecitabine (1250 mg/m^2) daily for 21 days [21]. This is standard practice for care of these patients in the UK. For the trial, this regimen was coupled with IV infusion of omega-3 PUFAs as Omegaven® (FreseniusKabi, Bad Homburg, Germany). Omegaven® was infused once weekly at a rate of 2 ml/kg body weight for 4 hours (i.e., 140 ml over 4 hours in a 70 kg patient). Omegaven® is a 10% fish oil lipid emulsion described by the manufacturer as containing 1.25 to 2.82 g/100 ml EPA and 1.44 to 3.09 g/100 ml DHA. Chemical analysis by gas chromatography revealed the EPA and DHA contents of the batch of Omegaven® used in the current study to be 2.0 and 2.3 g/100 ml, respectively. Thus, patients received 0.04 and 0.046 g EPA and DHA/kg body weight during each 4 hour infusion; in a 70 kg patient this would equate to 2.8 g EPA and 3.2 g DHA during each infusion. Omegaven® was administered via a peripheral venous line immediately after the chemotherapy treatment on day 1 of each cycle and then again on days 8 and 15 of the cycle. Blood samples were collected prior to and immediately after each infusion for analysis of PUFAs in plasma NEFAs and plasma PC.

Outcome measures

The fatty acid composition of plasma NEFAs, plasma PC and RBC membranes was analysed over the entire treatment period of six months. Blood samples were taken immediately prior to and within 15 minutes of completion of each cycle of Omegaven® infusion (Figure 1). Plasma was prepared from all blood samples while RBCs were prepared only from the pre-infusion blood samples. Blood was collected into EDTA and plasma isolated by centrifugation at 1300 x g for 10 minutes. RBC membranes were isolated from the pellet by addition of serial dilutions of phosphate buffer saline (PBS) and centrifugation after each at 1300 x g for 10 minutes. All

samples were stored at -80°C until analysed. Clinical outcomes will be reported separately.

Fatty acid analysis by gas chromatography

Total lipid was extracted from plasma and RBC membranes with chloroform:methanol (2:1 vol/vol); butylated hydroxytoluene (50 mg/l) was added as an antioxidant. NEFAs and PC were isolated from the plasma lipid extract by solid phase extraction (SPE) on Bond-Elute cartridges. The lipid extract was loaded onto the SPE cartridge and triacylglycerols and cholesteryl esters were eluted with chloroform and discarded. Next, PC was eluted with chloroform:methanol (60:40, vol/vol) under vacuum suction. Finally, NEFAs were eluted with chloroform:methanol:glacial acetic acid (100:2:2, vol/vol/vol) under vacuum suction. Plasma NEFAs, plasma PC and RBC membrane lipids were dried down under nitrogen at 40°C and then redissolved in 0.5 ml of dry toluene. Then fatty acid methyl esters (FAMEs) were formed by reaction with methanol containing 2% (vol/vol) sulphuric acid and heating at 50°C for two hours. After cooling and neutralisation with $KHCO_3$ and K_2CO_3, FAMEs were extracted into hexane.

FAMEs were separated and identified by gas chromatography on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 column (30 m x 0.22 mm x 0.25 μm). The inlet temperature was 300°C. The oven temperature was initially 115°C and this was maintained for 2 min after injection. The oven temperature was programmed to increase to 200°C at the rate of 10°C/min to hold at 200°C for 16 min and increase to 240°C at the rate of 60°C/min to hold at 240°C for 2 min. The total run time was just longer than 29 min. Helium was used as the carrier gas. FAMEs were detected by using a flame ionization detector held at a temperature of 300°C. The instrument was controlled by, and data collected, with HPChemStation software (Hewlett-Packard). FAMEs were identified by comparison of retention times with those of authentic standards run previously. The following omega-3 and omega-6 PUFAs were identified - omega-3 PUFAs: α-linolenic acid, eicosatetraenoic acid, clupanodonic acid (aka docosapentaenoic acid), EPA and DHA; omega-6 PUFAs: linoleic acid,

gamma linolenic acid, eicosadienoic acid, dihomo-γ-linolenic acid, adrenic acid and arachidonic acid.

