A Randomised Control Trial Investigating the Effects of Parenteral Fish Oil on Survival Outcomes in Critically Ill Patients with Sepsis

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by

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Abstract

Introduction
Sepsis is a leading cause of mortality in critically ill patients on the intensive care unit (ITU). Death from sepsis in the ITU is frequently preceded by the development of multiple organ failure as a result of uncontrolled inflammation. Treatment with omega-3 (fish oil) has been demonstrated to attenuate the effects of uncontrolled inflammation and may be clinically beneficial in reducing morbidity from organ dysfunction.

Method
A randomised control trial investigating the effects of parenteral omega-3 (Omegaven^{TM}), given early in the course of sepsis, was carried out in a single institution. Consecutive patients diagnosed with sepsis were entered into the study. Patients were randomised to receive either parenteral fish oil and standard medical care or standard medical care only.

The primary outcome measure was a reduction in organ dysfunction using the SOFA score as a surrogate marker. The secondary outcome measures were mortality, length of stay, mean C-reactive protein (CRP), days free of organ dysfunction/failure and fatty acid (FA) analysis.

Results
Sixty patients were included in the study. The baseline demographics were matched for the two cohorts. Patients treated with parenteral fish oil were associated with a significant reduction in new organ dysfunction (delta-SOFA 2.2±2.2 vs. 1.0±1.5, p=0.005 and maximum-SOFA 10.1±4.2 vs. 8.1±3.2, p=0.041) and mean CRP (186.7±78 vs. 141.5±62.6, p=0.019).

There was no significant reduction in the length of ITU and total hospital stay between cohorts. Patients treated with fish oil in the strata of less severe sepsis had a significant reduction in mortality (p=0.042).

Conclusion
The treatment of critically ill septic patients with parenteral fish oil is safe. N-3 FAs are rapidly taken up by circulating white cells. It is associated with a significant reduction in organ dysfunction and CRP. It may be associated with a reduction in mortality in patients with less severe sepsis. A multi-centre trial is justified as a result of this trial.

Abbreviations

AA	Arachidonic acid
ALI	Acute lung injury
APACHE	Acute physiology and chronic health evaluation
APC	Activated protein C
ARDS	Acute respiratory distress syndrome
CARS	Compensatory Anti-inflammatory response syndrome
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CRP	C-reactive protein
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic
FA	Fatty acid
FO	Fish oil
GCS	Glasgow coma score
HDU	High dependency unit
IL	Interleukin
ITU	Intensive therapy unit
LODS	Logistical organ dysfunction score
LOX	Lipoxygenase
MI	Myocardial infarction
MODS	Multiple organ dysfunction score
NEFA	Non-esterified fatty acid
PC	Phosphatidylcholine
PMN	Polymorphonuclear leukocytes
PUFA	Polyunsaturated fatty acid
RCT	Randomised controlled trial
ROC	Receiver operating characteristic
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
TNF	Tumour necrosis factor
TPN	Total parenteral nutrition

Publications (papers and abstracts) and Presentations Arising from this Thesis

Presentations

A randomised control trial investigating the effects of parenteral omega-3 in critically ill patients with severe sepsis in ITU. Hall TC, Bilku DK, Al-Leswas D, Metcalfe MS, Dennison AR. East Midlands Surgical Society. 05/2012. Awarded first prize for best oral presentation

A randomised control trial investigating the effects of parenteral omega-3 in critically ill patients with severe sepsis in ITU. Hall TC, Bilku DK, Al-Leswas D, Metcalfe MS, Dennison AR. ESPEN 2013 Conference, Leipzig. Poster presentation

A randomised control trial investigating the effects of parenteral omega-3 in critically ill patients with severe sepsis in ITU. Hall TC, Bilku DK, Al-Leswas D, Metcalfe MS, Dennison AR. Royal Society of Medicine, Surgical section. Competitively selected for presentation. October 2013

Publications

The Difficulties of Clinical Trials Evaluating Therapeutic Agents in Patients with Severe Sepsis. Hall TC, Bilku DK, El-Leswas D, Horst C, Dennison AR. Ir J Med Sci. 2012 Mar;181(1):1-6

Biomarkers for the differentiation of sepsis and SIRS: The need for standardisation of diagnostic studies. Hall TC, Bilku D, El-Leswas D, Horst C, Dennison AR. Ir J Med Sci. 2011 Dec;180(4):793-8

A Randomized Controlled Trial Investigating the Effects of Parenteral Fish Oil on Survival Outcomes in Critically Ill Patients With Sepsis: A Pilot Study. Hall TC, Bilku DK, Al-Leswas D, Neal CP, Horst C, Cooke J, Metcalfe MS, Dennison AR. JPEN J Parenter Enteral Nutr. 2014 Jan 9

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1 Introduction

1.1 The burden of sepsis in critically ill patients

Mortality rates in intensive therapy units (ITUs) continue to be high despite advances in critical care medicine. Sepsis is a serious and complex inflammatory process that is characterised by a systemic inflammatory response to the presence of an infection. Sepsis is the leading cause of death in non-cardiac ITUs. The high mortality has persisted despite an improved understanding of the pathophysiology of sepsis and the wealth of available antimicrobials. Mortality rates can be as high as 60% in septic patients who account for approximately 40% of ITU expenditure [1,2].

The introduction of sepsis care bundles in the management of these critically ill patients has demonstrated that improvements can still be attained when compared to historical controls [3,4]. However attempts to reduce mortality rates further are likely to be hindered by the development of bacteria resistant to an increasingly wide range of antibiotics. The majority of the patients admitted to ITU have a sepsis syndrome triggered by various pathogens, trauma, surgery, burns, or cancer. The inappropriate host response and hyper-inflammatory state is costly financially and in terms of patient morbidity. The development of severe sepsis within 24 hours of ITU admission increases mortality by 15% [5]. Worldwide mortality from severe sepsis and septic shock are 26.5% and 38.9% respectively [6]. The mean cost per case of severe sepsis in ITU is £18,173 compared to £3,828 in non-septic cases [5].

As a consequence of the high associated mortality, numerous studies have attempted to identify novel treatment strategies in septic patients, often with inconclusive results

[7]. Many large-scale, multicentre clinical studies have been performed but despite initial optimism, the outcomes have been largely disappointing. Improvements in our understanding of the pathophysiological mechanisms driving sepsis have sparked interest in agents targeting specific mediators in the inflammatory pathway. Despite retrospective analysis suggesting some subgroup efficacy, the majority of trials show no clear evidence of an overall benefit [8].

The lack of level I evidence in ITU medicine relates to the difficulty in designing trials which can accommodate the inherit heterogeneity of the ITU population. This observation not only applies to trials in sepsis but to all aspects of ITU care [9]. The intensive care literature includes more negative than positive trials [10] and indeed some interventions such as the use of TNF receptor antagonists in severe sepsis have resulted in an increased morbidity [11]. Three trials have, however, demonstrated evidence of a beneficial effect in severe sepsis for steroids [12], intensive insulin therapy [13] and most recently, activated protein C [14]. The beneficial effects of these have been challenged by subsequent clinical trials [15,16]. The use of steroids and intensive insulin therapy, in particular, has largely been discredited. The Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study was an international, multicentre, randomised, placebo-controlled Phase 3 trial. It was conducted to demonstrate if the administration of activated drotrecogin alpha reduced the 28-day mortality in patients with sepsis. As a result of the PROWESS study investigating activated protein C, the drug has been given Food and Drug Administration approval. More recently it has been removed from the market due to concerns over increased bleeding [17].

Evidence is lacking due to a number of ubiquitous factors found in the critically ill patient. The design of trials and interpretation of results in a patient population with widely varying pathologies, ages and responses to the initial insult will always be difficult. In addition, there are significant ethical barriers conducting RCTs investigating life-saving strategies, such as the use of vasopressors in septic shock. Before the widespread adaptation of evidence based medicine, it has previously been suggested that as few as 15% of all medical interventions had been adequately validated [18]. In critically ill patients, the potential to do harm and generate substantial costs is particularly high and therefore intensivists should always question unproven dogma.

1.2 The difficulties of trials evaluating critical illness in sepsis: why are further more rigorous trials needed?

1.2.1 Heterogeneity of critically ill septic patients

Severe sepsis is the final common pathway following an uncontrolled state of systemic inflammation in the presence of bacteraemia. Sepsis is a complex and dynamic process and the initial insult varies in terms of pathogen, foci, virulence, resistance, the production of toxic products and host factors including age, background and pre-existing co-morbidities. This heterogeneity leads to difficulties in the interpretation of trial results.

Mortality varies significantly depending on the infectious foci, for example, mortality from urosepsis is lower than that of intra-abdominal sepsis [19,20]. Host variables, such

as co-morbidities interact further to exacerbate disease processes and are not infrequently the primary cause of death [21]. Trial results should be interpreted through an intention-to-treat analysis in an attempt to account for this (analysis based on the initial treatment intent, not on the treatment eventually administered).

1.2.2 Differences between Gram-negative and Gram-positive sepsis

The nature of the causative organism may be an important variable in determining the responsiveness to therapeutic agents in sepsis and these organisms differ widely in terms of the inflammatory response elicited in the host [22]. The conventional views on the molecular pathways of septic shock originated from studies investigating Gram-negative endotoxic shock, which was believed to be quantitatively of greater importance to the pathophysiology of septic shock [23]. However, because of the widespread use of surgically implanted foreign material and changes in microbial resistance and virulence [24], Gram-positive organisms are increasingly prevalent and mortality rates from these may now exceed those from Gram-negative organisms [25,26]. The increasing prevalence of Gram-positive pathogens in critically ill patients has been emphasised in several reviews [27,28].

It was believed that the pathogenesis of septic shock from Gram-negative and Gram-positive organisms shared similar mechanisms. These views have been challenged in the last decade and there is accumulating evidence to suggest that pathogenetic mechanisms underlying Gram-negative and Gram-positive sepsis differ significantly [27,29]. The previous understanding that Gram-negative bacteria caused shock by endotoxin based mechanisms and Gram-positive shock was secondary to exotoxins, has been demonstrated to be over simplistic and this has had consequences for trial outcomes. For example the differences in outcome with an anti-cytokine agent were

demonstrated in the TNF receptor study where the detrimental effects were confined largely to Gram-positive organisms [11]. The anti-cytokine (TNF receptor Fc fusion protein in this trial) disrupts the hosts' innate immune response mechanisms and this effect adversely affects the body's ability to deal with Gram-positive organisms. This may be in part due to the fact that Gram-positive organisms are readily killed in the intracellular space by neutrophils and macrophages but Gram-negative pathogens may also be killed in the extracellular space by antibody and complement [30].

Differences also exist between cytokine responses. It has been shown that the peak response can be delayed for 50-75 hours following a challenge with Gram-negative pathogens compared to only 1-5 hours with Gram-positive pathogens [31]. Whilst these differences can be demonstrated easily in vitro, it would be extremely difficult to control for these variations in clinical trials. For example when measuring cytokine responses following a septic insult, where timing of the initiation is impossible to identify.

1.2.3 The variety of genetic background

Studies have demonstrated a strong genetic component to fatal infectious diseases [32]. The familial risk of death from an infective process is even greater than that for atherosclerotic disease [33]. The complex host response pattern is controlled by numerous cytokines and cellular receptors which have been demonstrated to display substantial individual genetic variation. Single nucleotide polymorphisms have been shown to affect susceptibility (and the clinical course) of numerous diseases with a large number of genes (and their products) involved in the host reaction to sepsis [11,34]. Genetic variation in these molecules alters the course of the event. Variability in a number of systems such as the macrophage-membrane proteins and interferon-gamma

receptors have been identified as important determinants of specific microbial susceptibility [35,36]. Despite the array of therapies targeting different components and pathways in the inflammatory response there have been, to date, no significant advances (regarding genetics) which could allow specific tailoring of therapy.

1.2.4 The issues of sample size

Trials designed with a primary outcome of mortality will require many hundreds or thousands of patients to be adequately powered. Although a well-defined physiological abnormality is present in sepsis syndrome, critically ill patients do not represent a homogenous cohort. Even in conditions where interventions can be clearly related to outcomes (within a homogenous cohort) e.g. myocardial infarction (MI), large sample sizes are required to demonstrate statistical significance. It has been calculated that in order to demonstrate a 5% reduction in mortality from an intervention or drug to treat MI; a sample size of 10,000 patients would be required [37]. Due to the fact that the mortality is higher in sepsis than for an MI, the numbers needed for a trial in critically ill septic patients is likely to be lower [38], but nevertheless is still likely to be problematic. Some ITU trials have demonstrated reduced mortality with a few hundred patients [39,40], however the significant heterogeneity of ITU patients could be a major confounding factor in interpreting any positive results [41].

The initial success of the PROWESS group study is largely attributable to its design; incorporating an adequate sample size, which allowed it to be sufficiently powered to detect even a modest improvement in survival. As a result of the trial's size, exploratory analysis of subgroups could be undertaken to identify those patients who would optimally benefit from therapy. It identified that only patients with severe

sepsis and a high risk of death should be treated. Further trials have shown no benefit and also an increase in bleeding complications in those patients with a low mortality risk [42].

Multi-centre trials are frequently used to obtain sufficient data by recruiting adequate numbers of patients. They also offer other advantages such as avoiding 'investigator fatigue' which may happen in single centre trials due to trial novelty wearing off, new products emerging and lengthy time spent on the same trial. The dangers of a multi-centre approach are the heterogeneity (both nationally and internationally) in intensive care practice and the availability of resources and hence access to the trial. For example there are fewer intensive care beds available in the UK when compared to the USA and Europe; which means that in the UK patients admitted to intensive care have higher severity scores and as a consequence a higher mortality [43].

A further problem with multicentre trials is the potential to increase the study group's heterogeneity and also lead to violations. The variability between units stems from deviations in physician-based management decisions, adequacy of supportive care, timing of surgical intervention and choice of antimicrobial and source control [44]. The almost ubiquitous nature of these variables means that therapeutic agents need to be extremely effective for their benefits to be detectable.

1.2.5 Difficulties of inclusion criteria

1.2.5.1 Problems with the clinical definitions of SIRS, sepsis and severe sepsis

It is important that trials use definitions that will help to improve homogeneity and increase power. The inclusion criteria of some trials only require a 'clinical suspicion

of infection' as opposed to an objective 'clinical evidence of infection'. The definition of sepsis as stated by The American College of Chest Physicians and the Society of Critical Care Medicine is frequently used as the standard for consistent terminology [45]. This is defined as the proven or suspected source of infection together with at least two of the four systemic inflammatory response syndrome features, 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. At present, there is no satisfactory objective biochemical marker with sufficient specificity or sensitivity to be routinely employed in clinical practice and hence the need for such definitions [46,47].

The definition of SIRS has been criticised as being overly sensitive and non-specific to be used in trial inclusion criteria [48]. The SIRS criteria are found to be met by more than two thirds of intensive care patients and 95% of unselected patients admitted to a general medical ward [49]. Trial entry based on sepsis syndrome has been criticised for an over prediction of mortality rates and it is also difficult to identify organ dysfunction in the presence of sedative usage and inotropes [50,51]. The SIRS definition also does not allow precise staging of septic patients in terms of baseline risk and potential benefit from therapeutic agents. It is also clear that clinical definitions used in isolation do not predict patients who are at high risk of mortality. At the International Sepsis Definitions Conference in 2001, a staging system similar to the TNM system for malignant tumours was developed. This classification scheme for sepsis, called PIRO, stratifies patients based on their **P**redisposing conditions, the nature and extent of the **I**nsult (infection), the nature of the host **R**esponse and the degree of **O**rgan dysfunction [45]. Preliminary studies have demonstrated that this may be useful as a triage tool, although at present it requires further testing to accurately determine its clinical usefulness and to refine the classification parameters [52].

1.2.5.2 Inclusion criteria

Scoring systems are frequently employed to measure disease severity and overcome the issues encountered using the overly sensitive definitions of SIRS. Such scoring systems have been criticised for lacking a physiological basis, being misleading, complex and including criteria unrelated to the septic process [48,53]. More recent scoring systems such as the Sepsis-related Organ Failure Assessment (SOFA) [54] and the PIRO [45] that incorporate the degree of organ failure may prove useful. In an attempt to ensure an independent level of inclusion criteria consistency, the PROWESS study group used a clinical coordinating centre which was available 24 hours a day (Vanderbilt Coordinating Centre), to assess recruitment eligibility and safety [42].

1.2.5.3 Exclusion criteria

The PROWESS trial, in addition, used strict exclusion criteria for patients unlikely to benefit from the APC. Moribund patients and those with prolonged organ dysfunction were excluded [42]. It is unlikely that patients with such severe disease could obtain benefit from an experimental agent.

