The Impact of an Omega-3 Fatty Acid Rich Lipid Emulsion on Fatty Acid Profiles in Critically Ill Septic Patients

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Conflicts of Interest: PCC advises and receives speaking fees from Fresenius Kabi, manufacturer of Omegaven™. The other authors declare no conflict of interest.

Summary

Death from sepsis in the intensive therapy unit (ITU) is frequently preceded by the development of multiple organ failure as a result of uncontrolled inflammation. Treatment with omega-3 fatty acids (FAs) has been demonstrated to attenuate the effects of uncontrolled inflammation. A study investigating the effects of parenteral nutrition providing fish oil (FO) was conducted. Septic ITU patients were randomised to receive either parenteral FO and standard medical care or standard medical care only.

FA composition of plasma phosphatidylcholine (PC), plasma non-esterified FAs (NEFAs) and peripheral blood mononuclear cells (PBMCs) was determined by gas chromatography. EPA and DHA were rapidly incorporated. There was a reduction in the arachidonic acid (AA) to EPA+DHA ratio in plasma PC and NEFAs. Fewer patients died in the FO group compared with the control group although this was not statistically significant. A reduction in the AA/(EPA+DHA) ratio in PBMCs and plasma PC was associated with improved survival.

Abstract

Background: Death from sepsis in the intensive therapy unit (ITU) is frequently preceded by the development of multiple organ failure as a result of uncontrolled inflammation. Treatment with omega-3 (n-3) fatty acids (FAs), principally eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been demonstrated to attenuate the effects of uncontrolled inflammation and may be clinically beneficial in reducing mortality from organ dysfunction. Fish oil (FO) is a source of EPA and DHA.

Methods: A randomized trial investigating the effects of parenteral (intravenous) nutrition providing FO (0.092 g EPA+DHA/kg body weight/day) was conducted. Sixty consecutive ITU patients diagnosed with sepsis were randomised to receive either once daily parenteral FO and standard medical care or standard medical care only.

Results: Forty one patients (21 received fish oil; 20 controls) consented to blood sampling and blood was taken on days 0, 1, 2, 3, 5, 7, 10 and 13; because of deaths, patient discharge and withdrawal of consent, the number of blood samples available for analysis diminished with time. FA composition of plasma phosphatidylcholine (PC), plasma non-esterified FAs (NEFAs) and peripheral blood mononuclear cells (PBMCs) was determined by gas chromatography. EPA and DHA were rapidly incorporated into all 3 lipid pools investigated. There was a reduction in the arachidonic acid (AA) to EPA+DHA ratio in plasma PC and NEFAs. Fewer patients died in the FO group (13.3% (n=4)) compared with the control group (26.7% (n=8)) but this difference was not significant. A reduction in the AA/(EPA+DHA) ratio in PBMCs and plasma PC was associated with significantly improved survival. Plasma PC, plasma NEFA and PBMC FA profiles are rapidly altered by FO infusion in critically ill septic patients.

Conclusion: The provision of high dose n-3 FAs resulted in a rapid and significant increase in EPA and DHA and a reduction in AA/(EPA+DHA) ratio. This latter reduction is associated with improved survival.

Keywords: Sepsis, Intensive care unit, Intensive therapy unit, Fish oil, Omega-3, Fatty acids

1 Introduction

Intensive therapy units (ITUs) will inevitably contain the sickest, most metabolically stressed patients in any care setting. Consequently, mortality rates in ITUs are high, sometimes as high as 60%, despite the improved understanding of the pathophysiology of sepsis [1,2]. Death from sepsis in the ITU is frequently preceded by the development of multiple organ failure as a result of uncontrolled inflammation [3-5] . Sepsis is a serious and complex inflammatory process that is characterised by a systemic inflammatory response to the presence of an infection.

Omega-3 (n-3) fatty acids (FAs), principally eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown in cell and animal models to have anti-inflammatory effects [6-8]. We recently reported that parenteral administration of n-3 FAs is associated with a significant reduction in organ dysfunction and C-reactive protein (CRP) concentration and may be associated with a reduction in mortality in patients with less severe sepsis [9].

Two recent systematic reviews and meta-analyses have been published investigating the effects of n-3 FAs in the critically ill patient but these did not demonstrate definitively improved outcomes [10,11]. Major confounding factors in the studies analysed included FO being given in differing amounts, as a bolus versus a slow infusion, by different routes (enteral and parenteral) and often in combination with other immuno-modulating nutritional support. In order to optimise the parenteral use of FO for improved patient outcome it seems important to understand more about the incorporation of its bioactive fatty acids, EPA and DHA, in ITU patients. Factors such as the timing/duration of parenteral FO and patient factors (such as age and sex) may influence the efficacy of FO and have been hitherto poorly explored.

The aim of this present study was to examine the FA composition of various blood lipid pools in septic patients treated with parenteral FO, to relate these to mortality and to investigate factors that might affect n-3 FA incorporation (age and sex). The lipid pools measured, which are all pertinent to sepsis,

were plasma phosphatidylcholine (PC), representing the major phospholipid in the circulation, plasma non-esterified FAs (NEFAs), which represent a direct route of exposure of bioactive fatty acids to cells and tissues, and peripheral blood mononuclear cells (PBMCs), representing cells with a functional role of particular relevance to inflammation, critical illness and sepsis. Thus, our measurements of the FA composition of plasma PC, plasma NEFAs and PBMCs are indicative of the potential of the infused lipid emulsion to modulate cell and tissue function, which in turn may influence clinical course and outcome. It is for this reason that we were interested in the time course of FA composition changes, because it may be desirable in some clinical settings to provide n-3 FAs quickly. The main clinical outcomes from this trial have been published recently[24].

2 Materials and Methods

2.1 Study design

The study was performed in a 9-bed general and surgical ITU and a 4-bed general and surgical high dependency unit (HDU) in a single tertiary-referral hospital. The study protocol was reviewed and approved by the National Research Ethics Service (South East Coast Research Ethics Committee (reference number 09/H1102/111)) and the study was conducted in accordance with the Helsinki declaration. From May 2010 until July 2012 sixty consecutive adult patients admitted to the ITU or HDU with sepsis or who developed new sepsis whilst on the ITU for other non-infectious pathologies were prospectively enrolled into the study.

Sepsis was defined as a proven or suspected source of infection together with at least two of the four markers of the systemic inflammatory response syndrome (SIRS), namely temperature >38°C or <36°C, heart rate >90 beats/min, white cell count >12 or <4 x 10^9, or respiratory rate >20 or $PaCO_2$ < 4.2 kPa. Septic patients were enrolled into the study within 12 hours of

admission to the ITU or within 12 hours of new onset sepsis, as diagnosed by the intensivists. Written informed consent was taken from the patient where possible or from a legal/professional representative if the patient lacked capacity. A 12-hour window was allowed for the intensivists to establish a clinical diagnosis of sepsis, to obtain the necessary written consent and to randomise the patients. Patients were randomised using sealed envelopes to receive either standard care or standard care together with infusion of a lipid emulsion based upon FO (Omegaven™; Fresenius Kabi, Bad Homburg, Germany). Full details of the trial methodology may be found elsewhere [9].