Statistical analysis

As data were not distributed normally, they are shown as median and interquartile range. Data were log transformed prior to analysis using SPSS version 21. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Of the 21 patients recruited, 20 received at least one Omegaven® infusion and are included in the intention-to-treat analysis (Table 1). These patients comprised 16 men and 4 women diagnosed with advanced oesophagogastric adenocarcinoma. Patients were aged 47 to 80 (median 67) years. Eighty four Omegaven® treatments were administered. As reported elsewhere[22], no patient experienced grade 3 or 4 hypertriglyceridemia related to Omegaven® infusion. Two patients required cisplatin instead of oxaliplatin chemotherapy due to peripheral neuropathy.

Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC following a single 4 hour infusion of Omegaven®

Data for the first Omegaven® infusion are shown in Table 2. There was a significant increase in the content of both EPA and DHA, and also of AA, in plasma NEFAs during the infusion (Table 2). Thus, there was a significant increase in total omega-3 PUFAs and a significant decrease in the ratio of omega-6 to omega-3 PUFAs in the NEFA fraction (Table 2). There was a strong trend for EPA content of plasma PC to increase during Omegaven® infusion and there was a small, but significant, decrease in the ratio of omega-6 to omega-3 PUFAs in the PC fraction (Table 2).

Repeatability of the increase in EPA, DHA and AA in plasma NEFAs following Omegaven® infusion

The increases in EPA, DHA and AA in plasma NEFAs were examined after each of the 24 infusions with Omegaven®; the results are shown in Figure 2. It is evident that the increases in EPA of about 3.5%, in DHA of about 5% and in AA of about 0.5% seen with the first infusion (Table 2) are highly repeatable across each of the later infusions (Figure 2).

Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC over the entire period of Omegaven® treatment

Blood samples collected prior to each Omegaven® infusion allowed the change in PUFAs in plasma NEFAs and plasma PC to be determined over the entire treatment

period of 6 months. Overall there was limited effect on the fatty acids in NEFAs. However, the content of EPA in plasma PC increased with increasing number of infusions (i.e., with time) ($p < 0.001$), as shown in Figure 3. In contrast, there was no significant change in plasma PC DHA or AA over time (Figure 3). Consequently, the total omega-3 PUFA content of plasma PC increased and the ratio of omega-6 to omega-3 PUFAs decreased with increasing number of infusions, although this did not reach statistical significance (data not shown).

Omega-6 and omega-3 PUFAs in RBC membranes over the entire period of Omegaven® treatment

Blood samples collected prior to each Omegaven® infusion allowed the change in PUFAs in RBC membranes to be determined over the entire treatment period of 6 months. Table 2 compares the data prior to the first and the final infusion. There was a significant increase in the content of EPA, but there were no significant changes in the content of DHA or AA in RBC membranes.

DISCUSSION

Fish, fatty fish and omega-3 PUFAs may have a role in prevention of some cancers [23, . 24]. Furthermore omega-3 PUFAs may have a role in cancer therapy. For example, DHA supplements taken orally during chemotherapy of breast cancer led to reduced toxicity and improved outcome of chemotherapy and chemosensitized breast tumours [25]. A combined EPA and DHA oral supplement increased efficacy of chemotherapy in patients with advanced non-small cell lung cancer which improved the response rate and had other clinical benefits [26]. The use of omega-3 PUFAs, either in capsules or as components of oral nutrition supplements, in the palliative management of gastrointestinal cancer has been investigated mainly in colorectal and pancreatic cancers [27-33]. In some other studies when omega-3 PUFAs were combined with palliative chemotherapy there was a reduction in chemotherapy related toxicity and better preservation of lean body weight [15,16,34]. Fish oil resulted in improved survival in three studies [28,30,33], while improvement in quality of life was seen in four studies. [15,28,32,34]

In the studies described above, fish oil was provided either as capsules or in oral nutrition supplements to patients with colorectal, breast, lung or prostate cancer, conditions in which swallowing is not compromised. However, the use of capsules or oral supplements would be more challenging for patients with advanced oesophageal cancer due to luminal obstruction and resultant dysphagia. In these patients, IV administration of omega-3 PUFAs (as fish oil) could be advantageous. Not only would this circumvent problems with swallowing, but omega-3 PUFAs were reported to be more easily incorporated into plasma, blood cells and tissues when infused intravenously in rats compared to when given orally [19]. Furthermore higher doses of EPA and DHA can be given intravenously than can be consumed orally and the IV route assures compliance. Non-compliance has been reported to be a problem in some studies of oral supplements in cancer patients [35,36]. It was considered that repeated short infusions over a period of several months could be a strategy for supplying omega-3 PUFAs to patients with advanced oesophagogastric cancer to increase their status of EPA and DHA.