1.2.6 Difficulties in translation from animal to human models

It is evident that animal models provide unrealistic evidence of survival benefit from therapeutic agents when compared to clinical trials [22,30]. Whilst animal models are useful in obtaining preliminary data, they are used in a homogenous population of standardised young and healthy animal breeds, which minimises the impact of confounding factors on outcomes. This needs to be taken into consideration if the results of such animal trials are used in power calculations for human trials. It is likely

that the failure to take this into consideration may be the cause of past trials failing to detect significant improvements with agents, which had been very effective in animal models.

In animal models, the therapeutic agent under investigation is often given at a set and predetermined time soon after the onset of sepsis. The sepsis is initiated by a known event and the time course is thus known very accurately. In reality, the clinical onset of infection is rarely known and patients frequently have significant comorbidities. The initiator of infection in animal models is often a bolus of organisms or endotoxin given intravenously, thus inducing a severe and overwhelming sepsis which, (with the exception of meningococcal sepsis) is not analogous to the pathophysiology of clinical sepsis [55]. Many animal studies also measure the cytokine response to a purified bacterial component with known characteristics and standardised infective dose.

Whilst animal trials are clearly informative the controlled nature, from the initiation of the septic insult to the use of the therapeutic agent at a defined time point, cannot be duplicated in human studies. The PROWESS study overcame some of these issues with extensive Phase I and II preclinical and clinical testing to ensure its pharmacokinetics, pharmaceutical effect and dosing were thoroughly understood and could be optimised for a phase III trial [14] . APC had the added advantage that standard coagulation markers can easily measure its pharmacologic activity.

It is anticipated that the frequency of sepsis will increase, especially with the advances in medical care generating large numbers of immunocompromised patients [56]. Sepsis is a dynamic process with complex pathophysiological processes, which remain

incompletely understood. Trials that fail to account for the array of variables in sepsis outcomes are unlikely to succeed.

1.3 Outcome measures in critically ill septic patients on the ITU

Experience from clinical trials evaluating intervention in septic patients has challenged the paradigm that mortality is the gold standard end point in the evaluation of treatment efficacy. Although mortality represents an easy to define, highly relevant and measurable end point it possesses some significant drawbacks [57]. Mortality is an appropriate endpoint when the mechanisms of death for a particular condition are completely understood. The pathophysiology of organ failure associated with sepsis is poorly understood but likely to involve multiple factors including disturbed microcirculation and tissue oxygenation, deranged apoptosis and direct cellular toxicity of cytokines and other sepsis-related compounds. Issues exist in the commonly used 28-day (or 30-day) mortality also since patients who die after 28 days could be considered a treatment success even if the cause of death is still probably related to the disease treated in ITU. Conversely patients may die early in the course of sepsis secondary to coexisting disease unrelated to the septic insult. Awareness of these issues have led to a shift towards more relevant end-points such as length of stay, morbidity secondary to organ dysfunction/failure and secondary nosocomial infection development.

Morbidity has sparked a great deal of interest and has called for an objective and simple way to describe individual organ dysfunction/failure in a continuous form, from mild dysfunction to complete failure, which can be used to measure the evolution of individual (or aggregated) organ dysfunction. Length of stay may be an

unreliable indicator of treatment success due to confounding factors and hence organ dysfunction maybe more reliable [58].

As a consequence trials increasingly use organ dysfunction as the end-point for clinical trials in ITU patients [59-62] particularly as this cohort of patients typically die from multiple organ failure [63]. The relationship between mortality and multiple organ failure in septic patients is well established [64-66]. Studies have demonstrated the importance of initiating treatment early in the first few days of diagnosing sepsis. Worsening organ dysfunction in the first 24-72 hours of diagnosis increases mortality [58,67,68]. Independent of initial organ failure status, an increase in organ dysfunction in the first 24 hours can increase mortality by more than 50% [68].

1.3.1 The introduction of scoring systems to critically ill patients

Scoring systems have become an important means to predict risk and outcome in medicine and particularly in critical illness. Since the first scoring system came into mainstream use, namely the APGAR score for new-born vitality [69], there has been a growth in both general and disease-specific scoring systems. Scoring systems are particularly useful to predict outcomes in ITU patients, where costs of treatment are so great and intervention can be directed towards specific groups. Some are used only in the first 24 hours of ITU admission such as the Acute Physiology and Chronic Health Evaluation (APACHE) and Simplified Acute Physiology Score (SAPS) score [70,71]. More recently a model based on the UK critical care units has been developed by the Intensive Care National Audit & Research Centre (ICNARC) based on data from

a large, multicenter, high quality clinical database. This new scoring system has demonstrated better discrimination in mortality prediction than other previously published models [72].

These systems do not take into account organ dysfunction that develops after the first 24 hours. As the different organ systems can be affected at varying timepoints in the course of the disease [73] a daily prediction model can miss the total organ dysfunction sustained by the patient and, therefore, underestimate risk. Death from multiple organ dysfunction depends on the number, severity, duration and combination of organ failures that cannot be measured using variables measured at isolated single time-points [74-77].

Organ failure needs to be expressed on a continuum of development and resolution as opposed to being an absolute binary measure. Expressing whether organ failure is absent or present misses out important information on dysfunction severity. Newer scoring systems such as the Sequential Organ Failure Assessment (SOFA) score [78] and Multiple-Organ Dysfunction Score (MODS) [75] are used over time to measure the evolution of individual (or aggregated) organ dysfunction. Using a sequential score for outcome prediction more effectively represents the dynamics of illness, including the effects of therapy compared with traditional outcome prediction models at the time of ITU admission.

1.3.2 The Multiple Organ Dysfunction Score (MODS)

The MODS was based on extensive literature reviews and past experience and was evaluated for its ability to predict mortality in an incremental manner. The scores were then validated on a separate group of patients. The score represents the most aberrant data for the entire ITU stay. The scoring is simple to apply but the MODS has been criticised for problems in evaluating the circulatory function score; measured as the cumbersome pressure-adjusted heart rate (PAR), which is treatment (vasopressor) independent [79].

1.3.3 The Logistic Organ Dysfunction Score (LODS)

The LODS is another scoring system predicting risk based on organ failure. It was the first of the organ dysfunction scores and was derived from multivariate regression analysis of a large data-base of more than 13 000 patients [80]. It is defined by twelve variables for six organ systems recorded as the most aberrant data in the first 24 hours. The original LODS was for the first 24 hours and was not intended for monitoring the disease progression; however modifications of the scoring have resulted in its use for serial monitoring. In addition to scoring the number of failing organs, a validated estimate of the severity of organ dysfunction can be calculated by a logistical regression equation using mortality as a surrogate marker.

1.3.4 The Sequential Organ Failure Assessment (SOFA) score

The SOFA score uses routinely collected data for the calculation of a graded score of 0–4 for each organ indicating the level of organ dysfunction (Table 1: Sequential Organ Functional Assessment (SOFA) score). A higher number equates to more severe failure. SOFA comprises separate daily scores for respiratory, renal, cardiovascular, central nervous system, coagulation and hepatic failure. The scores can be used in several ways:

- as individual scores for each organ
- as the sum of scores on one single ITU day
- the mean of the worst scores per day during the ITU (mean SOFA) stay
- total 'maximum SOFA score' minus 'admission total SOFA' (delta-SOFA)
- or the sum of the worst scores during the ITU stay (max SOFA)

The admission SOFA reflects the degree of failure already present when the patient enters the ITU. This can be used to stratify patients according to severity of illness, for example, for inclusion in clinical trials based on the admission SOFA score. Prospective [54] and retrospective [78] scores in the first 24 hours of admission to ITU have demonstrated good correlation with mortality. Other studies have demonstrated that the delta-SOFA and maximum-SOFA scores also correlate to outcome [81]. The delta SOFA measures the progress of the patient during their ITU stay and can potentially be influenced by an intervention. Moreno and colleagues demonstrated that the delta-SOFA was a good prognostic indicator after controlling for the admission SOFA score, suggesting that strategies directed at the prevention of further organ dysfunction will have a significant impact on prognosis, independent of the

physiological condition of the patient on admission to the ITU [81]. The area under the receiver operating characteristic (ROC) curve (AUC) was 0.742 (SE 0.017) for delta SOFA in predicting mortality.

Table 1: Sequential Organ Functional Assessment (SOFA) score

Organ System	0	1	2	3	4
Respiration PaO_2/FiO_2	>400	<400	<300	<200 with respiratory support	<100 with respiratory support
Coagulation Platelets ($10^3/mm^3$)	>150	<150	<100	<50	<20
Liver Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6-11.9	>12
Cardiovascular [#] (hypotension)	No hypotension	MAP <70	Dopamine ≤5 or dobutamine (any)	Dopamine >5 or norepinephrine ≤0.1	Dopamine >15 or norepinephrine >0.1
Central Nervous System (GCS)	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) or urine output (ml/d)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

 # adrenergic agents administered for at least 1 hour (doses are given in µg/kg/min)
MAP mean arterial pressure; GCS Glasgow Coma Scale

The same study also demonstrated that maximum-SOFA can be used to quantify the impact of therapeutic interventions on overall or organ-specific morbidity. Some but not all of those interventions may have an impact on mortality. The total maximum SOFA score had an AUC value of 0.847 (SE 0.012) in their study. For individual organ system scores, the best discriminative power was seen for cardiovascular score. In multivariate analysis, the impact on outcome of organ dysfunction/failure was higher for cardiovascular (odds ratio 1.68) and renal (odds ratio 1.46) scores [81]. This finding is supported by other studies [82]. Other work has shown good correlation with mortality from the mean-SOFA score with AUC values as high as 0.88 (SE 0.03) [58].

1.3.5 The limitations of the SOFA score

The use of the delta- and maximum- SOFA score does have its limitations [83]. A high delta-SOFA was poor at predicting survivors from non-survivors, perhaps because the delta-SOFA score would be relatively low if the admission SOFA was very high and did not take into account any improvement in organ dysfunction. In an attempt to negate these limitations some studies have used combinations of admission scores with sequential scores for example the SAPS score together with maximum-SOFA in order to improve predictive power between survivors and non-survivors, but these are yet to be validated in larger studies [84,85].

Further drawbacks exist for the scoring systems as many use a single parameter as a surrogate marker of the severity of any particular organ's dysfunction. With advances in supportive care, these markers used in isolation may not correctly represent the degree of organ dysfunction at the time of evaluation. For example, continuous renal replacement therapy will lower serum creatinine, which is the marker in many scoring systems to measure renal function, and as a consequence underestimate the true extent of deranged organ physiology. The SOFA score incorporates both urine output and creatinine in combination in an attempt to ameliorate this fault. The developers of the SOFA score believed it was only a very small subset of patients who would have preserved urine output and normal creatinine in the presence of acute kidney injury [86]. Further disparities exist when introducing mechanical ventilation, positive end expiratory pressure, vasoactive drugs in cardiovascular assessment and the use of sedative drugs in assessing the Glasgow Coma Scale (GCS)[87]. The original

description of the SOFA score stated that it was not clear whether the actual or assumed GCS was optimal for scoring CNS function [78].

The SOFA score does, to an extent, make allowances for some of these supportive care adjuncts by including within the scoring matrix the use of catecholamines, mechanical ventilation and urine output as parameters [78]. Despite these limitations, the SOFA score is the most commonly used organ dysfunction/failure score in practice [88].

The different forms of scoring systems have become a necessary tool to describe ITU populations and to explain differences in mortality [83,89]. It must be borne in mind, however, that these scoring systems may show a lack of fit when evaluated in different critical care populations [90] and their exclusion criteria may exclude up to 15% of admissions, thus introducing bias into a risk-adjusted analysis [91]. As there are several potential areas of error related to the interpretation of the numbers supplied by the systems, they must only be used with knowledge of the science of severity scoring. Whilst some are calculated using only data in the first 24 hours of admission, others are sequential over the entire ITU and can provide information over the course of the disease and thus monitor therapeutic efficacy. The use of dynamic sequential measurements over the course of the disease more closely reflects the clinical perception that mortality is not dictated by the degree of illness at admission to the ITU but rather is dependent on the patient's response (or lack of response) to therapeutic interventions.

1.4 Nutrition in the critically ill patient

The ITU will inevitably contain the sickest, most metabolically stressed patients in any care setting. Up to 43% of ITU patients are malnourished and this poses a risk of complications including muscle loss and weakness leading to ventilator dependence [92]. Although the most appropriate method of feeding critically ill patients is not straightforward, nutrition has been shown to improve outcomes [93].

In the initial course of sepsis, a massive hyper-inflammatory or acute phase response (systemic inflammatory response syndrome—SIRS) triggered by the microbial invasion and/or direct tissue injury, takes place. It is mediated by the pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), eicosanoids and cortisol. Its purpose is to mobilise nutrients including glucose and amino acids for the production of white cells, collagen, fibroblasts and acute phase proteins. Whilst this hyper-inflammatory and catabolic response is necessary; a prolonged or excessive response can be detrimental and lead to multi-organ dysfunction.

Following the period of hyperinflammation, an anti- inflammatory phase (Compensatory Anti-inflammatory Response Syndrome—CARS) comes into action to antagonise the initial reaction. Here, anti-inflammatory acting cytokines are synthesised and lymphocytes and monocytes become apoptotic. Lymphocytes show impaired proliferation and produce low levels of the T-helper 1-type cytokines (associated with the host defence against bacteria and viruses) but high levels of the T-helper 2-type and regulatory T-cell-type cytokines (associated with inhibition of the

host defence against bacteria and viruses) [94-96]. All these reactions may lead to a further weakening of the host and facilitate the acquisition of secondary infections.

1.4.1 The introduction of lipids providing nutrition to the critically ill

ITU patients are prone to developing a negative nitrogen balance because of catabolism and can lose significant amounts of skeletal muscle leading to prolonged mechanical ventilation [97,98]. Lipids have frequently been used in the ITU setting to provide nutrition to the critically ill patient. They may in addition abrogate the effect of the catabolic and hyper-inflammatory state in sepsis or after surgery [99]. They can be applied orally, enterally, or parenterally depending on bowel function. Oral or enteral administration is often preferred, but may not be possible in ITU patients due to incapability to swallow, failure of gut peristalsis or gut-blood barrier transport. Total parenteral nutrition (TPN) has been used for sustained periods in critically ill patients in intensive care units to provide nutritional support [100] and has been shown to improve survival compared to no nutrition [101].

In order to provide full calorific support, lipids and lipid emulsions play an essential role and are also crucial for cell membrane composition. The lipid traditionally used in parenteral nutrition regimens is soybean oil, in which approximately 54% of the fatty acid (FA) component is linoleic acid (LA, an n-6 FA) [102]. Concern has been expressed that a lipid emulsion high in LA might be potentially harmful due to the perceived risk that it is pro-inflammatory, pro-coagulatory and immunosuppressive. Clinical trials using LA rich emulsions have not supported this hypothesis [103]. A meta-

analysis of two studies in which TPN was administered in critically ill patients suggested that although there was no difference in mortality rate; TPN with standard lipids resulted in a higher infectious complication rate than TPN infused without lipids [104,105]. Newer formulations of TPN are available containing FAs with other lipids, such as medium-chain triglycerides, olive oil, or fish oil [103].

Intravenously applied lipid emulsions result in a high concentration of free FAs due to the initiation of the lipoprotein lipase on the endothelium. These free FAs subsequently translocate into the vessels after activation [106]. FA availability is further increased by heparin, which activates lipoprotein lipases, [107]. In addition, the metabolic stress caused by underlying disease processes can also increase free FA levels [108]. Free FAs from parenteral application, in addition to the stress of critical illness, means that much higher concentrations of plasma lipids are found in these patients [109-111].

1.4.2 Lipid synthesis and implications in the pathophysiology of critical illness

The cell membranes of all cells are composed of phospholipids and the polyunsaturated fatty acid (PUFA) components, omega-3 and omega-6 FAs are metabolised to produce a bewildering array of biologically highly active products. FAs are composed of hydrocarbon chains with a carboxyl group at one end. The carbons are connected by single bonds in saturated FAs. In unsaturated FAs some carbons are connected by double bonds in varying locations. N-3 and n-6 FAs are differentiated and named according to the position of the first double bond. N-FAs

have the first double bond three carbons from the methyl end of the carbon chain and n-6 fatty acids have the first double bond six carbons from the methyl end. N-3 and n-6 are regarded as essential fatty acids (EFAs) because humans cannot endogenously desaturate the n-3 or the n-6 bond. Both n-3 and n-6 FAs must, therefore, be obtained from dietary sources especially cold-water fish that in turn derive n-3 and n-6 FAs from consumed plankton and algae.