2.2 Fish oil infusion

A FO based lipid emulsion (Omegaven™) was given according to the manufacturer's guidelines. Omegaven™ is a 10% lipid emulsion i.e. it contains 100 g lipid/l. The FA component is provided by FO and this comprises 96.3% of the lipid; there is also 2.5 g glycerol/l and 1.2 g egg phospholipid/l. The FA composition of the emulsion is shown in Table 1. Because FO is a natural product, its FA composition can vary and therefore the FA composition of Omegaven™ can vary (see Table 1**).** We measured the FA composition of a typical batch of Omegaven™ used in the current study and found that it contained 25% of FA as EPA and about 21% as DHA (see Table1). Omegaven™ was infused daily at 2 ml/kg body weight/day (i.e. 0.2 g lipid/kg body weight/day) at a rate of 0.5 ml/kg/hour. Omegaven™ was given daily until death or discharge from the ITU/HDU.

Table 1: Fatty acid composition of Omegaven™

	Concentration (g/l)	
Fatty Acid	**As provided by manufacturer**	**As measured**
Myristic acid (14:0)	1.0–6.0	4.5
Palmitic acid (16:0)	2.5-10.0	13.2
Palmitoleic acid (16:1n-7)	3.0–9.0	8.2
Stearic acid (18:0)	0.5–2.0	3.3
Oleic acid (18:n-9)	6.0–13.0	10.6
Linoleic acid (18:2n-6)	1.0–7.0	3.3
Alpha-linolenic acid (18:3n-3)	~2.0	1.2
Arachidonic acid (20:4n-6)	1.0–4.0	1.7
Eicosapentaenoic acid (20:5n-3)	12.5–28.2	25.0

Docosapentaenoic acid (22:5n-3)	1.5–4.5	2.1
Docosahexaenoic acid (22:6n-3)	14.4–30.9	20.8
Total fatty acids	96.3	-

2.3 Blood sampling and plasma and PBMC isolation

Blood samples were collected in all patients on days 0, 1, 2, 3, 5, 7, 10 and 13. In those patients randomised to receive FO, day 0 refers to the time pre parenteral infusion. In addition, two patients consented to 4 hourly blood samples taken over 24 hours from the time at which the first FO infusion was commenced for an analysis of the FA response during a single dose of Omegaven^{TM}. Blood was collected heparin-coated vacutainers and centrifuged to obtain plasma which was stored at -80°C until analysis. PBMCs were isolated by centrifugation of blood on a density medium gradient (Histopaque-1077; Sigma-Aldrich, Poole, UK) using the manufacturer's instructions and as described in detail elsewhere [12]. PBMCs are a mix of lymphocytes (~85% of cells) and monocytes (~15% of cells) and, using the procedure outlined, are not likely contaminated with other cell types. Samples were stored at -80°C for 3 to 12 months until analysis.

2.4 Fatty acid analysis

PC and NEFAs were isolated from plasma by solid-phase extraction[28]. The FA composition of plasma PC, plasma NEFAs and PBMCs was determined by gas chromatography as described in detail elsewhere [13].

2.5 Clinical data collection

Baseline demographics and clinical data were recorded for 2 weeks or until death or discharge following enrolment in the study. In addition, date of discharge from ITU, discharge from the acute hospital and 28-day mortality were recorded. Microbiological cultures were taken as directed by the intensivists. Patients exited the trial when discharged from the ITU/HDU, at day 14, due to mortality or if they withdrew consent.

2.6 Statistical analysis

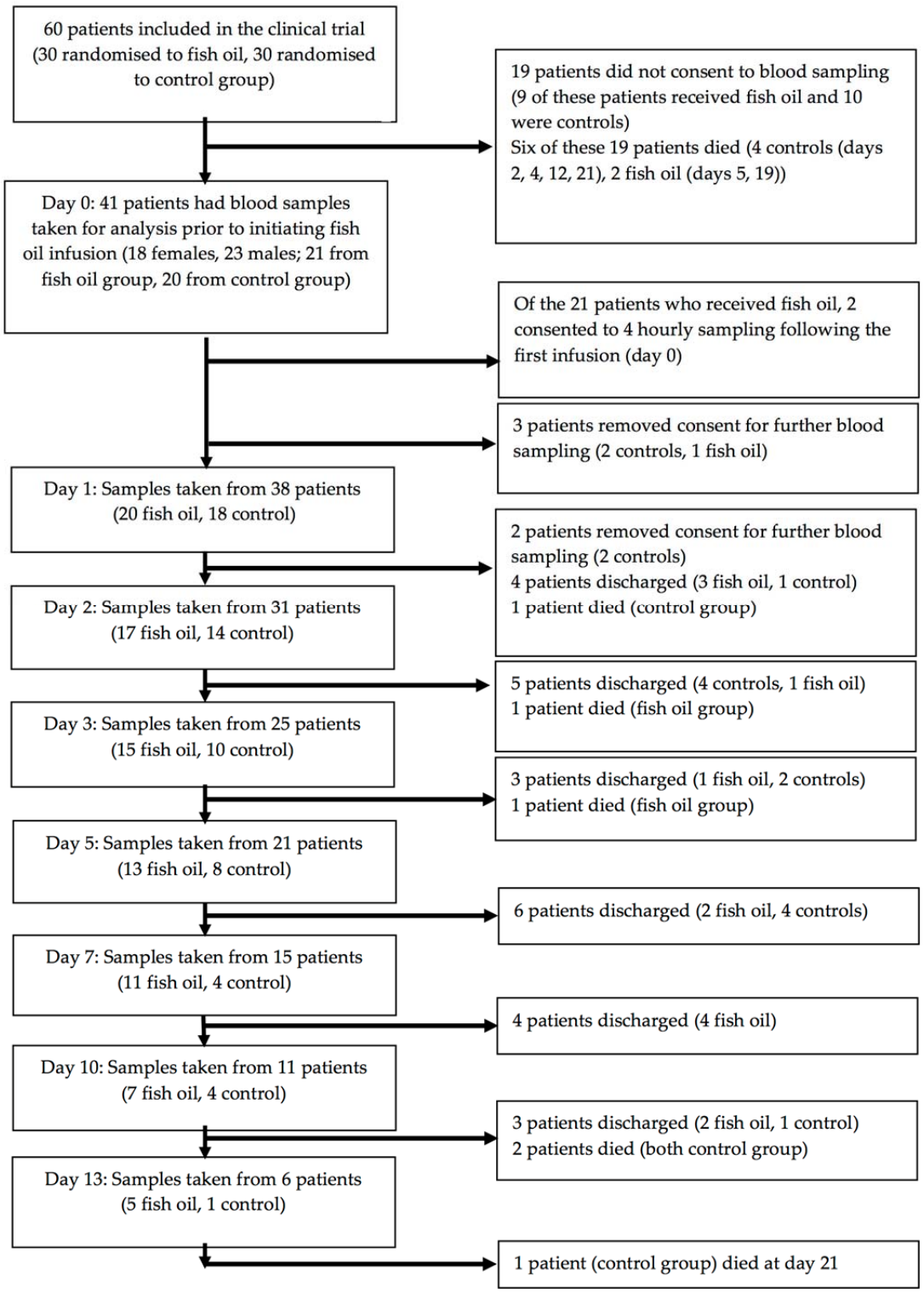
The Shapiro-Wilk test was used to determine if the continuous data variables were distributed normally or not. Normally distributed data are reported as mean and with standard deviation (SD). Categorical variables are expressed as numbers and percentages. Normally distributed data were analysed using the 2-tailed t-test and non-normally distributed data were analysed with the Mann Whitney U test. Categorical data were analysed using the Pearson Chi-square and Fishers exact test as appropriate. Analysis was conducted use of SPSS version 20. In all cases a value of $p < 0.05$ was taken to indicate statistical significance.