Omegaven® is a fish oil supplement emulsified with purified egg phosphatidate. The fatty acids in the fish oil are largely present in the form of triacylglycerols i.e., fatty acids esterified to glycerol; EPA and DHA contribute about 40 to 45% of the fatty acids present. Upon infusion, the triacylglycerols are hydrolysed in the circulation by lipases releasing NEFAs. During the course of a single infusion we observed a marked increase in EPA and DHA in the NEFA pool, an average of 18.5- and 7-fold increases, respectively. This is consistent with the aforementioned hydrolysis of the triacylglycerol component of the fish oil and is important because the released non-esterified omega-3 PUFAs would be made available to cells and tissues where they could elicit their biological effects. These would include host cells involved in inflammation, immune and metabolic responses but also cancer cells. These cells would take the fatty acids up by general free fatty acid uptake mechanisms [37], but in addition some cells, including inflammatory macrophages, express receptors that have some specificity for omega-3 PUFAs, particularly DHA [38]. Thus, this rapid release of non-esterified EPA and DHA would act to facilitate the functional activities of these fatty acids. There was also a 50% increase in non-esterified AA during infusion. This is most likely because Omegaven® contains AA (0.1 to 0.4 g per 100 ml according to the manufacturer) which would also be freed by lipases. Patients received repeated weekly infusions of Omegaven® for up to 6 months. The appearance of EPA, DHA and AA in the NEFA pool was very similar with each infusion. As far as we are aware, this is the first time that fatty acid changes with such a repeated regimen of fish oil infusion have been reported.

Plasma PC, EPA, but not DHA, increased by a small amount during infusion. PC acts as a monolayer "coat" on lipoproteins and the small increase in plasma PC EPA during the 4 hour infusion would suggest recycling of the non-esterified EPA that originated in Omegaven® into PC over that period. This most likely occurs in the liver. It is not clear why DHA does not appear in plasma PC during a four hour infusion, but appearance of DHA in plasma PC takes longer than appearance of EPA [39], probably reflecting different metabolic handling of the two omega-3 PUFAs.

Arshad *et al.* used a weekly short term (2 hour) infusion of a lipid emulsion providing omega-3 fatty acids in patients with advanced pancreatic cancer or three consecutive weeks followed by a rest week, and assessed pre-infusion fatty acid levels for up to six months. In that study, post-infusion levels were measured only for seven weeks [18]. The novelty of the current study is its use of repeated short-term infusions (a single infusion of 4 hours each week) over a long period of time (up to 6 months). This regimen resulted in increased EPA in plasma PC and in RBC membranes. These increases were progressive over time, suggesting a gradual accumulation of EPA in these pools. This demonstrates that this approach enables net accumulation of EPA in blood lipid and cell pools and this would be expected to influence cell and tissue function. Oral supply of EPA results in a time-dependent accumulation of EPA in plasma PC and in RBC membranes [39]. In the current study, DHA did not accumulate in the way that EPA did. It is not clear why this is the case, since DHA accumulates in plasma PC and RBC membranes when taken as a regular oral supplement over a period of time, although accumulation of DHA is slower than that of EPA [39]. Whatever the reason, the observation suggests that the DHA provided in each infusion is used by the body in a different way than the EPA and that it may not accumulate.

The regimen of repeated infusions of fish oil did not result in net accumulation of EPA or DHA in plasma NEFAs assessed prior to each infusion. In this state, most NEFAs would be derived from hydrolysis of triacylglycerols stored in adipose tissue. The absence of any accumulation of EPA or DHA in plasma NEFAs would suggest that there is very limited or no storage of infused EPA and DHA in adipose tissue. It is worth noting that oral supplementation with high doses of EPA and DHA for periods as long as one year results in only very small accumulation of those fatty acids in adipose tissue [39,40].