Most n-6 FA is consumed as linoleic acid (LA) [18 carbons, two double bonds, n-6 or 18:2 (n-6)]. This can be found primarily in vegetable oils, especially soybean oils, and meat (in addition to some arachidonic acid (AA) [20:4 (n-6)] also obtained from meat) [112]. LA is the shortest chain omega-6 FA and it is converted to γ-linolenic acid (GLA) [18:3 (n-6)] and AA. N-3 FAs may also be found in vegetable oils, especially canola and soybean oil, and green leafy vegetables as α-linolenic acid (ALA) [18:3 (n-3)] and in larger amounts in fatty cold-water fish as eicosapentaenoic acid (EPA) [20:5 (n-3)] or docosahexaenoic acid (DHA) [22:6 (n-3)]. ALA is the parent 18-carbon FA from which the human body synthesises the longer n-3 fatty acids such as EPA and DHA. The same enzymes use both n-3 and n-6 FAs as a substrate for the subsequent production of various pro- and anti- inflammatory eicosanoid metabolic products; specifically prostaglandins, thromboxanes, leukotrienes, lipoxins, and hydroxyl fatty acids, which are directly involved in inflammation [113]. All three major n-3 FAs (ALA, EPA and DHA) suppress the production of AA from LA by competing more successfully than LA for the activity of the Δ5 and Δ 6 desaturases [114]. Local-acting lipid mediators involved in the regulation of inflammation are derived from the semi-essential PUFA's AA, EPA and DHA.

1.4.3 The addition of omega -3 fatty acids to parenteral nutrition

The majority of older generation lipid emulsions used in parenteral nutrition are based solely upon soybean oil, which is rich in the n-6 FA linoleic acid, or a 50:50 mix of vegetable oil rich in medium-chain saturated fatty acids and soybean oil (often termed MCT/LCT to indicate the mixture of medium chain and long chain triglycerides). The eicosanoid product profile would, therefore, be expected to be proinflammatory and proproliferative. It has been postulated that adding n-3 FAs (principally EPA and DHA) to TPN could have profound anti-inflammatory effects aiding recovery in patients with conditions causing systemic inflammatory response syndrome or sepsis [115-117]. It has been suggested that the optimum dose of n-3 to attenuate the response is 3–10 g/d or approximately 0.1–0.2 g/kg [118].

The anti-inflammatory properties of n-3 FAs, therefore, may have therapeutic potential, but as only negligible amounts of EPA and DHA can be produced by human metabolism (only from the desaturation of ALA as mammals cannot re-esterify FAs and insert double bonds) they are unlikely to reach therapeutic potential. Thus, high quantities of parenterally administered n-3 FAs could have therapeutic potential.

1.5 Omega-3: A potential novel therapeutic addition to the sepsis armoury

Recently fish oil, containing long chain n-3 fatty acids, has been introduced into some lipid emulsions [103,119] with the rationale that n-3 fatty acids act to reduce inflammatory responses [120], which may be promoted by an excessive or unbalanced supply of n-6

fatty acids that TPN is more frequently composed of. Compared with n-6 fatty acid rich vegetable oil, fish oil reduces the metabolic signs of endotoxemia in animal models [121], lowers plasma cytokine concentrations [122] and serum procalcitonin, [123] as well as improving survival [121].

N-3 fatty acids are believed to act by four main anti-inflammatory mechanisms that could potentially be beneficial during sepsis and critical illness [124]:

- Metabolism into bioactive anti-inflammatory eicosanoid inflammatory mediators
- Alteration of membrane lipid rafts
- Inhibition of nuclear receptor activation (specifically NF-kB) to modulate production of inflammatory mediators
- Metabolism into novel pro-resolving and anti-inflammatory mediators named the resolvins and protectins.

1.5.1 The pro- and anti- inflammatory mediator profile

Most cellular membranes are rich in arachidonic acid (AA) derived from omega-6 fatty acids (n-6 FAs) since this constitutes the majority of fatty acids in a normal diet. Leukocyte membrane phospholipids are normally composed of 30% PUFAs [125]. When the inflammatory cascade is activated by a stimulus, a macrophage can mobilize 25% to 40% of its membrane lipid content to produce free AA [125]. Cyclooxygenase (COX) or lipoxygenase (LOX) and cytochrome p-450 activity on AA, cleaved from cell membrane phospholipids by phospholipase A2, produces a

prostaglandin, leukotriene, prostacyclin, thromboxane (eicosanoid) product profile that tends to be pro-inflammatory and pro-proliferative in most tissues. Namely these are the 2-series prostaglandins and thromboxanes and the 4-series leukotrienes and lipoxins (Figure 2). Eicosanoids are powerful ‘‘local hormones’’ acting only at the site of production. COX activity on EPA or AA results in the production of prostaglandins or thromboxanes, whilst LOX activity results in the production of leukotrienes (Figure 1: Human fatty acid pathways).

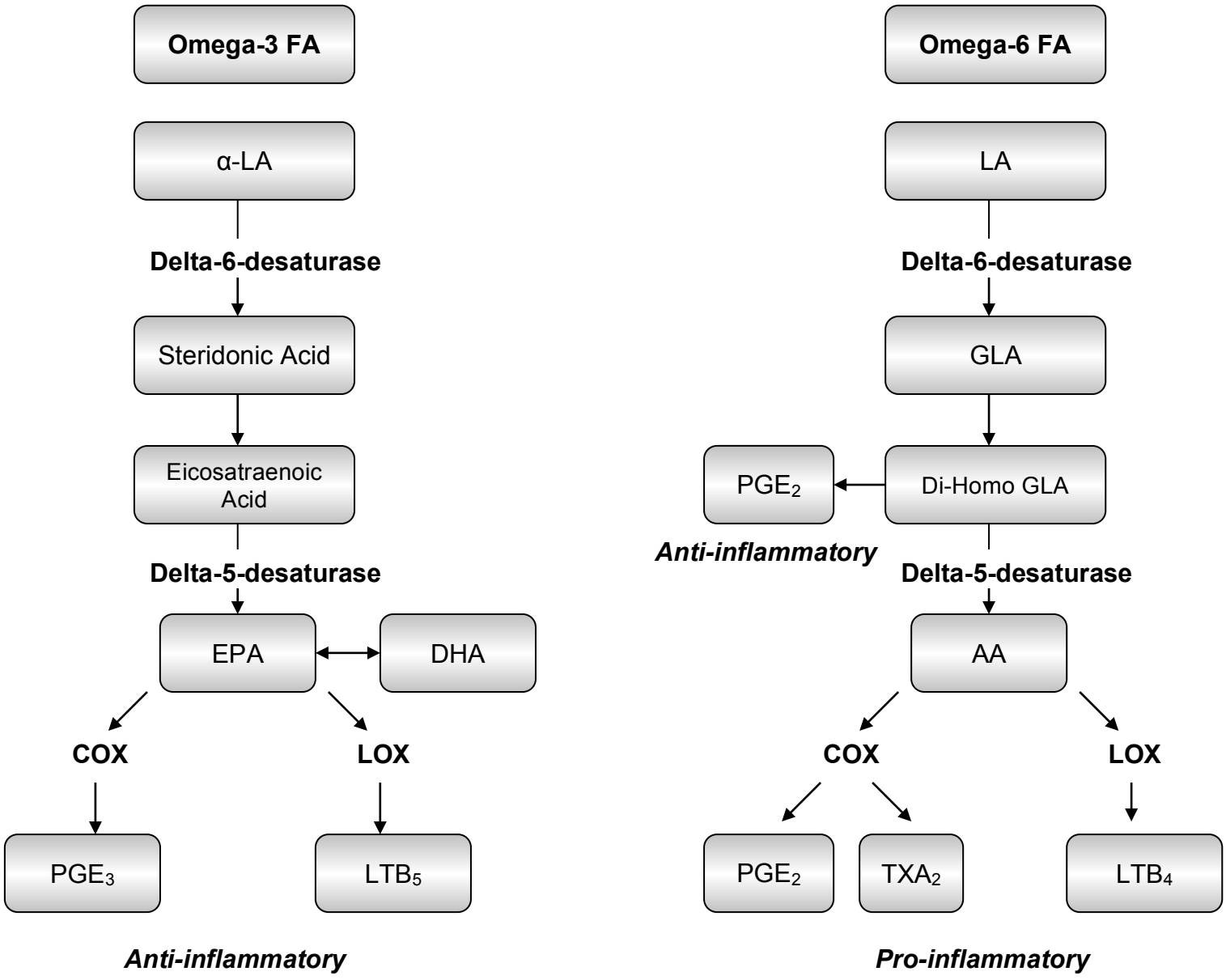
The effects of prostaglandin E_2 (PGE_2), thromboxane A2 (TXA2), and leukotriene B_4 (LTB_4) are well known in the pathophysiology of inflammation. Their clinical effects include inducing fever, chemotaxis, vasodilatation, increased vascular permeability, and enhanced pain sensation [126-128]. In addition, their molecule activity results in the generation of reactive oxygen species, release of proteases such as elastase and synthesis of lipid mediators [125]. TXA_2 has been demonstrated to increase vascular permeability, platelet aggregation and bronchoconstriction; it is also believed to have a role in organ dysfunction due to its pro-thrombotic effects causing local tissue ischaemia [125,129,130].

In contrast, COX or LOX activity on EPA produces a different series of eicosanoid products that tends to be less inflammatory and less promotional to proliferation in most tissues, namely the 3-series prostaglandins and thromboxanes and the 5-series leukotrienes (Figure 1: Human fatty acid pathways).

Products derived from the metabolism of n –3, significantly decrease the generation of pro-inflammatory cytokines such as TNF-a, IL-1, IL-6 and IL-8 [131-133] and are

associated with plasma biomarker levels, reflecting lower levels of inflammation and endothelial activation [134].

Figure 1: Human fatty acid pathways



Any adjustment of the diet which affects the ratio of n -3 and n -6 fatty acids in the cell wall has the potential to significantly influence the type of eicosanoids which are produced and it is this effect which is able to modulate the cells of the immune system. The aim of n -3 supplementation is thus to reduce the amount of substrate available for the synthesis of harmful inflammatory mediators by competing with AA for metabolism via COX and LOX. However, even with a balanced diet rich in fish, plasma concentrations are minimal. This is in sharp contrast to parenteral administration of a fish-oil lipid emulsion, which leads to a significant and rapid increase in EPA and DHA concentrations in plasma and platelet and leukocyte membrane phospholipids within hours [110,128,135,136].

One n -6 FA may provide benefit in critical illness via mechanisms that are incompletely understood, namely γ-linolenic acid (GLA), which may have an additive effect to DHA and EPA [137]. GLA incorporated into immune cell phospholipids as dihomo-GLA where it can reduce the availability of AA and AA's associated pro-inflammatory products [138]. Dihomo-GLA is converted to PGE_1 that has been shown to vasodilate the pulmonary and systemic circulation [139]. In animal models, nutrition containing DHA, EPA and GLA resulted in a reduction in alveolar concentrations of LTB_4, PGE_2, and TXB_2, a decrease in pulmonary capillary permeability and reduction in alveolar neutrophil accumulation [137,140].

1.5.2 Alteration of lipid rafts

N -3 FAs are rapidly incorporated into cell membranes where they affect many functions including the modulation of the production of cytokines and the control of microviscosity and fluidity. Fluidity refers to a complex property involving membrane permeability and components. It has a central role in influencing the activity of membrane-bound enzymes, surface receptor functions, and transporters as well as lipid-based second messenger systems [141].

Within the cell membrane, lipid rafts (composed of phospholipid bilayers) facilitate intercellular signaling and contain many receptors and signaling proteins [142]. FAs have been shown to affect lymphocyte membrane fluidity in a structure dependent manner [143]. N -3 FAs have been shown to displace acylated proteins from rafts in T cells and can, therefore alter cell function [144,145]. DHA, in particular, may significantly affect cellular signal transduction and inflammatory processes by influencing the basic properties of cell membranes, including fluidity, compressibility, and permeability [146,147].

1.5.3 Inhibition of transcription factors

FAs can affect cell responses through the regulation of gene expression by acting as ligands for nuclear receptors and controlling transcription factors such as peroxisome proliferator-activated receptors (PPARs) and sterol-regulatory element binding proteins [148]. FAs influence many genes and their effects on the cellular response are

wide ranging; from alterations in surface adhesion molecule expression to cytokine production.

N -3 FAs exert their effects due to a direct interaction on intracellular signaling pathways, which leads to activation of one or more transcription factors particularly nuclear factor kappa-ß (NF-κB) [149]. NF-κB is a key transcription factor involved in the upregulation of pro-inflammatory cytokine and adhesion molecule production [150]. It is activated by the phosphorylation of an inhibitory subunit (I-κB) triggered by extracellular inflammatory stimuli [151]. Recent studies have suggested that EPA and DHA may directly inhibit NF- κB activation and as a consequence down-stream inflammatory cytokine production, although the exact mechanism of inhibition remains unclear [152,153].

Animal models have shown that n -3 also suppresses inflammatory gene expression especially for TNF-α, IL-6 and IL-1ß as this gene expression is regulated by eicosanoids derived from AA. LA on the other hand may increase production of TNF-α and IL-6 through activation of NF-κB and may therefore play a pro-inflammatory role [154-156].

1.5.4 Metabolism of omega-3 into novel pro-resolving and anti-inflammatory mediators

Active resolution of acute inflammation is a previously unrecognised interface between innate and adaptive immunity. Once thought to be a passive process, the

resolution of inflammation is now shown to involve active biochemical mediators that enable inflamed tissues to return to homeostasis [157]. These mediators were named resolvins as they were first discovered during the resolution phase of acute inflammation [157-160].

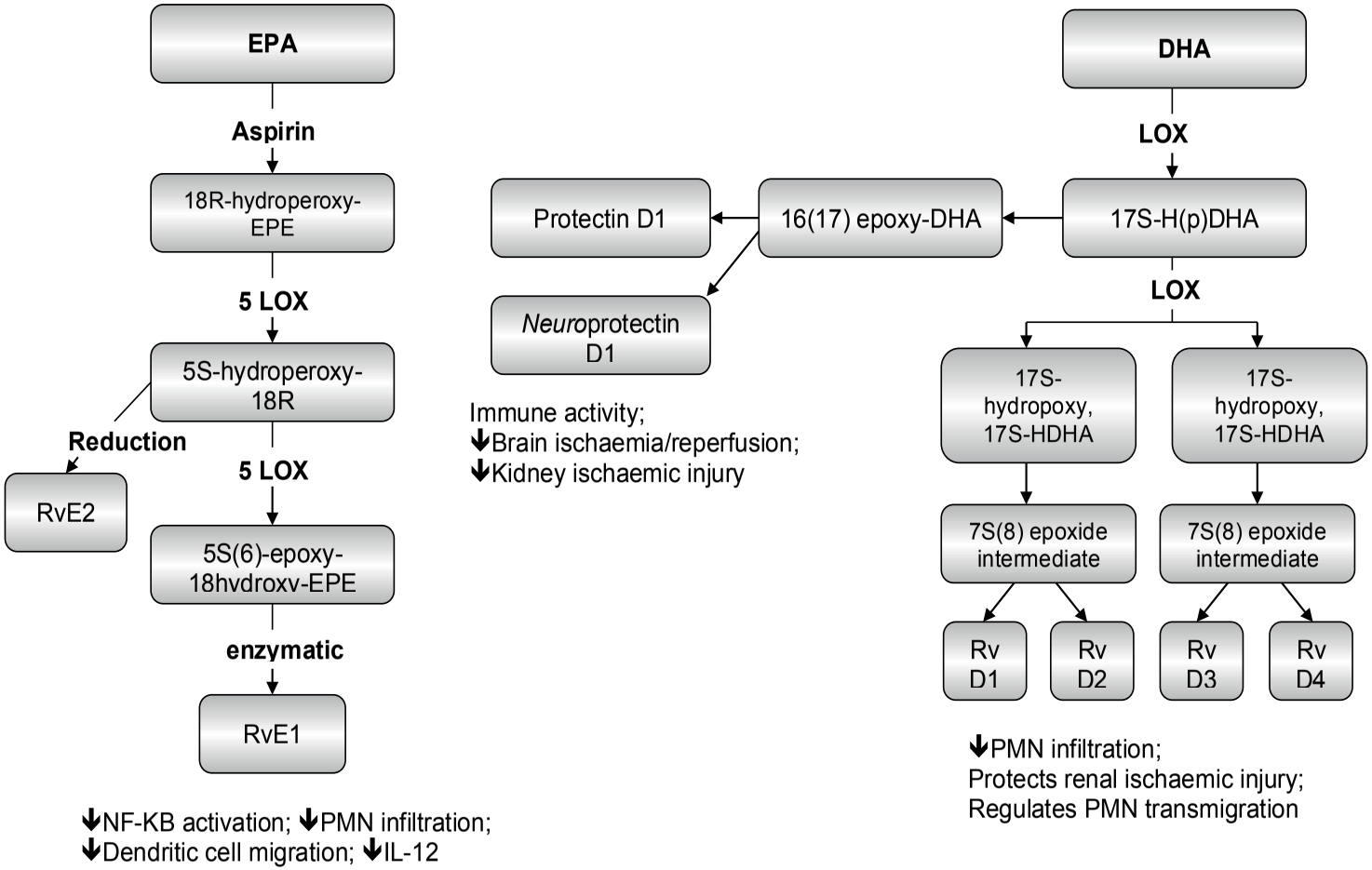
EPA derived compounds are designated as E-series resolvins, whereas the mediators derived from DHA were called D-series resolvins and (neuro) protectins (discovered initially in the brain). Resolvin (Rv) E1 has been found to affect cell response by reducing the activity of NF-kB [161]. At the locus of inflammation, the pro-inflammatory impact of neutrophils and production of pro-inflammatory cytokines (like TNF-a and IL-1) is alleviated due to the reduced activation of NF-kB. Furthermore, they are able to reduce the activity of proteases and reactive oxygen species (ROS), which leads to reduced tissue injury and oedema formation [162]. RvE1 and Protectin D1 are mediators that have been effective in resolving animal models of airway inflammation and colitis [161,163].