3 Results

3.1 Flow of patients through the study

Sixty patients were recruited into the study. These comprised 27 females and 33 males. The age range was 39 to 89 y with a mean (SD) age of 64.1 (12.2) y. The main causes for entry into the ITU were sepsis and post operative care. Thirty patients were randomised to each group. Figure 1 shows the flow of patients through the study, the reasons for loss at each time point, and the number of samples available for each type of analysis at each time point. Nineteen patients withdrew consent or did not permit consent for blood sampling. Therefore, blood samples from 41 patients were analysed for FAs at least once (n = 23 males and n = 18 females). Mortality in the group treated with FO (n=30) and in the control group (n=30) was 13.3% (n=4) and 26.7% (n=8), respectively (p=0.197). In the patients who consented to blood sampling, there were 2 deaths in the FO group (9.5%) and 4 in the control group (20%).

Figure 1. Flow of patients through the study



3.2 Study entry fatty acid levels

Mean FA levels for each of the 3 lipid pools at study entry (day 0) are shown in Table 2; there were no differences between patients who were to receive FO or not. The most abundant FAs differed depending on the pool analysed.

In plasma PC the most abundant FAs were 16:0 (palmitic acid), 18:2n-6 (linoleic acid) and 18:1n-9 (oleic acid). In plasma NEFAs the most abundant FAs were oleic acid and palmitic acid. In PBMCs the most abundant FAs were stearic acid (18:0), palmitic acid and oleic acid. Arachidonic acid (AA; 20:4n-6), EPA and DHA were present in smaller quantities. Absolute concentrations of FAs were also measured for the two plasma lipid pools. In PC, the baseline concentrations (ug/ml plasma) of AA, EPA and DHA were 54.51 ± 6.78, 5.40 ± 1.22 and 14.98 ± 2.34, respectively. The mean AA/(EPA+DHA) ratio was 2.51, 1.14 and 5.45 in plasma PC, plasma NEFAs and PBMCs respectively.

Table 2: Fatty acids in plasma phospatidylcholine (PC), plasma non-esterified fatty acids (NEFAs) and peripheral blood mononuclear cells (PBMCs) at study entry (day 0). Data are mean ± SD % total fatty acids in each pool and are for 41 patients.

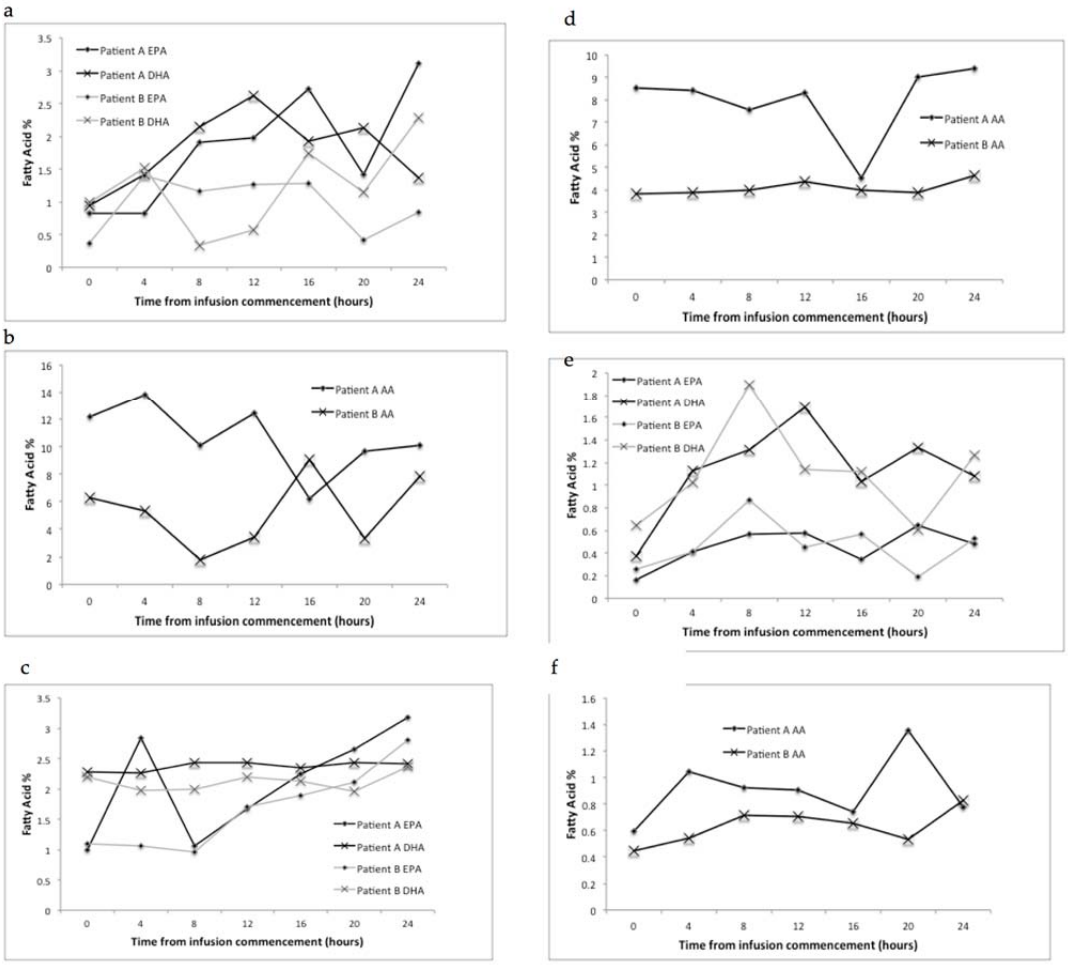
Fatty acid	Plasma PC	Plasma NEFAs	PBMCs
Myristic acid (14:0)	0.35±1.4	1.36±0.38	0.95±0.64
Palmitic acid (16:0)	32.87±2.04	24.77±0.78	25.13±5.19
Palmitoleic acid (16:1n-7)	1.16±0.58	2.79±1.09	1.28±0.70
Stearic acid (18:0)	12.62±1.79	15.99±4.99	25.25±7.75
Oleic acid (18:1n-9)	17.07±2.18	36.17±6.66	24.33±4.23
Vaccenic acid (18:1n-7)	2.33±0.70	2.29±0.70	2.07±0.58
Linoleic acid (18:2n-6)	19.64±3.78	9.22±2.82	7.24±3.97
Gamma-linolenic acid (18:3n-6)	0.10±0.06	0.31±0.19	0.43±0.32
Alpha-linolenic acid (18:3n-3)	0.31±0.19	1.30±0.38	0.77±0.57
Arachidic acid (20:0)	0.17±0.06	0.99±0.58	0.90±0.64
Gondoic acid (20:1n-9)	0.20±0.13	0.58±0.26	0.95±0.51
Eicosadienoic acid (20:2n-6)	0.27±0.13	0.34±0.19	0.43±0.64
Dihomo-γ-linolenic acid (20:3n-6)	1.98±0.64	0.61±0.38	0.93±0.51
Arachidonic acid (20:4n-6)	7.13±1.92	1.35±0.76	8.05±0.19
Behenic acid (22:0)	0.08±0.04	0.15±0.64	0.16±0.45
Eicosatetraenoic acid (20:4n-3)	0.22±0.13	0.18±0.13	0.19±0.69
Eicosapentaenoic acid (20:5n-3)	0.74±0.04	0.28±0.19	0.50±0.13
Adrenic acid (22:4n-6)	0.03±0.06	0.06±0.64	0.04±0.19
Docosapentaenoic acid (22:5n-3)	0.64±0.03	0.36±0.13	0.94±0.26
Docosahexaenoic acid (22:6n-3)	2.10±0.77	0.90±0.45	0.97±0.06