The ratio of omega-6 to omega-3 PUFAs in the diet, in blood lipids and in cells and tissues is thought to be important in influencing metabolism and cellular processes, including proliferation of cancer cells [12]. In the current study, the changes in the fatty acid content of plasma NEFAs during infusion and in plasma PC

following repeated infusions resulted in a lowered ratio of omega-6 to omega-3 PUFAs. This would likely be of functional significance.

The novelty of its design is a strength of the current study, as is its measurement of the fatty acid composition of two plasma lipid pools and one cellular (RBC) pool. A limitation is its small sample size. A control group not receiving intravenous fish oil was not necessary in the current study because in the absence of an exogenous supply of EPA and DHA, their concentrations in plasma NEFAs, plasma PC and RBC membranes do not change, as shown by previous long term oral supplementation studies [39,40] and in short term intravenous infusion studies [41,42]

In the current study, an omega-3 PUFA containing lipid emulsion was administered IV in patients receiving platinum-based chemotherapy. The aim was to provide the two biologically active omega-3 PUFAs EPA and DHA. Roodhart et al. [43] demonstrated that another omega-3 PUFA, 16:4n-3, could be generated in the presence of cisplatin and other platinum compounds and that it could induce resistance to those compounds in some model systems. More recently, Daenen et al. [44] reported that 16:4n-3 is present at variable concentrations in many fish oils and that its concentration in human blood plasma is increased following consumption of fish oil supplements. So far it has not been possible to measure 16:4n-3 in Omegaven® or in the human blood plasma samples generated as part of this study. It is important to note that the analysis of 16:4n-3 and other platinum-induced fatty acids is technically challenging; that common techniques for fatty acid analysis, such as gas chromatography used in the current study, lack the sensitivity required to detect the low concentrations of 16:4n-3; and that standards of so-called platinum-induced fatty acids are not yet commercially available. It will be important in future studies to gather information on the 16:4n-3 content of blood and blood cells and to better understand its roles in cancer patients.

In conclusion, a 4 hour infusion with Omegaven® enriched plasma NEFAs with EPA and DHA in patients with advanced oesophagogastric cancer receiving palliative chemotherapy, while repeated 4 hour infusions once a week for several months enriched plasma PC and RBC membranes with EPA.

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STATEMENT OF AUTHORSHIP

AM Eltweri, AL Thomas, DJ Bowrey, A Arshad and AR Dennison contributed to the study design and planning. AM Eltweri and DJ Bowrey as lead investigators sought research ethics committee approval for the study and coordinated the study on an ongoing basis. AM Eltweri and AL Thomas recruited trial participants. AM Eltweri and A Arshad supervised all Omegaven infusions and performed all blood sampling. AM Eltweri and HL Fisk conducted all laboratory analyses under the supervision of PC Calder. AM Eltweri conducted the data analysis. AM Eltweri, PC Calder and DJ Bowrey drafted the initial manuscript. AR Dennison, AL Thomas, HL Fisk and A Arshad revised the initial manuscript. All authors have seen and approved the final draft.

CONFLICT OF INTEREST

AME, ARD and DJB received departmental grant support for this work from Fresenius-Kabi. PCC and ARD have received speaking honoraria from Fresenius-Kabi. DJB has received departmental grant support for unrelated work from Nutricia. The other authors have no conflicts to declare.

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536 **Table 1: Characteristics of patients included in the trial**

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Characteristic		n=21
Sex	Male	16
	Female	5

Age	Median age in years (range)	67 (47-80)
	Number aged >60 years	16
	Number aged <60 years	5
WHO/ECOG Performance status	0	8
	1	9
	2	4
Baseline Weight	Median weight in kg (range)	76.5 (49.0-110.6)
Tumour site	Oesophagus	11
	Gastro-oesophageal junction	5
	Stomach	5
UICC Stage	Stage 3	3
	Stage 4	18
Site of metastasis	Local or lymph node metastasis	5
	One distant organ	11
	Two or more distant organs	5
Total number of chemotherapy cycles delivered (median)		91 (6)
Number of patients completing 4 cycles of chemotherapy (%)		12 (60%)
Number of patients completing 6 cycles of chemotherapy (%)		11 (55%)

538 **ECOG = Eastern Cooperative Oncology Group; UICC = Union for International**
539 **Cancer Control; WHO = World Health Organisation**

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Table 2: Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC prior to and at the end of the first Omegaven® infusion