Resolvins dampen the course and enhance resolution of inflammation via several different mechanisms. By attenuating expression of adhesion molecules and stimulation of endothelial nitric oxide synthatase (NOS) they reduce neutrophil extravasation and invasion to inflammatory sites [164].

A special role of resolvins takes place during the resolution phase of inflammation. Resolvins increase local recruitment of monocytes to sites of inflammation by chemotaxis. They reduce neutrophil extravasation and invasion to inflammatory sites by the expression of adhesion molecules such as E-selectin, ICAM and VCAM and by

decreasing Il-1 levels [165]. The subsequent uptake of apoptotic neutrophils by macrophages is therefore accelerated. At the end of the inflammatory process, resolvins facilitate the removal of macrophages via lymphatic vessels [158]. Results of experiments in animals imply that they may bring new treatment options [161,166,167]. These treatments include those for acute lung injury (ALI) and pneumonia [168], chronic airway inflammation [169], asthma [170], or peritonitis and sepsis [164]. Until recently, few clinical studies concerning the role of resolvins in patients have been published, it is possible that resolvins may be able to prove beneficial by accelerating the resolution of inflammation [164,168].

Figure 2: Resolvin and protectin synthesis



1.6 Omega-3 in critically ill patients: The evidence so far

Fish oil containing parenteral nutrition has been used in surgical patients and has demonstrated possible improvements in immune function [171,172] and reduced inflammation [133,171]. These findings have been linked to a shorter stay in the ITU [171] and in hospital [171,173]. Parenteral fish oil has been demonstrated to be well tolerated in critically ill patients [174], and may be associated with better liver function and improved antioxidant status in patients requiring parenteral nutrition [175]. In addition, it has been demonstrated that they result in improved morbidity (as predicted by risk scoring models such as APACHE and SAPS) [123].

Studies have also investigated the effects of n -3 on critically ill patients with other diseases associated with hyper-inflammation. One study was able to show the beneficial effects of a lipid emulsion enriched with FO in patients with severe pancreatitis [176]. The authors showed a reduction in the hyper- inflammatory response using parameters such as lower C-reactive protein (CRP), better oxygenation index and a reduced period of continuous renal replacement therapy following five days of parenteral nutrition with addition of FO. The authors postulated that n-3 FAs were able to diminish the hyper- inflammatory systemic response triggered by pancreatitis, resulting in decreased pro-inflammatory cytokine production and attenuated organ injury.

Heller and colleagues [177] reported a dose-response effect of parenteral fish oil on antibiotic demand, length of hospital stay and mortality (using doses of >0.05 g and >0.1 g fish oil/kg/day respectively) in critically ill patients. The need for antibiotic treatment was higher in the patients receiving fish oil doses of <0.15 g/kg/day

suggesting a threshold dose for n -3 efficacy. The study by Khor and colleagues supported this by finding that organ dysfunction could be improved by fish oil supplementation in their blinded RCT of 28 patients [123]. They found no difference in length of stay or serum TNF-α levels.

1.6.1 Omega-3 in septic patients

There is a paucity of studies investigating the effects of fish oil containing lipid emulsions in critically ill septic patients in the ITU. Several studies have investigated the role of immune-modulating additives to nutrition but have used a cocktail of ingredients including fish oil, arginine, glutamine and antioxidants. It is difficult to elucidate the exact contribution of fish oil to the demonstrated clinical benefits. Nevertheless, studies investigating the effects of immunomodulation have demonstrated a reduction in ventilator days and a reduction in mortality [139,178,179].

In a single-centre prospective, double-blind, placebo-controlled, randomised study recruiting 115 septic patients, Pontes-Arruda and colleagues [139] were able to show that patients fed with an enteral diet (which included EPA, GLA and antioxidants) exhibited a reduction in mortality when compared to a control diet. Intention-to-treat (ITT) analysis demonstrated that patients fed the EPA/GLA diet developed less severe sepsis and/or septic shock than patients fed the control diet (26.3% versus 50%, respectively; p =0.0259). The ITT analysis demonstrated that patients in the study group had a reduced incidence of cardiovascular failure (36.2% versus 21%, respectively; p = 0.0381) and respiratory failure (39.6% versus 24.6%, respectively; P = 0.0362). Similarly, when considering only the evaluable patients, fewer patients

developed cardiovascular failure (20.7% versus 37.7%, respectively; p = 0.03) and respiratory failure (26.4% versus 39.6%, respectively; p = 0.04). Whilst there was no significant reduction in 28-day all-cause mortality, there were significant reductions in length of stay on ITU and in patient stay. The improvements in respiratory function have been supported by another study by Gadek and colleagues in a randomised study investigating the effects of enteral EPA and GLA [180]. The group also found improvements in oxygenation, fewer days requiring supported ventilation, reduced organ dysfunction and reduced length of stay in the treated cohort.

Two meta-analyses were published dealing with this topic. Pontes-Arruda and colleagues in their meta-analysis of three studies [181] and 411 patients, demonstrated that a diet enriched in FO and GLA (in patients with acute lung injury and acute respiratory distress syndrome) lead to a significant reduction in mortality risk, onset of new organ failures, improved oxygenation, a shorter duration of mechanical ventilation and an overall better clinical outcome. Marik and colleagues analysed 21 studies incorporating 1,918 patients in their meta-analysis of high-risk patients undergoing elective surgery who received FO and arginine. They confirmed a significant reduction in the risk of acquired infections length of stay and risk of wound infection [182].

Since these meta-analyses were published, studies investigating similar immuno-modulating enteral feed have demonstrated conflicting results. A study by Grau-Carmona found that although length of ITU stay was reduced in the fish oil-treated group, there was no difference in organ dysfunction or improvement in gas exchange [183]. Bertolini and colleagues, who recruited critically ill patients from thirty-three general intensive care units in Italy, also reported negative results. In their study, the

trial patients received an enteral immuno-nutrition including FO, arginine, vitamin E compared to a group receiving a standard parenteral nutrition. In the subgroup of patients with severe sepsis they evaluated the mortality rate. Interim analysis demonstrated excess mortality in the group treated with immuno-nutrition (44.4% vs. 14.3%; p= 0.039) leading to premature cessation of the trial [184]. In support of these negative findings, Friesecke and colleagues [185] reported that the use of a mixed MCT/LCT/fish oil lipid emulsion in critically ill ICU patients had no effect on the expression of inflammatory marker IL-6, monocyte expression of HLA-DR (a marker of immune competence) or on clinical outcomes measures (including infections, ventilation requirement, or ITU or hospital stay) compared with MCT/LCT. The authors speculated that the failure to see positive results could have been because patients entered the trial after the inflammatory process was fully activated, in contrast with studies investigating therapy in surgical patients who receive n -3 prior to surgical trauma [186].

In two further studies, Mayer and colleagues [110,136] reported diminished inflammation, including reduced TNF-α, IL-1β, IL-6, IL-8 and IL-10 production by cultured monocytes, in septic patients receiving soybean oil together with fish oil mix compared to those receiving soybean oil alone. The administration of the n -3-rich emulsion induced an increase in ω -3-free FA's in plasma and reversed the n -3/ n -6 ratio, favoring EPA and DHA over AA. These changes reached a maximum effect in 3 days [136]. There was no difference in serum cytokine levels between groups. A trend towards reduced ventilation dependence associated with a lower CRP and leukocytes count in the ω -3 treated group was observed, but did not reach statistical significance [110]. An increase in LTB_5 (an anti-inflammatory leukotriene) was observed in the group receiving n -3 lipids. Trends were also seen with increased plasma n -3-free FA's, the

TXA_3/TXA_2 ratio, and platelet-activating factor (PAF) synthesis in the group receiving the n -3 rich lipid emulsion.

Barbosa and colleagues reported the results of a randomised clinical trial investigating the effects of fish oil containing lipid emulsion on patients with sepsis in a single unit [187]. Twenty-five patients with systemic inflammatory response syndrome or sepsis, and who were predicted to need parenteral nutrition were randomised to receive either a 50:50 mixture of medium-chain FAs and soybean oil or a 50:40:10 mixture of medium-chain FA's, soybean oil and fish oil. They demonstrated that parenteral fish oil increased plasma EPA, lowered IL-6 and improves gas exchange. These changes were associated with a trend towards shorter length of hospital stay.

1.6.2 The efficacy of omega-3 in sepsis: clinical equipoise

There is only limited, and occasionally contradictory, information on the influence of fish oil containing parenteral nutrition in septic ITU patients regarding markers of inflammation and clinical endpoints. The studies published thus far, do show that n -3 is safe in critically ill patients [103]. A major confounding factor in the studies is that fish oil is given in differing amounts, by different routes (enteral and parenteral) and is often combined with other immuno- modulating nutritional support. Absorption of individual lipids at the intestinal level can be drastically affected by the presence or absence of other nutrients [188]. Similarly, FO emulsion is frequently given together with MCTs, which may reduce efficacy.

Other limitations of the published studies in n -3 are that they were often underpowered, incorporated heterogeneous lipid emulsions, often lacked an intention to treat analysis of their data and used a control formula, which was lower in ω -6 fatty acids due to the presence of MCTs [189]. This would reduce the amount of LA available and may mean that the control lipid emulsion was less pro- inflammatory than that used in standard clinical practice [185]. Although much has been discovered about the mechanisms of action behind fatty acids, the inconclusive evidence presented thus far does not recommend its routine use and it is unclear if the provision of n -3 after the onset of a severe inflammatory response is beneficial.

1.7 Statement of aims

The study has several primary outcomes both dependant and independently associated with fish oil. Primarily, the study aims to look at whether the addition of parenteral FO therapy to critically ill patients with sepsis affects outcome. Studies, thus far, have been inconclusive due to the inherent difficulties previously discussed, small numbers and heterogeneous nature of both ITU patients and sepsis.

The efficacy of fish oil may also be multifactorial, based on both patient and infection related differences. The study therefore also aimed to permit subgroup analysis in an attempt to identify, which patients, if any, are most likely to benefit from this therapy, and likewise, to identify any group of patients to whom it may be detrimental.

In support of these conclusions, fatty acid levels, in a range of lipid pools will be measured in order for mechanistic inferences to be made and also to permit the previously un-investigated pharmacodynamic assessment of FO infusions. The fatty

acid levels will also be analysed for any associations with survival outcomes. Fatty acids can also be analysed against patient specific factors (such as age and gender) to measure any influence of these.

The vast amount of physiological data collected will permit trends in organ dysfunction/failure to be assessed in both surviving and non-surviving patients. This data will allow the patterns of organ system deregulation in multi-organ failure to be measured and compared. The appropriateness of scoring systems, such as APACHE, SOFA and SAPS will also be tested. An attempt at improving the currently available scoring systems will also be made by combining systems to improve the predicting power of mortality in the study population.

All of the data collected will be used, if parenteral fish oil is associated with advantageous clinical outcomes, to form the largest pilot study and basis for a multi-center randomised control trial.

2 Methods

The study was performed in a 9-bed general and surgical ITU and a 4-bed general and surgical high dependency unit (HDU) in one tertiary-referral hospital. The study protocol was reviewed and approved by the national ethics committee and was conducted in accordance with the Helsinki declaration. From May 2010 until July 2012 consecutive 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.

2.1 Difficulties encountered during the trials ethical approval

The fish oil emulsion used in this trial (Omegaven^{TM}; Fresenius Kabi) is licensed for use a supplement to complementary parenteral nutritional support. The intention of this study was to investigate the effects of omega-3 as a medicinal product in its own right, separate to that of nutritional support. It was also the study's intention to investigate its effects on critically ill patients who likely lacked capacity to consent for themselves. There are only three ethics committees in the UK able to assess the merits and ethics of the trial protocol, including London, where this trial/s protocol was evaluated.

Further difficulties were encountered to ensure that patients were recruited within the narrow therapeutic window from admission to the ITU (as outlined below). The ethics committee had suggested that professional representatives be enrolled (who were separate from the studies interests) who could act as the patient's advocate in the

absence of immediately available relatives. After a training session was given to the anaesthetic consultants in the ITU; a number of consultants gave signed consent to be the named professional representatives for the trial. The gaining of approval for this ethically complex study to start caused considerable delays at the outset.

2.2 Definitions

Sepsis was defined as a proven or suspected source of infection together with at least two of the four 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. Acute lung injury (ALI) was defined by the presence of acute hypoxemia (defined as a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of ≤300, or ≤200 for acute respiratory distress syndrome (ARDS)) and new bilateral infiltrates on a frontal chest radiograph that were not attributable to left atrial hypertension [190].

2.3 Study Patients

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, obtain the necessary written consent and randomise the patients. Patients were randomised to receive either standard care or standard care together with parenteral n -3 (Omegaven™; Fresenius Kabi). Sealed envelopes were used for the randomisation

process and were assigned in blocks of 20. The anaesthetic team directed routine medical care and made decisions on suitability for discharge to lower levels of care. All included patients were treated in accordance with the "Surviving Sepsis Campaign Guidelines" for the management of severe sepsis and septic shock [4]. This care included goal directed therapy with adequate initial fluid resuscitation administered to all patients with sepsis-induced tissue hypoperfusion [191]. Broad-spectrum antibiotics were administered within the first hour of ITU admission or had already been initiated on the ward prior to ITU admission.

Inclusion criteria were all consecutive patients deemed to require level II or III care with sepsis. Patients were excluded for the following reasons:

- Planned ITU/HDU admission post elective surgery
- Hypersensitivity to fish, egg or soy protein
- Uncontrolled haemorrhage
- Uncontrolled hyperlipidaemia
- Severe primary blood coagulation disorder
- Acute pancreatitis accompanied with hyperlipidaemia
- Ketoacidosis
- Acute thromboembolic disease
- Severe liver failure
- Acute phase of myocardial infarction or stroke
- Pregnancy
- Patient not expected to survive more than 24 hours
- Patients were also excluded if they developed sepsis when the intensivists had withdrawn treatment and palliative care plan had been instigated.

2.4 Omega-3 Infusion

Omega-3 (Omegaven™; Fresenius Kabi) was given as per manufacturers guidelines as an independent drug and not as a nutritional supplement. FA content of the emulsion was as shown in Table 2.

Table 2: Contents of Omegaven™

Fatty Acid	Concentration (g/L)
EPA (C22:6 n-3)	12.5–28.2
DHA (C22:6 n-3)	14.4–30.9
myristic acid (C14:0)	1–6
palmitic acid (C16:1 n-7)	3–9
stearic acid (C18:0)	0.5–2
linolenic acid (C18:3 n-3)	≤2
oleic acid (C18 n-9)	6–13
linoleic acid (C18:2 n-6)	1–7
stearidonic acid (C18:4 n-3)	0.5–4
eicosanoic acid (C20:1 n-9),	0.5–3
arachidonic acid (C20:4 n-6)	1–4
docosanoic acid (C22:1 n-9)	≤1.5
docosapentanoic acid (C22:5 n-3)	1.5–4.5
other fatty acids	10.51

Omega-3 was delivered as per the manufacturer's guidance at 2mls/kg/day and given at a rate of 0.5ml/kg/hour. It was given daily until day 14 or until discharge from the ITU/HDU. All paired days (e.g., day 1 to day 2) represented changes from one full calendar day to the next, with the exception of the period referred to as day 0 of the study. Reference to the day 0 term in this study was used to describe the period from baseline to day 1.

The energy supplied by Omegaven™ is negligible at 112 kcal/100ml. Nutrition was assessed by the intensivists and dieticians who commenced oral, naso-gastric (enteral) or parenteral nutrition as directed by the underlying pathology. The emulsion was

given via a dedicated central line lumen of a quin port central line or a large peripheral cannula.

Patients were monitored for any adverse effects from the infusion. The infusion was discontinued if these were encountered or if was felt by the anaesthetic team that the omega-3 was contributing towards any adverse effects. Serum lipids were monitored during the infusion to exclude the presence of fat overload syndrome. Overdose leading to fat overload syndrome may occur when the triglyceride level during lipid infusion rises above 3 mmol/l, acutely, as a result of too rapid infusion rate, or chronically at recommended rates of infusion in association with a change in the patients clinical condition e.g. renal function impairment or infection.