3.3 Acute appearance of FA after the first FO infusion

Two patients consented to have blood samples taken every 4 hours to allow for analysis of FA appearance in the three blood pools being studied following the start of the first FO infusion until the beginning of the next infusion, 24 hours later. The patients weighed 72 kg and 86 kg and received 144 ml and 172 ml of Omegaven™, respectively, both over 4 hours. This would have provided ~6.6 and ~7.9 g EPA+DHA, respectively.

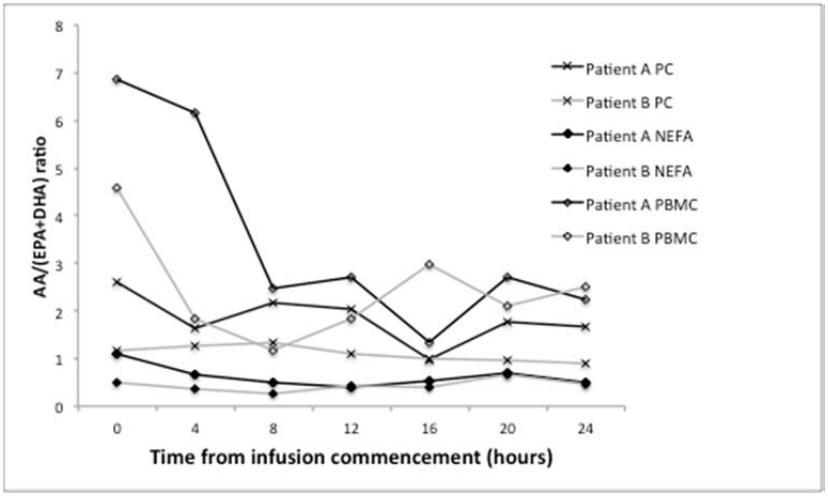
Both EPA and DHA were incorporated into PBMCs (Figure 2a). This was particularly evident in patient A, who also demonstrated a decline in PBMC AA (Figure 2b). EPA was incorporated into plasma PC, with an increase in content over the 24 h period (Figure 2c). At the end of the 24 h period the EPA level in plasma PC had trebled from baseline in both patients A and B. In contrast, both DHA and AA were stable in plasma PC with little change over the 24 h (Figure 2c and 2d). Levels of EPA and DHA in plasma NEFAs increased up to 8 to 12 h, trebling from baseline, and then declined (Figure 2e), AA also appeared to increase in plasma NEFAs over 8 to 12 h before declining (Figure 2f).

Figure 2. Acute appearance of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (a, c, e) and arachidonic acid (AA) (b, d, f) in peripheral blood mononuclear cells (a, b), plasma phosphatidylcholine (c, d) and plasma non-esterified fatty acids (e, f) in patients receiving a single short infusion (4 hours) of Omegaven™. Data are from two patients (A and B).



There was decrease in the AA/(EPA+DHA) ratio in PBMCs in both patients (Figure 3). Whether the ratio changed in plasma PC and NEFAs over this time period is not clear (Figure 3).

Figure 3. The ratio of arachidonic acid to eicosapentaenoic acid plus docosahexaenoic acid (AA/(EPA+DHA)) in plasma phosphatidylcholine (PC) and non-esterified fatty acids (NEFAs) and in peripheral blood mononuclear cells (PBMCs) in patients receiving a single 4 h infusion of Omegaven™. Data are from two patients (A and B).



3.4 Fatty acid profile over 14 days

Marked effects of Omegaven™ infusion were seen for EPA and DHA in all lipid pools studied (see below), but other FAs, including AA, were largely unaltered in any pool (data not shown).

3.4.1 FAs in PBMCs

Both EPA and DHA increased in PBMCs in the FO treated group (Figures 4a and b), such that EPA was higher in the FO group than in the control group at days 1, 2 and 3 and DHA was higher at days 3 and 7. PBMC EPA increased more quickly than DHA; EPA almost doubled within the first day whilst the DHA did not significantly increase until day 3. At peak incorporation, EPA and DHA had increased by approx. 200% and 50% from baseline, respectively. There was no significant change in the percentage of AA in PBMCs throughout the study period and no differences between groups (Figure 4c).

3.4.2 FAs in plasma PC

Both EPA and DHA increased in the FO treated group (Figures 5a and b), such that EPA was higher in the FO group than in the control group at days 1, 2, 3, 5, 7, 10 and 13 and DHA was higher at days 1, 2, 3, 5, 7 and 10. Plasma PC EPA increased more quickly than DHA; EPA more than doubled in

concentration within the first day. At peak incorporation, EPA and DHA had increased by approximately 400% and 100% from baseline, respectively. There was no significant change in the percentage of AA in plasma PC throughout the study period and no differences between groups (Figure 5c).

3.4.3 FAs in plasma NEFAs

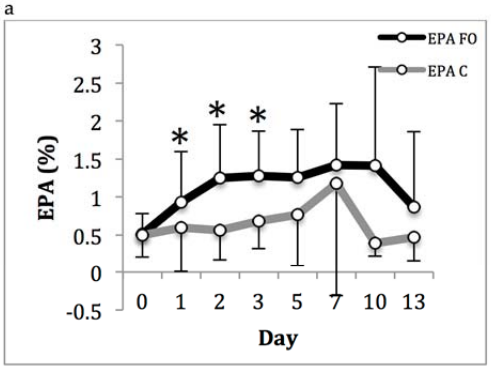
Both EPA and DHA increased in the FO treated group (Figures 6a and b), such that EPA was higher in the FO group than in the control group at days 2, 3, 7 and 10 and DHA was higher at days 2, 3, 7,10 and 13. EPA reached its peak level sooner than DHA. At peak incorporation, both EPA and DHA had increased by approx. 100% from baseline. There was no significant change in the percentage of AA in plasma NEFAs throughout the study period and no differences between groups (Figure 6c).

3.4.4 AA/(EPA+DHA) ratio in the different lipid pools

The ratio of AA/(EPA+DHA) did not change over time in the control group but declined in all three lipid pools in the FO group (Figure 7a-c). The ratio in PBMCs was different between groups at day 2, while in both plasma PC and plasma NEFAs it was different between groups at days 1, 2, 3, 5, 7, 10 and 13 (Figures 7a-c).