	Plasma NEFAs			Plasma PC		
Fatty acid	Pre-Omegaven® infusion	Post-Omegaven® infusion	P	Pre-Omegaven® infusion	Post-Omegaven® infusion	P
EPA	0.2 (0.1-0.6)	3.7 (2.8-4.3)	<0.001	0.9 (0.6-1.1)	0.9 (0.7-1.3)	0.067
DHA	0.9 (0.6-1.2)	6.3 (5.7-8.3)	<0.001	3.4 (2.8-3.8)	3.4 (2.8-3.7)	0.715
AA	1.2 (0.9-2.1)	1.8 (1.4-2.0)	0.035	8.6 (7.6-10.5)	8.5 (7.4-10.7)	0.315
Total omega-6 PUFAs	12.2 (11.1-13.0)	11.0 (9.7-12.6)	0.142	31.6 (30.3-34.4)	31.8 (30.1-33.2)	0.900
Total omega-3 PUFAs	2.4 (1.9-3.5)	12.6 (10.5-14.9)	<0.001	5.6 (4.9-6.3)	5.4 (5.0-6.1)	0.001
Omega-6 to Omega-3 PUFA ratio	3.5 (2.9-4.5)	1.0 (0.9-1.0)	<0.001	5.0 (4.4-5.4)	4.8 (4.5-5.1)	0.046

Data are median and interquartile range percentage of total fatty acids (n = 20 patients).

P values were calculated using paired sample t-test on log transformed data.

AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid (EPA); NEFAs = non-esterified fatty acids; PC = phosphatidylcholine; PUFA = polyunsaturated fatty acid

554 **Table 3: Omega-6 and omega-3 PUFAs in RBC membranes prior to the first and**
555 **last infusion of Omegaven®**

Fatty acid	Prior to first Omegaven® infusion	Prior to final Omegaven® infusion	*P*
EPA	0.4 (0.4 – 0.6)	0.9 (0.9 – 1.0)	0.027
DHA	3.4 (2.1 – 4.3)	2.5 (1.4 – 3.6)	0.534
AA	12.6 (8.9 – 16.4)	7.1 (3.2 – 10.9)	0.281

556 Data are median and interquartile range percentage of total fatty acids..

557 P values were calculated using paired sample t-test on log transformed data.

558 AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid;

559 PUFA = polyunsaturated fatty acid; RBC = red blood cell

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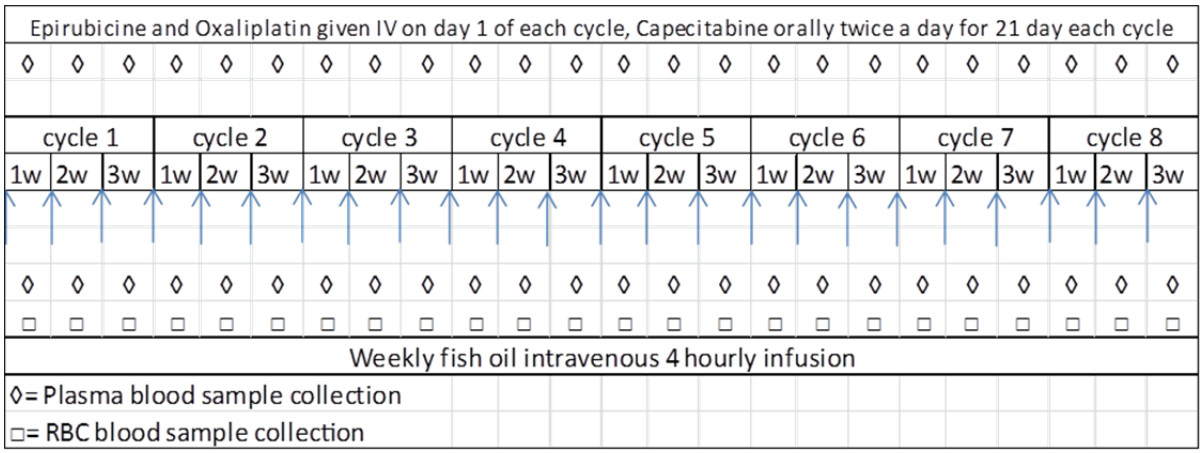

Figure 1: Overview of timing of Omegaven® infusion and blood sample collection. Chemotherapy (epirubicin, oxaliplatin, capecitabine) was administered in three weekly cycles, for up to eight cycles. Omegaven® was administered on a weekly basis, for up to 24 cycles.