2.5 Data Collection

The following variables were prospectively collected: age, gender, co-morbidities, admission diagnosis to the ITU, source of infection and available microbiology. The APACHE-II and SOFA score was calculated from the most aberrant clinical and laboratory parameters in the first 24 hours from admission to the ITU. The SOFA score was calculated daily from the most aberrant clinical and laboratory parameters. As described (11), the total SOFA score contains six individual organ SOFA scores namely, respiratory, coagulation (hematology), liver (hepatic), cardiovascular (CV), central nervous system (CNS) and renal. The issue of how best to score GCS in sedated/paralysed patients has been contentious for many years. In the original description of the SOFA score, the last GCS value prior to sedation/paralysis is used and is assumed to be unchanged until the cessation of such medication. In this study

many of the patients included in the study required sedation or were ventilated, and hence, GCS was omitted, as its measurement could be misleading.

Clinical data was recorded for 2 weeks or until discharge following enrolment in the study. In addition, date of discharge from ITU, discharge from the acute hospital and 28-day mortality were recorded. Routine blood tests and microbiology cultures were taken as directed by the intensivists. Patients exited the trial when discharged from the ITU/HDU, at day 14 or because of mortality.

2.6 Clinical end points

2.6.1 Primary end points

The primary end point of the study was to compare the degree of new organ dysfunction between study patients, who received parenteral n –3 (Omegaven^{TM}), and those who did not (control). To quantify organ dysfunction severity, the following scores were used:

- The delta-SOFA score (Sequential Organ Failure Assessment)
- Maximum-SOFA score

2.6.2 Secondary end points

Secondary end points were as follows:

- Length of ITU stay (days)
- Length of hospital stay (days)
- 28-day mortality
- Organ failure free days

- Specific day delta-SOFA scores (e.g. day 3 delta-SOFA represents total SOFA on day 3 minus baseline (day 0) SOFA)
- Development of new cardiac arrhythmias
- Duration of invasive mechanical ventilation and renal replacement therapy
- Mean plasma CRP
- Compare the FA levels of various lipid pools in the treatment group compared with controls
- Undertake pharmacodynamic assessment of the Omegaven^{TM} infusion.

Length of stay was defined as date of admission to ITU up until the day deemed fit for discharge (which could be shorter than actual discharge due to logistical issues). As previously described, a score of 1 or 2 points in each organ system was considered as evidence of organ dysfunction and a score of 3 or 4 points was considered as evidence of organ failure [61].

2.7 Routine laboratory measurements

Full blood count, biochemistry and coagulation were routinely assessed daily as per the intensivists. CRP was measured as directed by the intensivists.

2.8 Fatty acid analysis

Blood samples were taken from all consenting patients on days 0, 1, 2, 3, 5, 7, 10 and 13. On all days, the blood samples were taken prior to the initiation of the Omegaven™ infusion if the patient was in the treatment arm. Samples were taken on

these days until discharge from the ITU or on death. The samples were processed in the laboratory within 10 minutes of retrieval.

In addition, for pharmacodynamic assessment, a number of patients receiving Omegaven™ were selected for more intensive blood sampling. This involved the sampling of blood every 4 hours, from the commencement of the infusion, up until the next infusion (24 hours from the first). Whole blood was taken at these intervals and processed immediately for plasma and white cell isolation and storage at -80°C, until the samples were batch analysed.

The fatty acids in different locations (lipid pools or fractions) are considered to represent different roles. The PC (phosphatidylcholine) and NEFA (non-esterified fatty acid) fractions represent the transport roles whilst PMNs (polymorphonuclear leukocytes) represent the functional role with particular relevance in critical illness and sepsis. Fatty acids were therefore isolated and analysed in the PC, NEFA and PMN lipid pools by the methods outlines below.

In essence whole blood was separated into plasma (containing PC and NEFA) and white cells (PMN). The PC and NEFA pools were separated in plasma using solid phase extraction. All fatty acids were then analysed using gas chromotography.

2.8.1 Isolation and storage of plasma from whole blood

Specific steps for the isolation and storage of plasma from whole blood are detailed below. It is from these samples that PMN and PC lipid pools are measured.

1. Blood was drawn into vacutainer tube(s) containing ~1.8 mg K2EDTA per ml blood
2. The vacutainer tubes were inverted carefully 10 times to mix blood and anticoagulant and stored at room temperature until centrifugation.
3. Samples were centrifuged immediately. This was carried out for a minimum of 10 minutes at 1500 RCF at room temperature. The brake was not used to stop the centrifuge as this would result in layer mixing (see next step).
4. This gives three layers: (from top to bottom) plasma, leucocytes (buffy coat), erythrocytes.
5. The supernatant (plasma) is carefully aspirated at room temperature and placed in a centrifuge tube. Care is taken not to disrupt the cell layer or transfer any cells.
6. The plasma was inspected for turbidity. Turbid samples were centrifuged and aspirated again to remove remaining insoluble matter.
7. 0.5ml volumes of plasma were aliquoted into eppendorfs and store at –80 °C. The eppendorfs were appropriately labeled with the relevant information, including details of additives present in the blood.

2.8.2 Isolation of white cells

Specific Steps for PMN Separations:

1. Pipettes and other equipment were cleaned with 70% ethanol and the density gradient was prepared, using Histopaque™ (H-1077) solutions (Sigma-Aldrich).
2. For each 2 mL of whole blood, 5mL each of H-1077 was used.

3. The volume of H-1077 was dispensed into 15 mL tubes and warmed to room temperature.
4. Whole blood was gently layered onto this 5 mL layer of H-1077 using a pipette.
5. After the initial spin, blood will separate into three fractions, a red blood cell fraction (bottom), a white fraction (the so-called "buffy-coat", the middle layer), and a plasma fraction (clear and yellow, the top fraction).
6. The plasma fraction was gently extracted using a 1 mL pipettor.
7. Plasma was taken out until 1 mL above the buffy coat to extract a total of 2 mL and placed into the 15 mL tubes with the Histopaque™ density gradients. Care was taken when adding the blood to the Histopaque™ , to ensure it was added gently along the sides of the tube.
8. There was no shaking or excessive mixing of the gradient, before or after addition of cells.
9. The Histopaque™ tubes were spun for 25 minutes at 700g's.
10. After spinning, the tubes seperated into several fractions. The top fraction is leftover plasma. The next fraction is a large white band, and this band represents PMNs. The next fraction is clear and is the leftover Histopaque™ solution. Finally, red blood cells sink to the bottom fraction.
11. The plasma fraction was removed until 0.2 mL above the PMNs.
12. The PMN fraction was extracted. Typically, 3 mL of solution total was taken to remove all PMNs (a good number of PMNs is 10 million PMNs per 10 mL blood drawn).
13. The PMNss were placed into a clean 15 mL tube (put 4 mL PBMCs per 15 mL tube).
14. 8-10 mL of wash buffer was added (dPBS without Ca^{2+} and without Mg^{2+}

supplemented with 2% FBS) to the PMNs.

15. The sample was spun at 300g for 6 minutes.
16. The supernatant was extracted into waste using the autopipettor (taken out until 1 mL is left in tube). The cell pellet was not disturbed.
17. The cell pellet was resuspended in the 1 mL volume manually using the 1mL pipettor.
18. 10 mL wash buffer was added and spun again at 300g for 5 minutes.
19. The supernatant was poured into waste (turn over tube once, do not shake, do not lose pellet), the pellet resuspended, and washed once more (300g spin after adding wash buffer).
20. The clean up consisted of the following: 1) 10% bleach was added to the waste beaker for 20 min to kill any blood borne pathogens before rinsing in sink; 2) all pipettors and laminar flow hood were rinsed with 10% bleach; 3) gloves were disposed into the biohazard waste; 4) hands were washed vigorously with disinfecting soap.

2.8.3 Protocol of gas chromatography

The protocol used for the analysis of fatty acids in the three lipid fractions was as described initially by Burdge GC and colleagues[192] and is outlined in detail below.

2.8.3.1 Preparation of total lipid extraction

1. Internal standards (to allow for accurate calibration) were dissolved in 1 ml/mg of dry chloroform: methanol (2:1, v/v) containing butylated hydroxytoluene (BHT; 50 mg/l) as anti-oxidant were added.

2. Chloroform ± methanol (2:1, v/v) containing butyrated hydroxytoluene (50 m g/ml) was added, the preparation mixed briefly and then shaken for 15 min at room temperature.
3. 1 M -NaCl was added (1.0 ml), and organic and aqueous phases separated by centrifugation at 1125 g for 10 min at 48 C.
4. The aqueous phase was removed and the organic phase collected by aspiration.
5. The interfacial protein disc was homogenised in chloroform ± methanol (2:1, v/v) containing butyrated hydroxytoluene (50 m g/ml) and 1 M -NaCl (1.0 ml).
6. The organic phase was separated and collected as before, combined with the initial chloroform layer and dried under N_2 at 40°C.

2.8.3.2 Separation of lipid classes by solid phase extraction (SPE)

SPE was used to separate the PC and NEFA lipid pools from the plasma. This process allows the two lipid pools to be separated in a purified form according to their physical and chemical properties

1. The SPE tank was connected to vacuum pump and aminopropyl silica SPE cartridge was placed on the tank.
2. The total lipid extract was dissolved in 1.0 ml dry chloroform and vortex mix.
3. The sample was applied to the column using a glass Pasteur pipette and allowed to drip through into the screw-cap tube under gravity. When no further drips fall, the remaining liquid was removed by vacuum.

4. New screw-cap glass tube labelled PC were placed into the tank tray under the column.
5. The PC fraction was eluted under vacuum with the addition of 2 x 1.0 ml dry chloroform: methanol (60:40, v/v) until all liquid is removed from the column.
6. The PC fraction was removed and dried under nitrogen at 40°C. Samples were capped and stored at -20°C at this stage for up to a week.
7. New screw-cap glass tube labelled NEFA were placed into the tank tray and NEFA fraction eluted under vacuum by the addition of 2 x 1.0 ml washes of dry chloroform: methanol: glacial acetic acid (100:2:2, v/v/v).
8. Collected NEFA fraction were removed and dry under nitrogen at 40ºC. Samples were capped and stored at -20°C at this stage for up to a week.

2.8.3.3 Analysis using the Gas Chromatograph (GC)

Fatty acid methyl esters (PC, NEFA and PMNs) were prepared by incubation with acidified methanol. Lipids isolated by SPE were mixed with toluene (1.0 ml) by vortex mixing. Methanol containing 20 ml H_2SO_4 /l (2.0 ml) was added, mixed briefly and incubated at 50°C for 18 hours. The reaction mixture was cooled and neutralized with a solution (2.0 ml) containing a mixture of $KHCO_3$ (0.25 M) and K_2CO_3 (0.5 M). Fatty acid methyl esters were isolated by addition of hexane (2.0 ml), separation of organic and aqueous phases by centrifugation at 1125 g for 10 min at 14°C and collection of the hexane layer. Samples were transferred to GC autosampler vials, dried under N_2 and dissolved in dry hexane. In specimens used to determine lipid recovery, an equal mass of tricosanoic acid methyl ester recovery reference standard to the internal recovery standard was added.

Fatty acid methyl esters prepared from lipid fractions isolated by SPE were resolved on a Hewlett Packard 6890 GC equipped with an HP7686 GC autosampler using an Innowax fused silica capillary column (20m x 100 μm x 0.1 μm) (Hewlett Packard, Stockport, Cheshire, UK) with flame ionisation detection. Use the area under the peak data to calculate the contribution of individual fatty acids as a percentage of total fatty acids. Calculate absolute concentrations of fatty acids by dividing the area of internal standard by the amount added. Divide the area of each fatty acid by this result to obtain absolute concentrations of each fatty acid within the amount of tissue used. Detailed analysis of the fatty acid compositions both of fractions containing lipid standards and of those derived from plasma was carried out by GC±MS on a 6890 GC using an HP5-MS capillary column (30 x 250 μm x 0.25 μm; Hewlett Packard) connected to an HP5973 mass selective detector (Hewlett Packard). Peaks were identified by comparison of electron impact ionisation spectra.

2.9 Statistical Analysis

It was calculated that 140 patients were required for enrolment and randomisation in order to detect a 50% reduction in new organ dysfunction and a two-sided alpha error of 0.05 and a power of 80%. This 50% reduction in new organ dysfunction was based on a trial examining enteral n -3 in critically ill patients with sepsis [139]. An audit carried out by ITU the previous year (unpublished data) looking at reasons for admission to ITU discovered that more than 160 patients filtered through the unit annually. This suggested that the study could be completed within around 12 months.

The Shapiro-Wilk test of normality was used to determine if the continuous data variables were parametric or non-parametric. Parametric data was analysed using the 2-tailed t-test and non-parametric data with the Mann Whitney U test. Categorical data was analysed using the Pearson Chi-square and Fishers exact test as appropriate. Estimates of survival curves during a 28-day follow-up period were calculated according to the Kaplan-Meier product limit method and compared by using the log-rank test. Normally distributed data are reported as means with standard deviations (SDs). Categorical variables are expressed as numbers and percentages.

Because of the complex and heterogeneous nature of sepsis and to account for any imbalances between the two treatment groups at baseline, a logistic-regression procedure and significant covariates that predicted outcomes were used to adjust raw values for 28-day mortality. Age, illness severity (as predicted by the APACHE-II and SOFA score), serious co-morbidities and other baseline covariates that predicted outcomes (at a threshold p value of ≤ 0.20) were entered into the model.

Patients were also assessed according to the priori strata of "less severe" and "more severe sepsis" as defined by the median predicted mortality as per the APACHE-II score on admission. The treatment effect within each subgroup was assessed according to the within-stratum analysis, with the use of the chi-square test. Binary logistic regression analysis was then used to test for an interaction between stratum and treatment in order to determine whether there was a differential effect on the in-hospital mortality.

Analysis was conducted with the use of SPSS, version 20 software, and all p values were two-sided.

3 Results

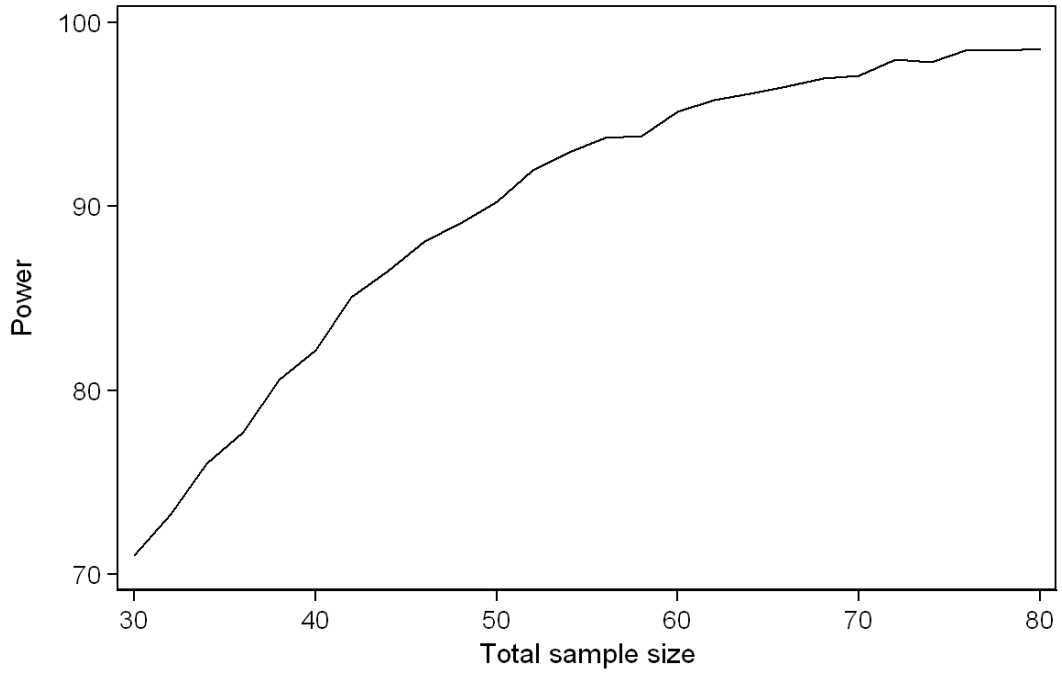
3.1 Introduction

3.1.1 Difficulties of trial recruitment

Due to reconfigurations of service provision at the single institution used to recruit patients (acute medical admissions moved to a different site within the Trust and with it, many of the acute medical specialties), ITU recruitment fell dramatically short of anticipated. In addition, two separate ITU trials were running synchronously with the fish oil trial. Patients could only be enrolled in a single trial and therefore numbers were reduced. A system was in place to alternate suitable patient recruitment between trials.