Figure 4. Peripheral blood mononuclear cell content of eicosapentaenoic acid (EPA; a), docosahexaenoic acid (DHA; b) and arachidonic acid (AA; c) in patients in the Omegaven™ (FO) and control groups.
Data are mean ± SD. The number of patients at each time point varies as follows: control group day 0 n=20, day 1 n=18, day 2 n=14, day 3 n=10, day 5 n=8, day 7 n=4, day 10 n=4, day 13 n=1; FO group day 0 n=21, day 1 n=20, day 2 n=17, day 3 n=15, day 5 n=13, day 7 n=11, day 10 n=7, day 13 n=5. *indicates significantly different from control group.

a



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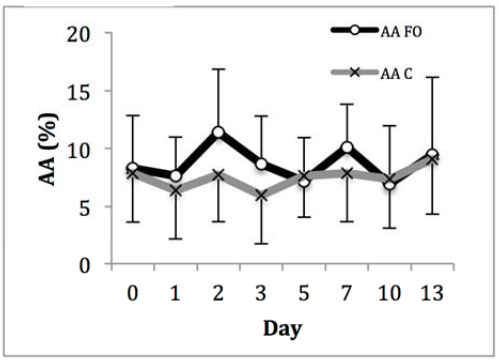

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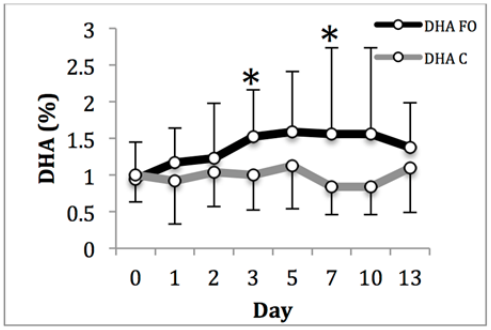


Figure 5. Plasma phosphatidylcholine content of eicosapentaenoic acid (EPA; a), docosahexaenoic acid (DHA; b) and arachidonic acid (AA; c) in patients in the Omegaven™ (FO) and control groups.
Data are mean ± SD. The number of patients at each time point varies as follows: control group day 0 n=20, day 1 n=18, day 2 n=14, day 3 n=10, day 5 n=8, day 7 n=4, day 10 n=4, day 13 n=1; FO group day 0 n=21, day 1 n=20, day 2 n=17, day 3 n=15, day 5 n=13, day 7 n=11, day 10 n=7, day 13 n=5. *indicates significantly different from control group.

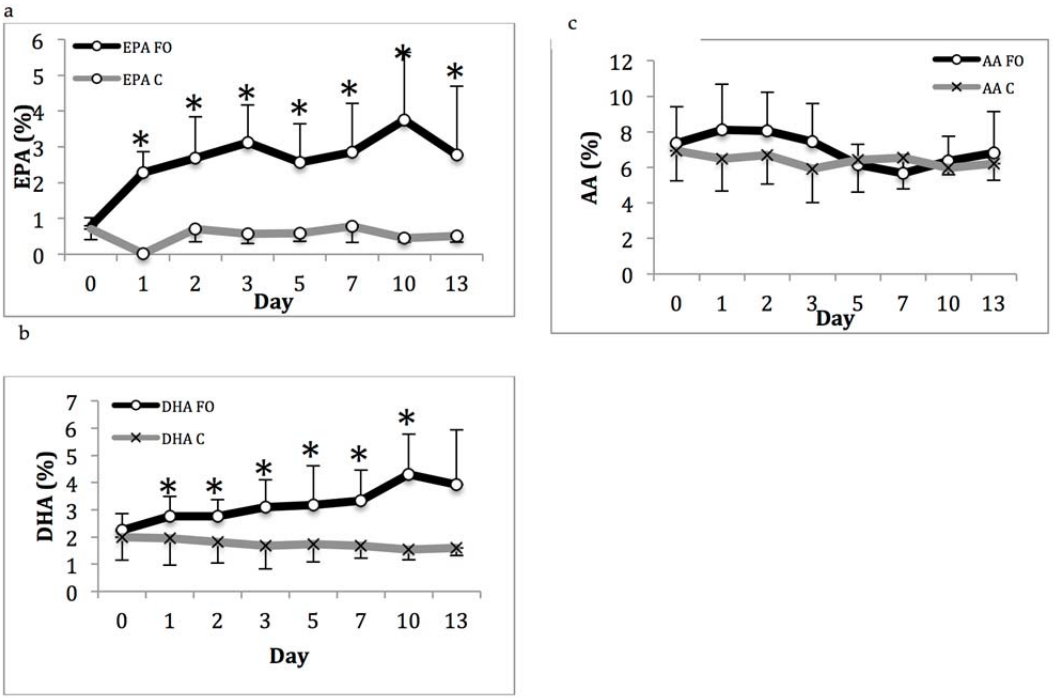
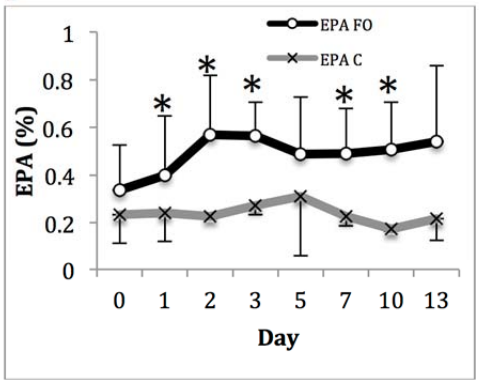


Figure 6. Plasma non-esterified fatty acid content of eicosapentaenoic acid (EPA; a), docosahexaenoic acid (DHA; b) and arachidonic acid (AA; c) in patients in the Omegaven™ (FO) and control groups.
Data are mean ± SD. The number of patients at each time point varies as follows: control group day 0 n=20, day 1 n=18, day 2 n=14, day 3 n=10, day 5 n=8, day 7 n=4, day 10 n=4, day 13 n=1; FO group day 0 n=21, day 1 n=20, day 2 n=17, day 3 n=15, day 5 n=13, day 7 n=11, day 10 n=7, day 13 n=5.*indicates significantly different from control group.

a



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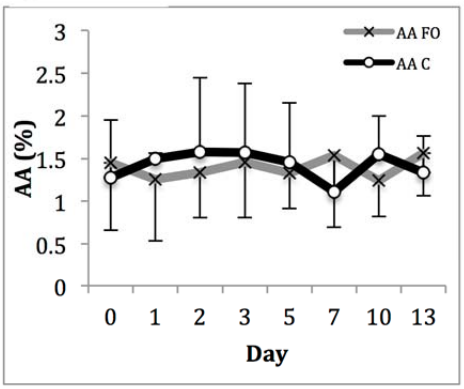