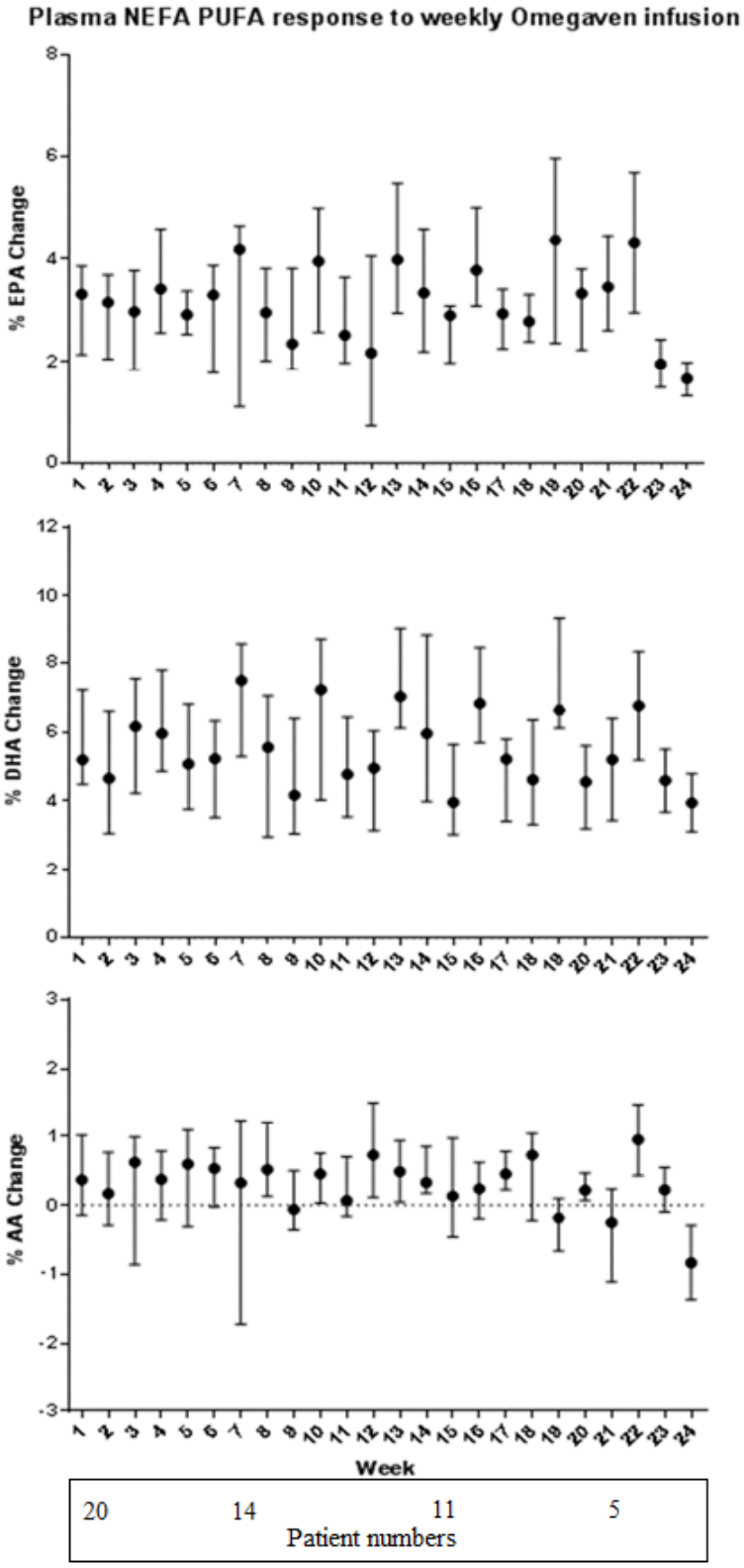

Figure 2: Percentage change in EPA, DHA and AA in plasma NEFAs for each weekly infusion of Omegaven® i.e., comparison of immediate post- with pre-infusion levels for each cycle. Data are median and interquartile range, and are for decreasing numbers of patients as the time increases.