In respect of the recruitment shortfall, and to ensure Omegaven^{TM} safety in the study population, a repeat power calculation was carried out. A power curve was produced from simulations based on extrapolating the data from the first 27 patients (Figure 2: Power calculations). For a power of 95% it was calculated that 60 patients would be required.

Figure 2: Power calculations



3.1.2 Patient enrolment and demographics

Of the 92 consecutive patients screened, 60 underwent randomisation after informed consent was provided by either the patient themselves or a representative (Figure 3: Enrolment and Outcomes). No patient withdrew consent to continue with the study. Of the 60 patients included, 30 received parenteral fish oil and 30 acted as controls. One patient had the fish oil infusion discontinued after 4 days due to a coagulopathy of indeterminate aetiology. Whilst it was thought most likely to be secondary to consumption from sepsis and heparin, the fish oil infusion was discontinued. No side effects of the fish oil were reported throughout the study apart from one patient who reported a fish-like taste in their mouth.

The demographics and baseline characteristics of the two cohorts are shown in Table 3: Patients enrolled were critically ill as indicated by the baseline APACHE II scores, the SOFA score, the serum lactate levels and the number of baseline organ dysfunctions and failures. There were no significant differences between the two cohorts with regards to demographics, co-morbidities, sepsis severity, haemodynamic, biochemical and respiratory variables, inflammatory markers, pathogen type and numbers of failed organs. The only significant baseline variant was the number of patients with a haematological dysfunction, which was more prevalent in the control than the fish oil cohort (46.7% vs. 10%, p=0.002).

Figure 3: Enrolment and Outcomes

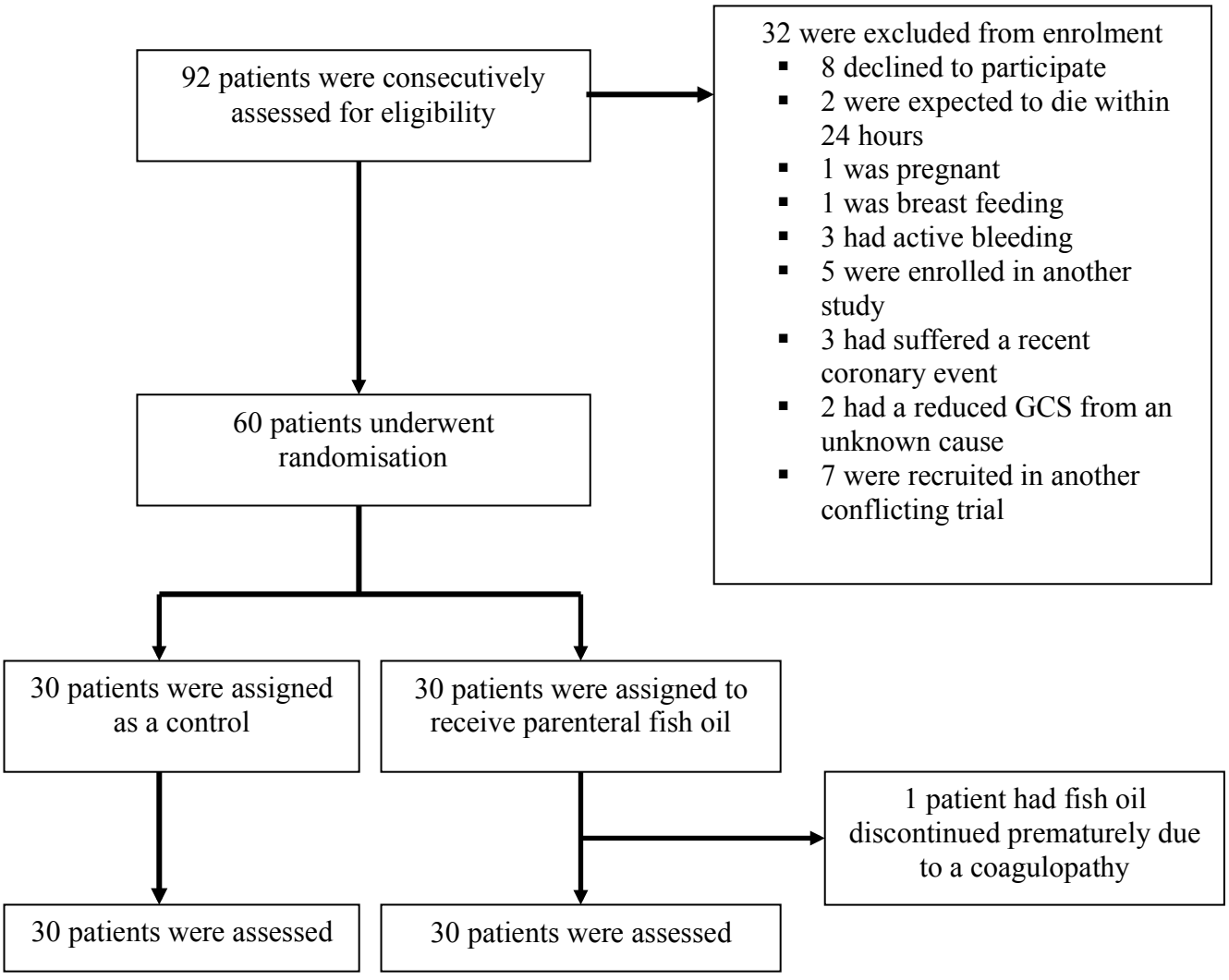


Table 3: Demographic and baseline characteristics of the patients

Characteristic	Control Group (n=30)	Fish oil Group (n=30)	p value
Age – yr	64.5 ± 13.4	63.8 ± 11.7	0.830
Gender – female;male	15:15	12:18	0.436
Recent surgery – no. (%)	12 (40)	18 (60)	0.121
Elective	5 (16.7)	9 (30)	0.222
Emergency	7 (23.3)	9 (30)	0.559
APACHE II	17.9 ± 6.2	19.1 ± 6.7	0.473
Corresponding mortality risk (%)	30.4 ± 15.8	33.1 ± 18.0	0.562
SOFA score	7.6 ± 3.2	7.2 ± 3.0	0.582
Co-morbidities – no. (%)			
Hypertension	16 (53.3)	20 (66.7)	0.292
Ischaemic heart disease	2 (6.7)	7 (23.3)	0.145
Congestive heart failure	1 (3.3)	2 (6.7)	1.000
COPD	11 (36.7)	8 (26.7)	0.405
Chronic renal failure	5 (16.7)	8 (26.7)	0.347
Diabetes	6 (20)	5 (16.7)	0.739
Liver disease	0 (0)	0 (0)	n/a
Alcoholism	0 (0)	2 (6.7)	0.492
Cancer	9 (30)	8 (26.7)	0.774
Immunocompromised	1 (3.3)	5 (16.7)	0.195
Steroid use	1 (3.3)	7 (23.3)	0.052
Solid organ transplant	1 (3.3)	4 (13.3)	0.353
Intravenous drug abuse	0 (0)	1 (3.3)	1.000
Baseline biochemistry			
Albumin	25.8 ± 6.0	25.9 ± 6.0	0.949
CRP	234.1 ± 95.0	180.2 ± 104.6	0.105
Blood glucose	8.18 ± 2.6	8.72 ± 2.56	0.348
Haemodynamic variables			
Mean arterial pressure (mmHg)	63.5 ± 7.6	67.3 ± 12.6	0.238
Arterial pH	7.27 ± 0.1	7.28 ± 0.1	0.704
Serum lactate (mmol/litre)	2.5 ± 2.4	2.4 ± 2.0	0.717
Respiratory variables			
PaO_2/FiO_2	227.9 ± 115	197.0 ± 111	0.304
Ventilated – no. (%)	11 (36.7)	18 (60)	0.071
Focus of sepsis – no. (%)			
Chest	8 (26.7)	8 (26.7)	0.774
Abdomen	16 (53.3)	18 (60)	0.436
Urinary tract	6 (20)	2 (6.7)	0.253
Skin	0	2 (6.7)	0.49
Pathogen type cultured – no. (%)			
Gram + alone	3 (10)	7 (23.3)	0.166
Gram – alone	10 (33.3)	4 (13.3)	0.067
Mixed	7 (23.3)	8 (26.7)	0.766
Other	2 (6.7)	1 (3.3)	1.000
No pathogen	8 (26.7)	10 (33.3)	0.573

Characteristic	Control Group (n=30)	Fish oil Group (n=30)	p value
Baseline Organ Failure - no. (%)			
Cardiovascular	17 (56.7)	16 (53.3)	0.795
Respiratory	13 (43.3)	18 (60)	0.196
Renal	6 (20)	7 (23.3)	0.754
Hepatic	4 (13.3)	2 (6.7)	0.671
Haematological	2 (6.7)	1 (3.3)	1.000
Baseline Organ Dysfunction - no. (%)			
Cardiovascular	9 (30)	7 (23.3)	0.559
Respiratory	16 (53.3)	10 (33.3)	0.118
Renal	13 (43.3)	11 (36.7)	0.598
Hepatic	11 (36.7)	8 (26.7)	0.405
Haematological	14 (46.7)	3 (10)	0.002

Abbreviation: CI confidence interval; CRP C reactive protein; MAP mean arterial pressure; BM blood sugar; COPD chronic obstructive pulmonary disease; IVDU intravenous drug user

3.2 Results I: Cohort results independent of fish oil

3.2.1 Baseline demographics of mortality outcomes across the cohort

The baseline demographic data for survivors and non-survivors was analysed and compared (Table 4). Non-survivors were associated with a significantly higher SAPS, SAPS mortality prediction and day 0 SOFA score. A higher APACHE score was not, however, associated with a non-survival. Hepatic failure was the only baseline organ failure that was significantly more frequently seen in non-survivors.

Table 4: Baseline demographics of survivors vs. non-survivors

Variable	Survivors (n=47)	Non-survivors (n=13)	p value
Age – yrs	63.87 ± 11.66	65.15 ± 15.51	0.746
Age ≥65	27	5	0.225
Gender - female: male	23:24	4:9	0.244
Sepsis source			
Abdomen vs. other	25	8	0.592
Respiratory vs. other	14	3	0.740

Skin vs. other	2	0	1.00
Urinary vs. other	6	2	1.00
Pre-existing condition– no.			
HTN	29	7	0.609
IHD	6	3	0.392
CCF	3	0	1.00
COPD	14	5	0.737
CRF	11	2	0.713
DM	8	3	0.690
Liver disease	0	0	N/A
EtOH abuse	2	0	1.00
Cancer	13	4	1.00
Immunocompromised	6	0	0.324
Steroid use	8	0	0.182
Solid organ transplant	5	0	0.575
IVDU	1	0	1.00
Recent surgery– no.			
Elective	13	1	0.264
Emergency	13	3	1.00
Any	26	4	0.117
APACHE II	18.45 ± 6.45	18.69 ± 6.47	0.904
Death risk	31.74 ± 16.18	31.92 ± 19.64	0.753
SAPS	37.53 ± 9.79	44.23 ± 10.30	**0.035**
Death risk	23.69 ± 16.95	35.96 ± 18.30	**0.033**
Day 0 SOFA	6.89 ± 2.81	9.15 ± 3.52	**0.018**
Day 0 CRP	214.28 ± 107.10	169.00 ± 65.03	0.326
Day 0 Albumin	26.38 ± 5.88	23.92 ± 6.03	0.190
Baseline organ failure– no.			
CV	23	10	0.073
Respiratory	25	6	0.653
Renal	9	4	0.450
Hepatic	2	4	**0.017**
Haematological	1	2	0.115

Variable	Survivors (n=47)	Non-survivors (n=13)	p value
Baseline organ dysfunction			
CV	14	2	0.481
Respiratory	20	6	0.817
Renal	20	4	0.443
Hepatic	16	3	0.522
Haematological	10	7	**0.035**
Number of systems with dysfunction	1.70 ± 1.18	1.69 ± 1.03	0.970
Number of failing systems	1.28 ± 0.90	2.00 ± 1.15	**0.024**
Number with failure or dysfunction	2.98 ± 1.13	3.69 ± 0.85	**0.035**
Pathogen type in cultures			
Gram positive alone	8	2	1.00
Gram negative alone	13	1	0.264

Mixed	11	4	0.719
Other	2	1	0.526
No pathogen	13	5	0.504
New micro			
Gram positive	15	3	0.736
Gram negative	28	10	0.338
Fungus	3	0	1.00
Where sustained sepsis			
Ward vs. ITU	41:6	11:2	1.00
Days sepsis prior to fish oil	2.24 ± 2.69	1.36 ± 0.67	0.450
Haemodynamic variables			
MAP mmHg	66.11 ± 11.06	62.85 ± 8.03	0.338
Arterial pH	7.28 ± 0.11	7.28 ± 0.08	0.970
Serum lactate	2.48 ± 2.07	2.41 ± 2.68	0.815
Respiratory variables			
PaO2/FiO2	210.15 ± 110.70	220.77 ± 126.12	0.950
Baseline BM	8.66 ± 2.52	7.86 ± 2.71	0.148

Abbreviation: CI confidence interval; CRP C reactive protein; MAP mean arterial pressure; BM blood sugar; COPD chronic obstructive pulmonary disease; IVDU intravenous drug user

3.2.2 Mortality outcomes across the cohort

Non-survivors had a significantly higher maximum SOFA score and a shorter length of hospital stay when compared to survivors. The SOFA score on days 1, 3, 7 and 13 were not significantly different between the two cohorts.

Table 5: SOFA score between survivors and non-survivors

Variable	Survivors (n=47)	Non-survivors (n=13)	p value
Max SOFA	8.51 ± 3.69	11.15 ± 3.91	**0.028**
Delta SOFA	1.47 ± 2.01	2.23 ± 1.79	0.082
Day 1 SOFA	0.66 ± 1.24	0.92 ± 1.32	0.363
Day 3 SOFA	0.65 ± 1.59	0.50 ± 0.76	0.396
Day 7 SOFA	0.21 ± 0.63	0.75 ± 0.96	0.085
Day 13 SOFA	0.30 ± 0.67	N/C	0.420
Mean CRP	170.45 ± 74.37	143.61 ± 71.50	0.267
New arrhythmia	6	1	1.00
ITU length of stay	10.81 ± 10.77	9.54 ± 9.19	0.487
Total Length of stay	35.23 ± 25.69	11.46 ± 9.18	**<0.001**

3.2.2.1 Organ dysfunction/failure across cohort as per organ system

Table 6: Organ dysfunction/failure as per SOFA score

Variable	Survivors (n=47)	Non-survivors (n=13)	p value
Day 0			
CV SOFA	2.33 ± 1.59	2.83 ± 1.59	0.322
PaO2/Fi02	2.41 ± 1.17	2.25 ± 1.29	0.705
Renal SOFA	1.20 ± 1.28	1.67 ± 1.56	0.346
Platelets	0.35 ± 0.71	1.08 ± 0.90	0.001
Bilirubin	0.59 ± 0.86	1.33 ±1.30	0.063
Total SOFA	6.87 ± 2.92	9.42 ± 3.53	0.013
Day 1			
CV SOFA	2.37 ± 1.61	3.25 ± 1.36	0.066
PaO2/Fi02	2.30 ± 1.24	2.25 ± 1.22	0.953
Renal SOFA	1.30 ± 1.30	1.50 ± 1.68	0.873
Platelets	0.33 ± 0.70	1.08 ± 1.08	0.008
Bilirubin	0.50 ± 0.86	1.17 ± 1.34	0.095
Total SOFA	6.80 ± 3.43	9.17 ± 3.81	0.042
Day 2			
CV SOFA	1.59 ± 1.80	2.33 ± 1.80	0.160
PaO2/Fi02	1.93 ± 1.37	2.56 ± 0.73	0.240
Renal SOFA	1.04 ± 1.19	1.22 ± 1.56	0.952
Platelets	0.37 ± 0.68	1.00 ± 1.00	0.045
Bilirubin	0.43 ± 0.83	1.33 ± 1.41	0.028
Total SOFA	5.39 ± 3.61	8.44 ± 3.50	0.041
Day 3			
CV SOFA	1.13 ± 1.56	2.57 ±1.81	0.045
PaO2/Fi02	1.62 ± 1.54	2.71 ± 0.76	0.096
Renal SOFA	0.87 ± 1.16	1.71 ± 1.89	0.259
Platelets	0.20 ± 0.50	1.00 ± 1.15	0.009
Bilirubin	0.22 ± 0.64	1.29 ± 1.11	<0.001
Total SOFA	4.04 ± 3.78	9.29 ± 3.20	0.003
Day 4			
CV SOFA	0.98 ± 1.47	3.17 ± 1.60	0.009
PaO2/Fi02	1.36 ± 1.52	2.83 ± 0.75	0.040
Renal SOFA	0.733 ± 1.12	0.83 ± 1.60	0.875
Platelets	0.27 ± 0.58	0.83 ± 0.98	0.199
Bilirubin	0.23 ± 0.74	1.67 ± 1.03	0.002
Total SOFA	3.53 ± 4.05	9.33 ± 3.01	0.004
Day 5			
CV SOFA	0.76 ± 1.40	3.00 ± 1.10	0.001
PaO2/Fi02	1.11 ± 1.42	2.83 ± 0.75	0.008
Renal SOFA	0.53 ± 1.06	0.33 ± 0.82	0.633
Platelets	0.16 ± 0.37	1.00 ± 1.10	0.016
Bilirubin	0.20 ± 0.69	1.50 ± 1.05	<0.001
Total SOFA	3.89 ± 3.78	8.67 ± 2.73	0.002