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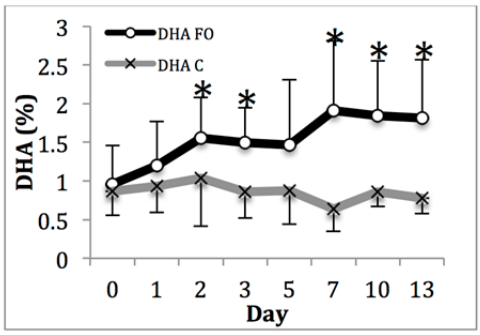
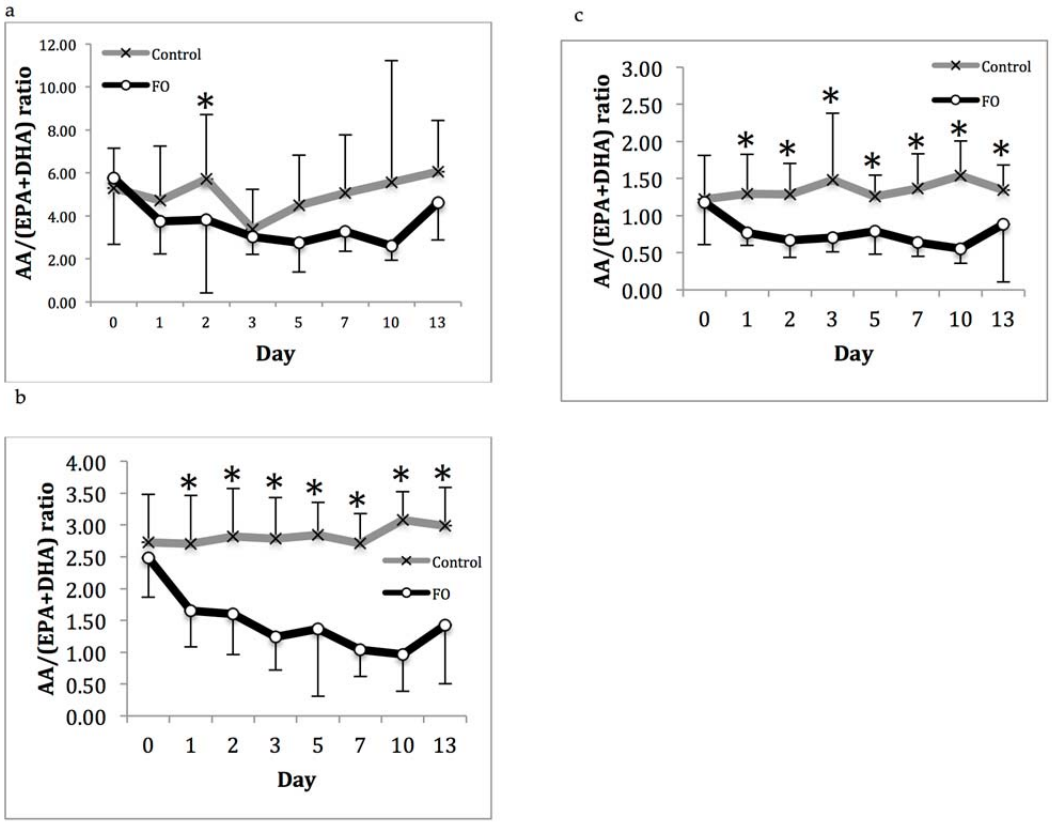


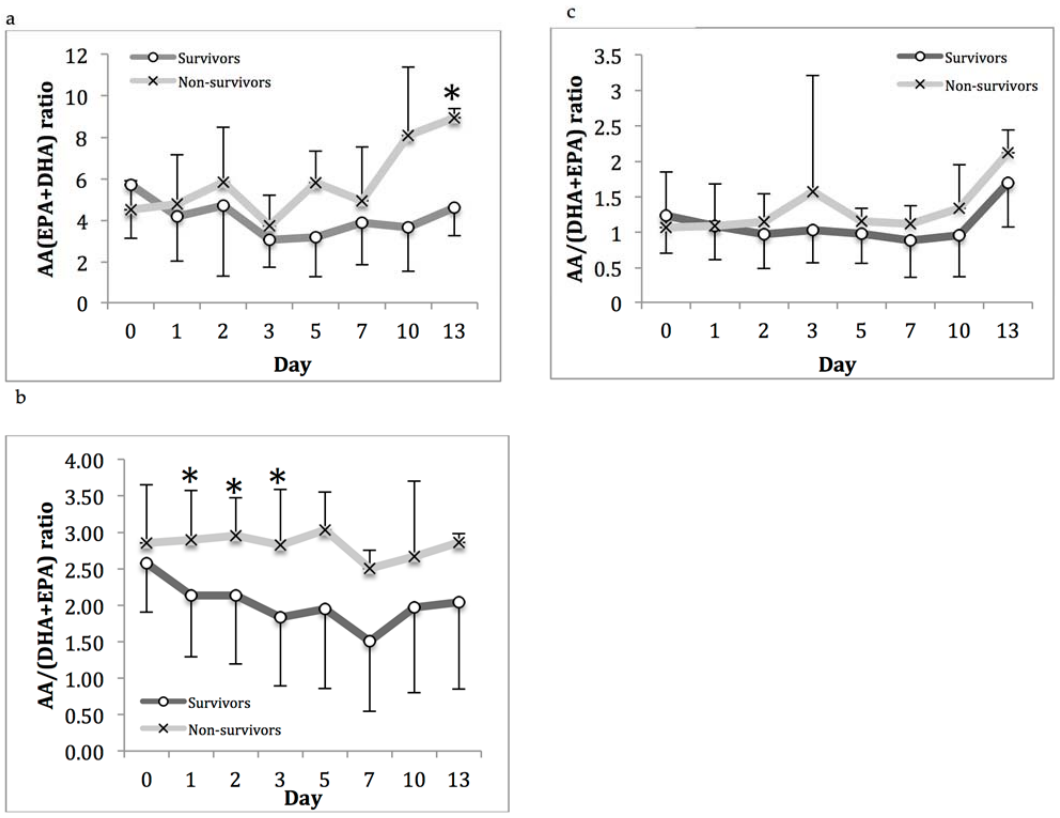
Figure 7. Ratio of arachidonic acid to eicosapentaenoic acid plus docosahexaenoic acid (AA/(EPA+DHA)) in peripheral blood mononuclear cells (a), plasma phosphatidylcholine (b) and plasma non-esterified fatty acids (c) in patients in the Omegaven™ (FO) and control groups. Data are mean ± SD. The number of patients at each time point varies as follows: control group day 0 n=20, day 1 n=18, day 2 n=14, day 3 n=10 day 5 n=8, day 7 n=4, day 10, n=4, day 13 n=1; FO group day 0 n=21, day 1 n=20, day 2 n=17, day 3 n=15, day 5 n=13, day 7 n=11, day 10 n=7, day 13 n=5. *indicates significantly different from Omegaven™ group.



3.5 AA/(EPA+DHA) ratio and mortality

Survivors had a lower AA/(EPA+DHA) ratio in PBMCs at day 13 and in plasma PC at days 1, 2 and 3 compared to non-survivors (Figures 8a-c).

Figure 8. Ratio of arachidonic acid to eicosapentaenoic acid plus docosahexaenoic acid (AA/(EPA+DHA)) in peripheral blood mononuclear cells (a), plasma phosphatidylcholine (b) and plasma non-esterified fatty acids (c) in survivors and non-survivors who provided blood samples. Data are mean + SD. The number of patients at each time point varies as follows: survivors day 0 n=35, day 1 n=32, day 2 n=26, day 3 n=21, day 5 n=18, day 7 n=12, day 10 n=8, day 13 n=5; non-survivors day 0 n=6, day 1 n=6, day 2 n=5, day 3 n=4, day 5 n=3, day 7 n=3, day 10 n=3, day 13 n=1. *indicates significantly different from non-survivors.