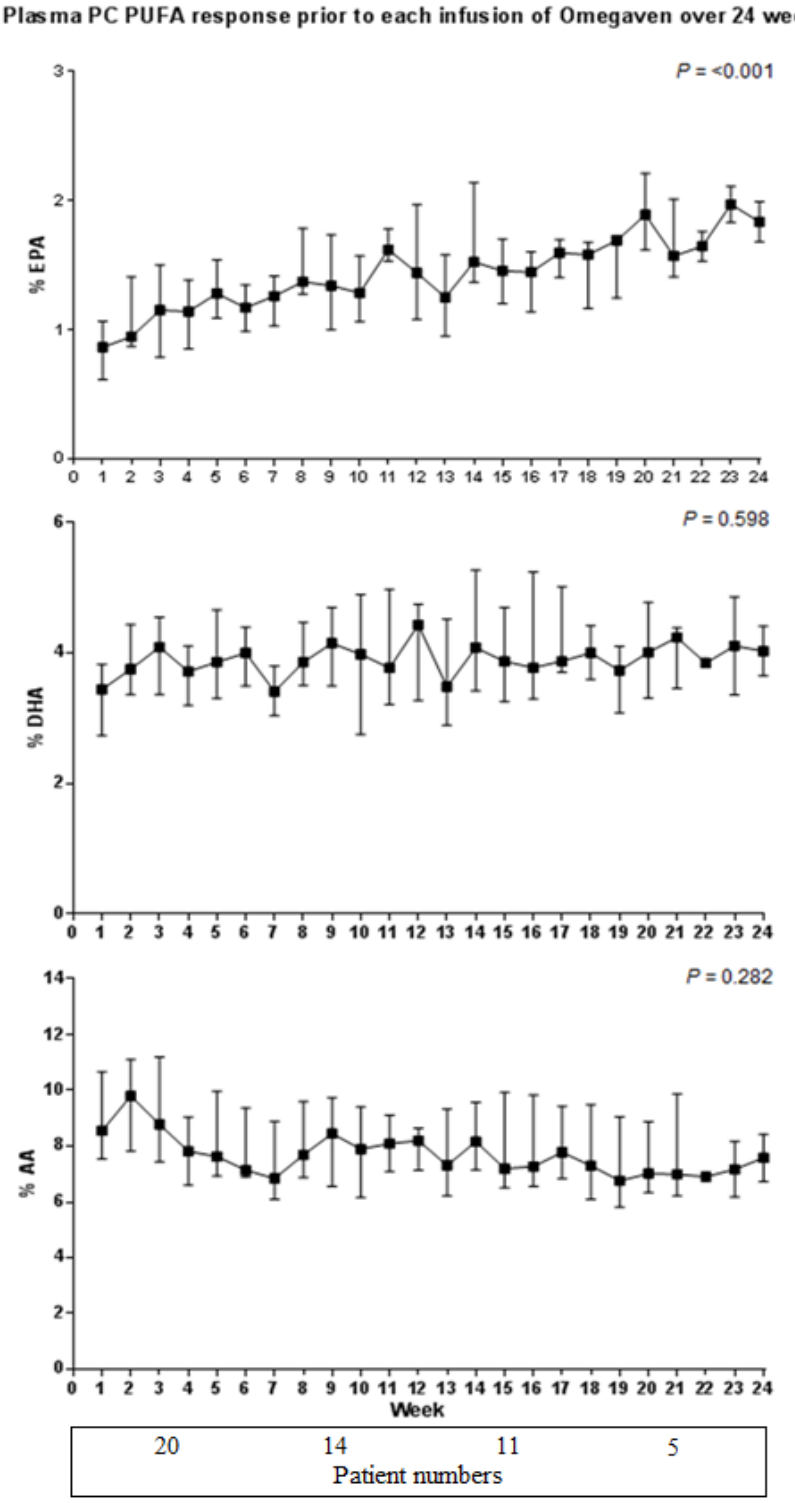

Figure 3: Percentage of EPA, DHA and AA in plasma PC prior to each infusion of Omegaven® i.e., comparison of each baseline pre-infusion level for up to 24 weeks. Data are median and interquartile range, and are for decreasing numbers of patients as the time increases.