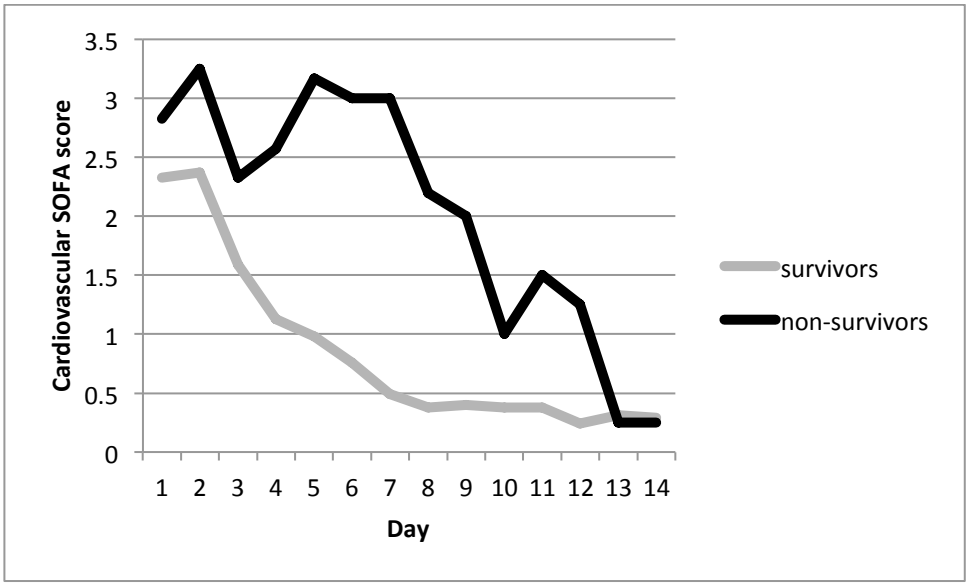
Day 6			
CV SOFA	0.49 ± 1.22	3.00 ± 1.10	<0.001
PaO2/Fi02	1.00 ± 1.41	3.17 ± 0.98	0.001
Renal SOFA	0.49 ± 1.01	0.33 ± 0.82	0.660
Platelets	0.09 ± 0.29	1.00 ± 1.10	0.003
Bilirubin	0.16 ± 0.64	1.67 ± 1.37	<0.001
Total SOFA	2.22 ± 3.52	9.17 ± 3.06	<0.001
Day 7			
CV SOFA	0.38 ± 1.03	2.20 ± 1.79	0.001
PaO2/Fi02	0.84 ± 1.36	2.60 ± 1.52	0.019
Renal SOFA	0.27 ± 0.69	N/C	0.310
Platelets	0.09 ± 0.36	1.00 ± 1.41	0.013
Bilirubin	0.13 ± 0.63	1.20 ± 0.84	<0.001
Total SOFA	1.71 ± 2.88	7.00 ± 3.46	0.002
Day 8			
CV SOFA	0.40 ± 1.01	2.00 ± 2.00	0.012
PaO2/Fi02	0.80 ± 1.34	2.40 ± 0.89	0.006
Renal SOFA	0.33 ± 0.98	N/C	0.390
Platelets	0.09 ± 0.36	0.60 ± 0.89	0.018
Bilirubin	0.04 ± 0.21	1.20 ± 0.84	<0.001
Total SOFA	1.67 ± 3.12	6.20 ± 2.77	0.002
Day 9			
CV SOFA	0.38 ± 1.01	1.00 ± 2.00	0.435
PaO2/Fi02	0.78 ± 1.31	2.50 ± 1.00	0.007
Renal SOFA	0.31 ± 0.95	0.25 ± 0.50	0.589
Platelets	0.11 ± 0.44	0.25 ± 0.50	0.233
Bilirubin	0.07 ± 0.33	0.75 ± 0.96	0.002
Total SOFA	1.64 ± 3.28	4.75 ± 2.36	0.007
Day 10			
CV SOFA	0.38 ± 0.98	1.50 ± 1.91	0.074
PaO2/Fi02	0.67 ± 1.21	2.50 ± 1.00	0.003
Renal SOFA	0.22 ± 0.77	0.25 ± 0.50	0.461
Platelets	0.09 ± 0.47	N/C	0.670
Bilirubin	0.11 ± 0.44	0.75 ± 0.96	0.007
Total SOFA	1.47 ± 2.86	5.00 ± 2.16	0.005
Day 11			
CV SOFA	0.24 ± 0.71	1.25 ± 1.50	0.044
PaO2/Fi02	0.58 ± 1.18	3.00 ± 0.82	0.001
Renal SOFA	0.11 ± 0.49	0.25 ± 0.50	0.218
Platelets	0.07 ± 0.33	N/C	0.670
Bilirubin	0.11 ± 0.44	0.75 ± 0.96	0.007
Total SOFA	1.13 ± 2.41	5.25 ± 2.22	0.001
Day 12			
CV SOFA	0.31 ± 0.90	0.25 ± 0.50	0.610
PaO2/Fi02	0.58 ± 1.20	3.25 ± 0.50	<0.001
Renal SOFA	0.11 ± 0.49	0.25 ± 0.50	0.218
Platelets	0.04 ± 0.30	N/C	0.766
Bilirubin	0.11 ± 0.44	0.75 ± 0.96	0.007
Total SOFA	1.16 ± 2.51	4.50 ± 1.29	0.002

Day 13			
CV SOFA	0.29 ± 0.87	0.25 ± 0.50	0.589
PaO2/Fi02	0.56 ± 1.14	3.25 ± 0.50	<0.001
Renal SOFA	0.11 ± 0.49	0.25 ± 0.50	0.218
Platelets	0.044 ± 0.30	N/C	0.766
Bilirubin	0.11 ± 0.44	0.75 ± 0.96	0.007
Total SOFA	1.11 ± 2.42	4.50 ± 1.29	0.002

3.2.2.1.1 Cardiovascular system

Survivors had a significantly reduced cardiovascular SOFA score on the majority of days during the study period. Survivors appeared to reduce the cardiovascular SOFA score significantly, and thereby have improved mean arterial pressure (MAP) and/or with reduced inotropic requirement, within 48 hours whilst non-survivors had a poor MAP until day 7.

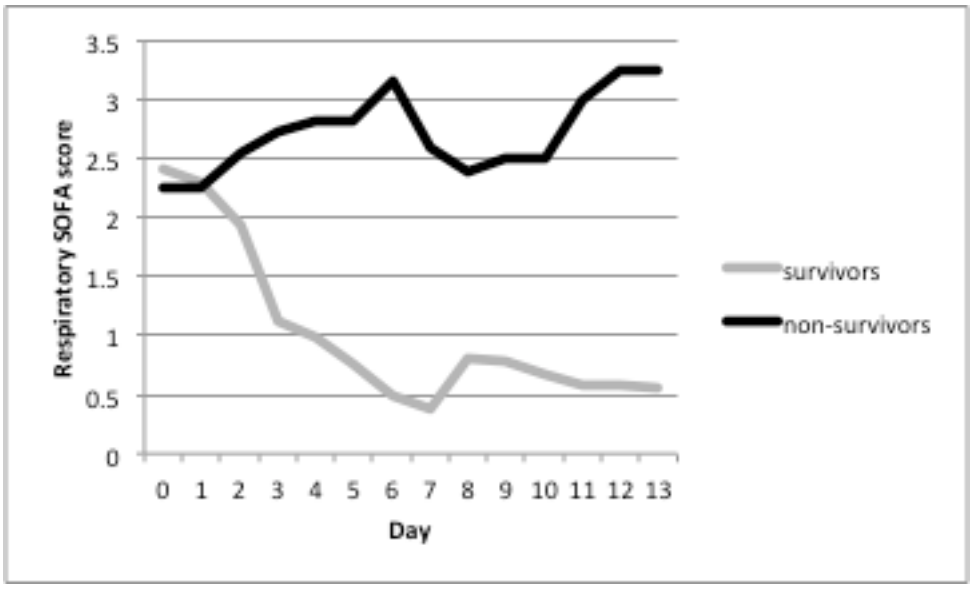
Figure 4: Cardiovascular SOFA score over study period



3.2.2.1.2 Respiratory system

From day 4 onwards the survivors had significantly reduced respiratory SOFA score and therefore improved oxygen exchange when compared to the non-survivors. Oxygen exchange demonstrated rapid improvement after 24 hours in the surviving cohort compared to the non-surviving cohort where the respiratory dysfunction never improved from the baseline.

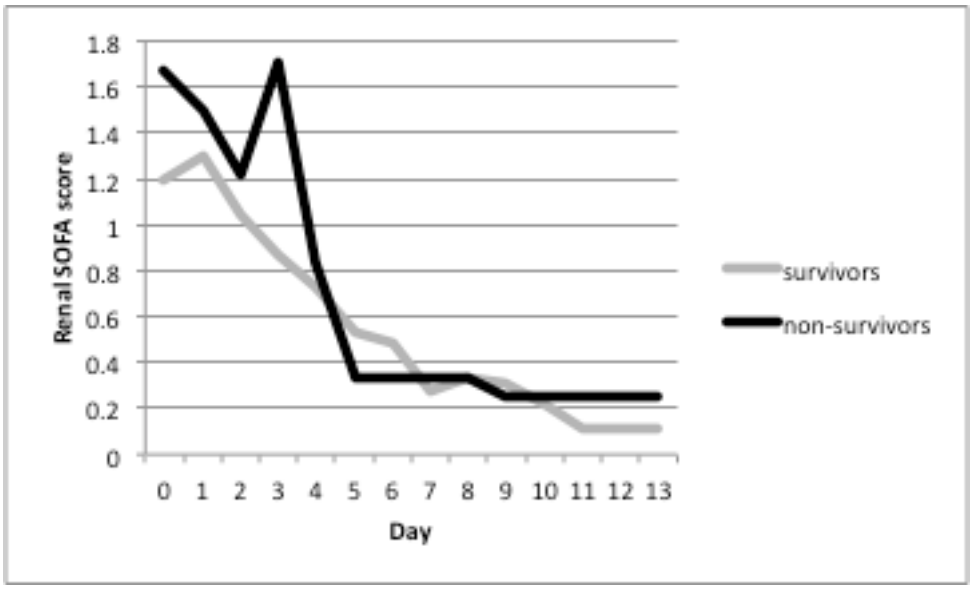
Figure 5: Respiratory SOFA score over study period



3.2.2.1.3 Renal system

Despite a trend towards survivors having improved renal function when compared to the non-survivors, this was not significant on any days. Similar to the cardiovascular system and respiratory system, renal function demonstrated rapid improvement after 24 hours in the surviving cohort compared to 4 days in the non-surviving cohort.

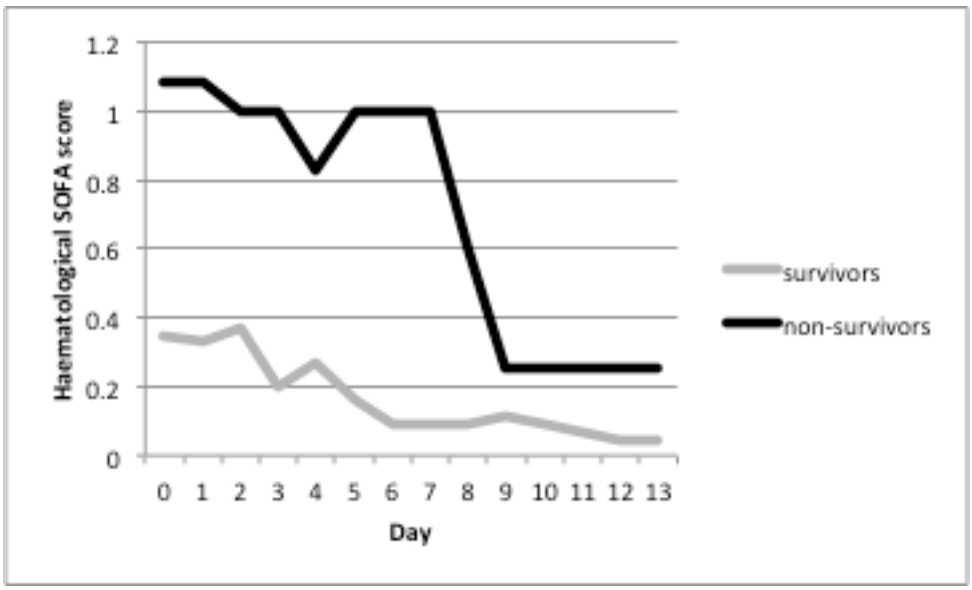
Figure 6: Renal SOFA score over study period



3.2.2.1.4 Haematological system

With the exception of days 4, 11, 12 and 13, the surviving cohort had a significantly higher platelet count than the non-survivors. The platelet count demonstrated gradual improvement throughout the study period in the surviving cohort compared to the non-surviving cohort who demonstrated little in the way of improved haematological function until day 7.

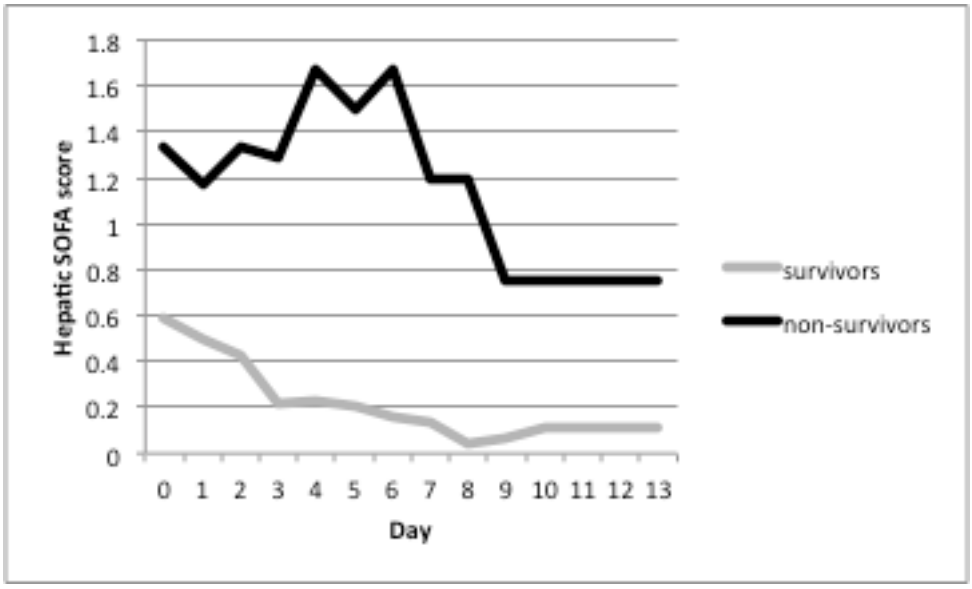
Figure 7: Haematological SOFA score over study period



3.2.2.1.5 Hepatic system

With the exception of days 0, 1 and 2 the surviving cohort had a significantly lower bilirubin count than the non-survivors. Similar to the cardiovascular, respiratory, haematological and renal system, bilirubin count demonstrated reduction after 24 hours and continued to improve in the surviving cohort. In the non-surviving cohort the hepatic function worsened until day 6 when an improvement was seen.

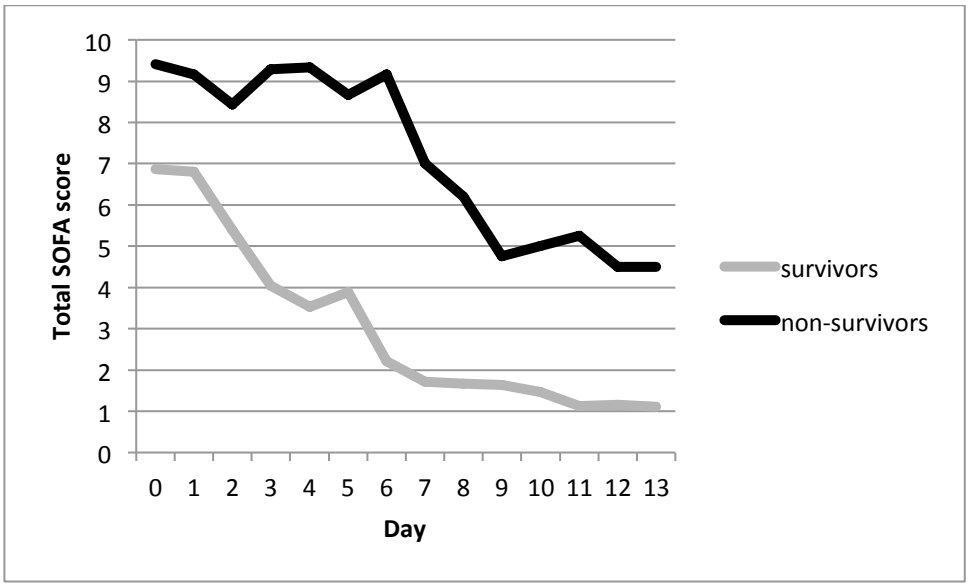
Figure 8: Hepatic SOFA score over study period



3.2.2.1.6 Total SOFA score

The surviving cohort had a significantly lower total SOFA score than the non-survivors on all days during the study. Similar to the cardiovascular, respiratory, haematological, hepatic and renal system, the total SOFA score demonstrated rapid reduction after 24 hours in the surviving cohort compared to 7 days in the non-surviving cohort.