3.6 Effects of sex and age on n-3 FA incorporation

At day 0 there was no significant difference in EPA or DHA levels between males (n = 23) and females (n = 18) in any of the three lipid pools (data not shown). Mean female and male age was 64.9 and 63.1 y, respectively. To investigate any sex difference in the incorporation of n-3 FA in the lipid pools comparisons from the baseline to the peak in FA were analysed. For PBMCs and plasma PC this was on day 10 for EPA and DHA. This allowed for samples from 8 patients (4 males and 4 females) who were still in the trial on day 10 and receiving Omegaven™ to be analysed. For plasma NEFAs this was on day 2 and 7 for DHA and EPA, respectively. This allowed for samples from 19 patients (10 males and 9 females) at day 2 and 14 patients (8 males

and 6 females) at day 7 who were still in the trial and receiving Omegaven™ to be analysed.

There was a consistent sex difference in the incorporation of EPA and DHA from Omegaven™ in all the lipid pools: the data suggest that males incorporate more n-3 FAs than females (Table 3). However, there was only a significant sex difference for EPA in the PC fraction (p=0.035).

Table 3: Maximum eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) change in peripheral blood mononuclear cells (PBMCs), plasma phosphatidylcholine (PC) and plasma non-esterified fatty acids (NEFAs) in males and females in the Omegaven™ group. Data are mean ± SD % change from baseline (day 0) in each pool.

		Fatty acid concentration change (% of total fatty acids)		
	FA	Female (n)	Male (n)	p
PBMCs	EPA	0.64±1.48 (4)	1.26±1.51 (4)	0.448
	DHA	0.77±1.85 (4)	1.12±1.26 (4)	0.686
Plasma PC	EPA	0.96±1.01 (4)	2.75±1.29 (4)	0.035
	DHA	0.29±0.90 (4)	1.37±0.84 (4)	0.058
Plasma NEFAs	EPA	0.13±0.63 (6)	0.20±0.56 (8)	0.195
	DHA	0.36±1.85 (9)	0.92±0.84 (10)	0.291

The median age of the entire study population was 65.5 y. The cohort was therefore divided into those aged > 65.5 and those aged < 65.5 y of age for the purpose of analysis of the effect of age on n-3 FA incorporation. In the subgroup aged < 65.5 y there were 18 men and 12 women, while in the subgroup aged > 65.5 y there were 16 men and 14 women at study entry. At day 0 there was no significant difference in the levels of EPA and DHA between those aged above and below 65.5 y in any of the lipid pools (data not shown).

To investigate any age difference in the incorporation of n-3 FA in the lipid pools comparisons from day 0 to the peak in EPA and DHA were analysed. For PBMCs and plasma PC (day 10 for both EPA and DHA) this allowed for samples from 8 patients (5 < 65.5 y and 3 > 65.5 y) who were still in the trial on day 10 and receiving Omegaven™ to be analysed. For plasma NEFAs

(day 2 for DHA and day 7 for EPA) this allowed for samples from 19 patients (11 < 65.5 y and 8 > 65.5 y) at day 2 and 14 patients (8 < 65.5 y and 6 > 65.5 y) at day 7 who were still in the trial and receiving Omegaven™ to be analysed. PBMCs from those aged > 65.5 y had significantly greater incorporation of EPA and DHA than those aged < 65.5 y (Table 4). No significant differences between age groups were seen in the other lipid pools examined (Table 4).

Table 4. Maximum eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) change in peripheral blood mononuclear cells (PBMCs), plasma phosphatidylcholine (PC) and plasma non-esterified fatty acids (NEFAs) in older and younger subjects in the Omegaven™ group. Data are mean ± SD % change from baseline (day 0) in each pool.

	FA	Fatty acid concentration change (% of total fatty acids)		p
		Age < 65.5 y (n)	Age > 65.5 y (n)	
PBMCs	EPA	0.73±2.30 (5)	1.33±0.72 (3)	0.048
	DHA	0.49±1.31 (5)	1.60±0.45 (3)	0.043
Plasma PC	EPA	1.82±3.44 (5)	2.06±1.98 (3)	0.877
	DHA	0.59±1.62 (5)	0.87±0.50 (3)	0.716
Plasma NEFAs	EPA	0.03±0.71 (8)	0.05±0.36 (6)	0.796
	DHA	0.56±0.66 (11)	0.57±0.62 (8)	0.964

4 Discussion

The study reports the FA profiles of selected blood lipid pools in septic patients and the effect of infusion of a commercially available FO based lipid emulsion (Omegaven™) on these profiles. The blood lipid pools studied were plasma PC and NEFAs, which represent transport pools, and PBMCs, which represent a functional pool. Plasma PC represents the phospholipid coat of lipoproteins, the major means by which fatty acids are transported in the bloodstream as complex lipids. PC fatty acids are available for uptake into cells and tissues, possibly by exchange of lipoprotein PC for cell membrane phospholipids and thus may have a functional effect through modification of

cell membrane composition. Plasma NEFAs arise from the release of free FAs from adipose tissue as a result of lipolysis by the action of hormone-sensitive lipase and from the incomplete entrapment of FAs released from lipoproteins, including those produced in the circulation from infused lipid emulsions, by lipoprotein lipase activity [14]. NEFAs are directly available to cells and may be taken up passively or by FA transporters for use as fuels. In addition, it is now known that there are receptors for some FAs on some cells (e.g. GPR120 on macrophages and adipocytes that responds to DHA [15]) meaning that FAs from within the NEFA pool may have functional effects controlling inflammatory and metabolic responses [15]. PBMCs are actively involved in the inflammatory response and altering their FA composition can influence the nature of their response and the type of lipid and peptide mediators they produce [16,17].

We found that EPA and DHA are rapidly incorporated into all three blood lipid pools investigated, even after a single 4 hour infusion, but with some differences in timing between EPA and DHA and among the different pools. Incorporation was increased further with additional infusions, reaching a new maximum within the time course studied here. We used the ratio of the pro-inflammatory n-6 FA AA to the sum of the n-3 FAs EPA and DHA as a marker of inflammatory potential. This ratio was decreased by n-3 FA infusion, especially in plasma PC and NEFAs, and was lower at some time points in PBMCs and plasma PC in those patients who survived compared with non-survivors.

As indicated above, an increase in EPA and DHA incorporation was seen soon after initiating Omegaven™ infusion, with an increase seen at the end of the first 4 h infusion, particularly for EPA. Over the next several h, in the absence of any further infusion, EPA and DHA content increased further in PBMCs and in plasma PC. The pattern seen for EPA and DHA in plasma NEFAs over this period is of interest. Plasma NEFA EPA and DHA increased up to 8 to 12 h (i.e. for 4 to 8 h after the end of the first Omegaven™ infusion) and then decreased. This suggests that the infused FO was largely hydrolysed over the first 4 h during its infusion and over the following 4 to 8 h,

releasing free EPA and DHA; over this 8 to 12 h period there was net appearance of free EPA and DHA in the circulation. After this time point there was net clearance of free EPA and DHA from the circulation, consistent with their uptake into cells and tissues. The early increases in EPA and DHA in PBMCs were reflected in a reduction in the AA/(EPA+DHA) ratio suggesting a potential impact on inflammation. In all three lipid pools the increase in EPA was greater than that of DHA, in line with other findings [18]. Despite its appearance in plasma NEFAs and in PBMCs, DHA content of plasma PC did not change during or immediately after the first FO infusion, an observation that is consistent with another study [19].

It is possible to compare the changes in EPA and DHA content seen here after a single infusion of Omegaven™ with those seen after chronic oral n-3 FA supplementation [20]. Browning et al. reported that with oral supplementation of 3.27 g/day of EPA+DHA EPA and DHA reached maximum levels after several weeks to months in plasma PC, plasma NEFAs and PBMCs [20]. Maximum contents of EPA were 3.5, 1.1 and 2.3% in plasma PC, plasma NEFAs and PBMCs, respectively. In the current study a single dose of FO (Omegaven™) resulted in a maximum EPA content of 3.0, ~0.5 and 2.5% in plasma PC, plasma NEFAs and PBMCs, respectively, but in just a few h. Likewise Browning et al. reported maximum contents of DHA of 3.1 and 3.3% in plasma NEFAs and PBMCs, respectively. In the current study maximum DHA content was ~2 and 2.5% in the two pools, respectively, again in just a few h. This rapid appearance of EPA and DHA with parenteral FO infusion is an advantage over the slower appearance of these FAs when FO is given orally. The parenteral administration of FO provides the FAs directly into the bloodstream, introducing them to lipid fractions like PC and directly exposing circulating cells, such as PBMCs, very quickly. Thus, rapid functional effects become a possibility.

Interestingly, there was also a possible increase in AA in the NEFA pool in patients receiving a single infusion of Omegaven™ (Figure 2f). Omegaven™ does contain some AA (Table 1) and it may be that the rise in AA reflects lack of entrapment of AA released by Omegaven™ hydrolysis. Alternatively the

appearance of free AA in the circulation may result from its replacement in more complex lipids and in cell membranes by EPA and DHA. Whatever the reason, the appearance of free AA is consistent with the report of Mayer et al. of appearance of AA in the NEFA pool after Omegaven™ infusion [21]. Any potential implications of this would seem mitigated by much larger rise in EPA and DHA causing an overall decrease in the AA/EPA+DHA ratio.

Repeated 4 h daily infusions of Omegaven™ for 14 days resulted in further enrichment of EPA and DHA in all three lipid pools investigated. The rate of incorporation into the different pools varied, likely reflecting the rates of turnover of those pools. Furthermore, in general, EPA was incorporated more quickly and to a greater extent than DHA. This is an interesting observation because Omegaven™ contains similar amounts of EPA and DHA (Table 1). This adds to the growing evidence that EPA and DHA are handled differently, however, the patterns of incorporation seen are consistent with previous reports [19,20,22].

Incorporation of DHA was less into PBMCs than into the plasma lipid pools. The relative resistance of immune cells to change in DHA content is reported by others [23-25]. The FA composition of immune cell phospholipids may not alter as much as blood lipid pools because these cells exert a significant level of control over their plasma membrane composition [26]. This finding may suggest that relatively minor changes in the FA composition of the immune cell membrane can have profound effect on cellular function in critical illness. The change in AA/(EPA+DHA) ratio was the least in the PMN fraction although a clear favourable trend for a lower ratio in survivors was seen. There is some evidence to suggest that EPA and DHA act differently within the immune system. One study has made an indirect finding that the anti-chemotactic effects of fish oil might be due to EPA rather than DHA [27], although no study has yet attempted to discriminate between the effects of the two n-3 FAs on leukocyte chemotaxis. Other studies have suggested that EPA, but not DHA, increased the attachment of bacteria to monocytes [28] and decreased the activity of natural killer cells [29]. Evidence also suggests that EPA may have a more suppressive effect on T cells than DHA [30,31] with the

rationale that its incorporation into the membranes disrupts the rafts and interferes with signalling platforms [30,31]. However, it has also been shown that DHA may have a stronger affinity for raft regions and therefore may have a more influential effect on lipid rafts leading to an increased fluidity and reduced order [30,31]. Both EPA and DHA give rise to resolvins that are now recognised as significant lipid mediators involved in resolution of inflammation and in immunomodulation [32-37].

The current study also addressed the impact of age and sex on incorporation of n-3 FAs from infused FO. Older patients (aged over 65.5 y) showed greater incorporation of EPA and DHA into PBMCs than younger patients (aged less than 65.5 y), but this difference was not seen in the two plasma lipid pools. It is well documented that immune function, including its inflammatory component, changes with age [38] but there are few reports examining the impact of age on n-3 FA incorporation patterns. Some studies have identified differences in FA profiles with ageing, including a higher n-3 FA status. For example, Crowe et al. reported a positive association between age and plasma EPA and DHA in both men and women [39]. Explanations for this might include differences in hormone status, physical activity, body composition, metabolism or diet across the age span. There are few reported investigations of the impact of age on incorporation of supplemented n-3 FA. However, Meydani et al. found larger increases in plasma EPA and DHA in older women compared to young women after oral n-3 FA supplementation [40]. The reasons for this finding were not clear although it was postulated that older people might have more efficient absorption of n-3 FAs and/or that there are hormonal differences with ageing that impact n-3 FA handling. The current study found no difference in FA profiles in any of the lipid pools between males and females at study entry. However, after Omegaven™ treatment males had a significantly greater incorporation of EPA into plasma PC than females. Other pools showed higher EPA and DHA in males than females but these differences were far from significant, perhaps because of the low numbers of patients in each group. Nevertheless, the lack of a generalised finding means this observation should be treated with caution.

It is important to recognize the limitations of the current study. First, although 60 patients entered the study and were followed for clinical outcome[24], only 41 of these patients consented to provide blood samples. Secondly, some patients withdrew consent for blood sampling or died, while some patients were discharged, meaning that the number of samples available for analysis decreased over the course of the study and only relatively low numbers of samples were available at the latter time points. This also resulted in relatively low numbers of samples available for the comparisons between age and sex of patients and between survivors and non-survivors. Thirdly, only two patients consented to blood sampling during and soon after the first FO infusion (day 0), meaning that those data are not amenable to statistical analysis. Finally, the FA composition of Omegaven™ varies among batches (Table 1) and it is not clear whether the same batch was used throughout the trial. If not, then the amount of EPA and DHA provided to different patients and at different time points in the study may have varied. All patients in the Omegaven™ group would have received substantial amounts of EPA and DHA irrespective of the batch used, but because the incorporation of these fatty acids into PBMCs, plasma PC and plasma NEFAs is known to be dose-dependent[34], some variation in the extent of incorporation would be expected between batches.

5 Conclusions

In conclusion, the current study demonstrates that n-3 FAs are rapidly incorporated from a FO-based lipid emulsion into plasma PC, plasma NEFAs and PBMCs in critically ill septic patients. This incorporation results in a decreased AA/(EPA+DHA) ratio that may be of clinical relevance. Indeed, the ratio tended to be lower in survivors compared with non-survivors.

6 References

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