Figure 9: Total SOFA score over study period



3.2.3 Mortality prediction of the scoring systems

There were 13 in-patient deaths in the study cohort (21.7%). The causes of death are given in the table below. The overwhelming cause of death was multi-organ failure secondary to sepsis in the majority of patients.

Table 7: Causes of all-patient deaths.

Patient ID	Cause of death
11	Multi-organ failure
14	Multi-organ failure
15	Multi-organ failure
23	Cerebrovascular accident
25	Multi-organ failure
41	Ischaemic bowel and multi-organ failure
45	Myocardial infarction and multi-organ failure
47	Multi-organ failure
52	Multi-organ failure
54	Multi-organ failure
57	Multi-organ failure
60	Multi-organ failure

3.2.3.1 Predictors of mortality

Area under the receiver operator curve (ROC) analysis confirmed that the number of failing organ systems at baseline was significantly associated with mortality (AUC 0.686, SE 0.095, p=0.031). Age, CRP and other physiological measurements, such as serum lactate and arterial pH, was not significantly related to mortality.

Of the scoring systems used, SAPS, day 0 SOFA and maximum SOFA were all significantly related to mortality (AUC 0.696, p=0.032; AUC 0.691, p=0.037; AUC 0.695, p=0.033 respectively). Of note, the APACHE II score was poorly related to mortality (AUC 0.504, SE 0.105, p=0.95).

Table 8: Efficacy of scoring systems

Variable	AUC	Standard error	95% CI	Significance
Age	0.506	0.105	0.301-0.711	0.950
APACHE2	0.504	0.095	0.319-0.690	0.964
SAPS	0.696	0.085	0.528-0.863	**0.032**
Day 0 SOFA	0.691	0.090	0.514-0.867	**0.037**
Max SOFA	0.695	0.084	0.530-0.860	**0.033**
Delta SOFA	0.653	0.084	0.489-0.817	0.093
Day 1 delta SOFA	0.570	0.092	0.390-0.750	0.440
Day 3 delta SOFA	0.570	0.112	0.350-0.790	0.543
Day 7 delta SOFA	0.684	0.162	0.366-1.000	0.256
Day 13 delta SOFA	0.600	0.176	0.254-0.946	0.612
Day 0 CRP	0.630	0.104	0.427-0.834	0.317
Day 0 Albumin	0.629	0.087	0.458-0.801	0.156
Number of failing systems	0.697	0.095	0.511-0.883	**0.031**
Number of dysfunctional systems	0.503	0.086	0.335-0.671	0.971
Number with failure or dysfunction	0.686	0.074	0.540-0.831	**0.042**
Days of sepsis before admission	0.563	0.092	0.383-0.742	0.523
mAP (mmHg)	0.586	0.088	0.414-0.758	0.346
Arterial pH	0.511	0.089	0.336-0.685	0.907
Serum Lactate	0.521	0.090	0.344-0.698	0.816
Pa02/Fi02	0.506	0.091	0.328-0.683	0.950
Baseline BM	0.632	0.088	0.460-0.804	0.149

3.2.3.1.1 ROC for mortality prediction

Figure 10: APACHE II ROC curve

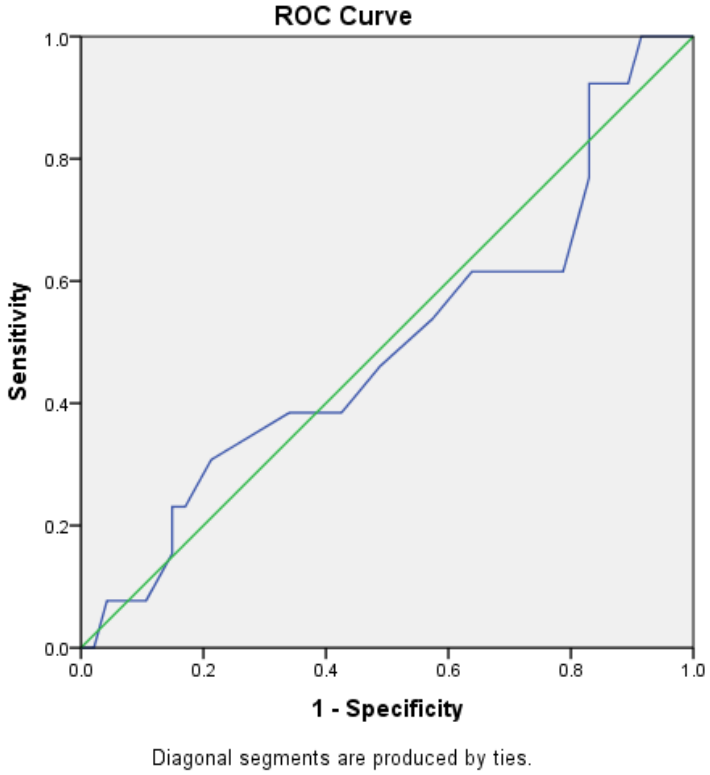


Figure 11: SAPS ROC curve

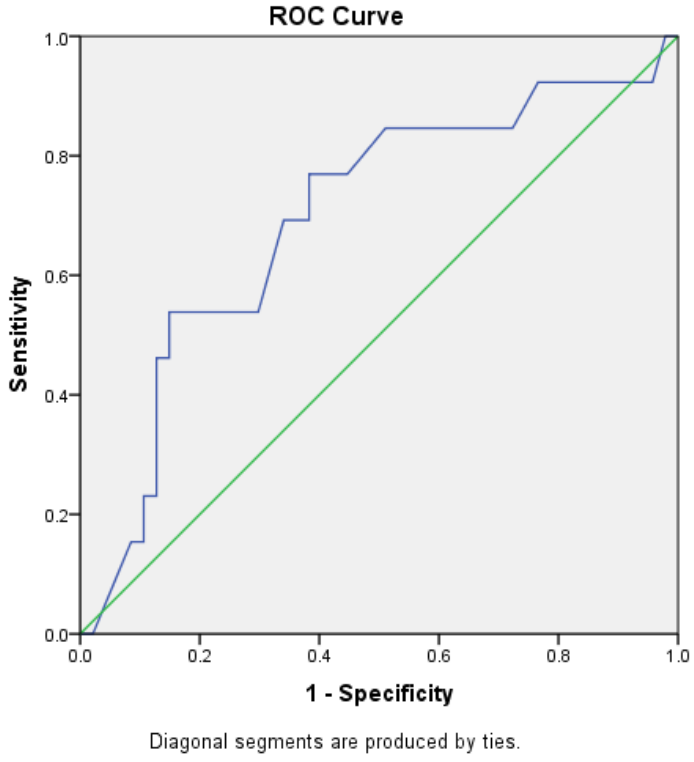


Figure 12: Day 0 SOFA ROC curve

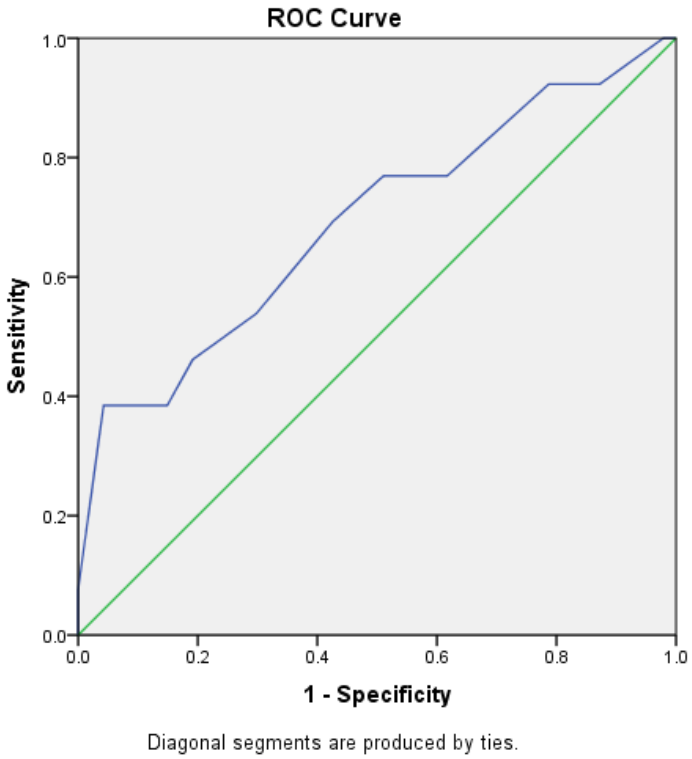


Figure 13: Sum of failing organs ROC curve

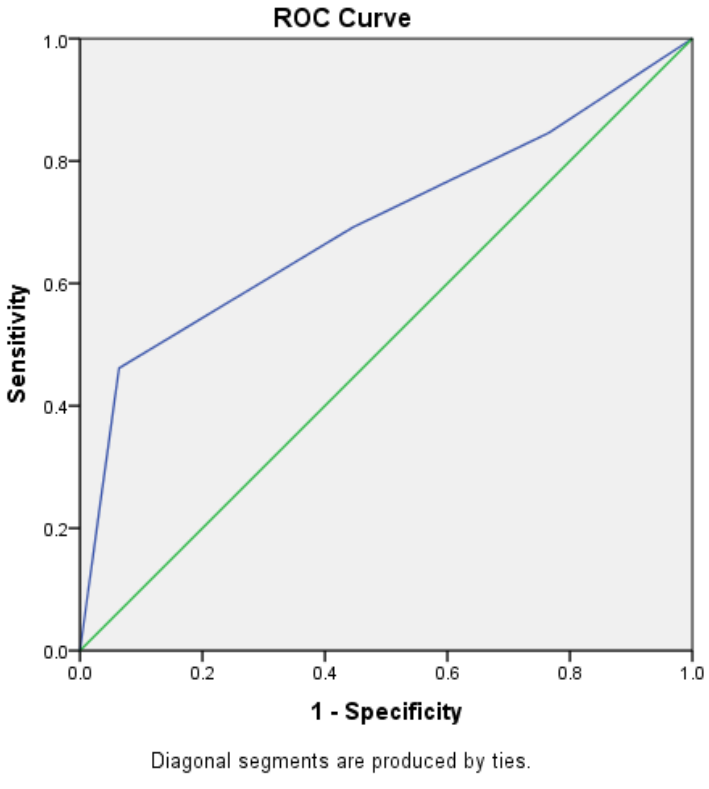


Figure 14: Sum of organs with dysfunction or failure ROC curve

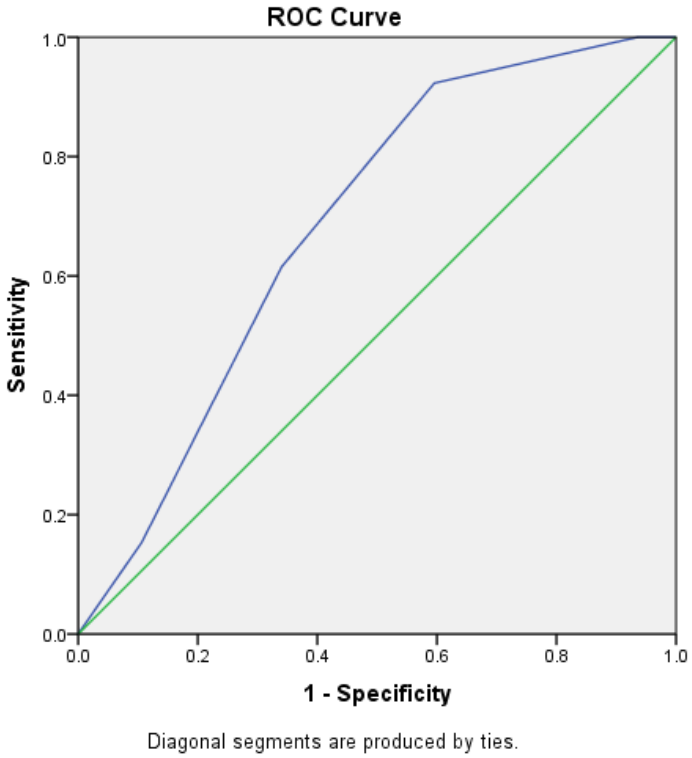


Figure 15: Max SOFA ROC curve

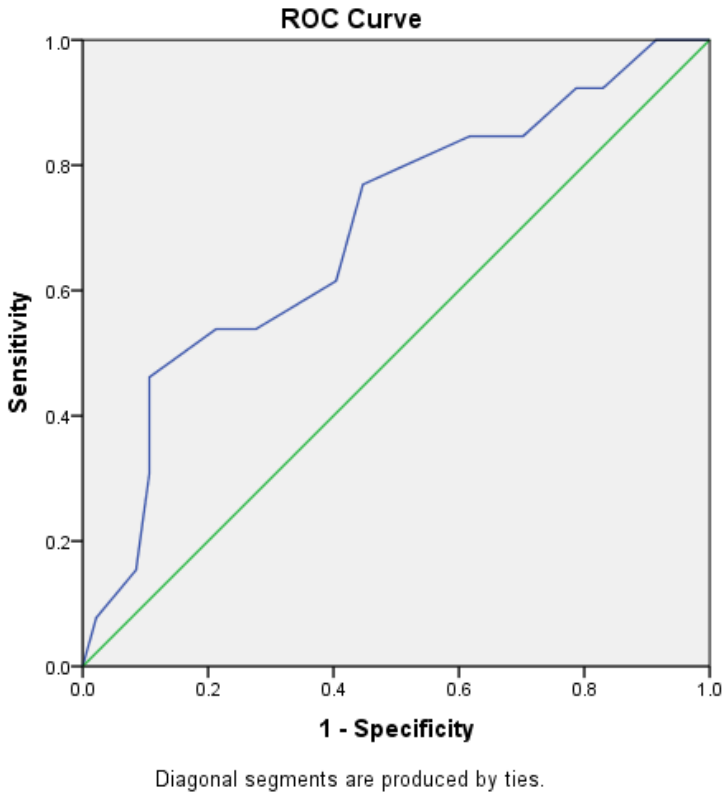
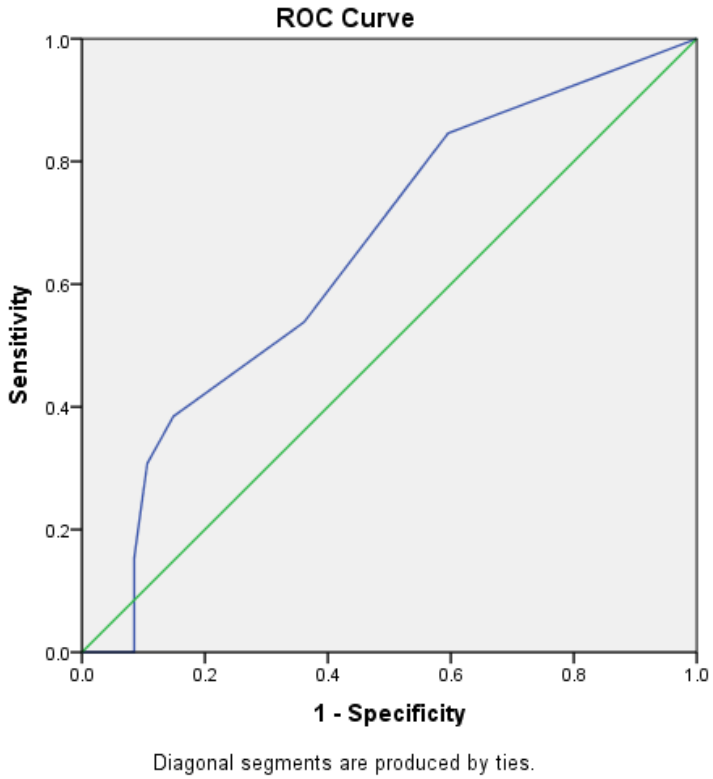


Figure 16: Delta-SOFA ROC curve



3.2.3.2 Effectiveness of combination scoring systems

Using pairwise comparison of the ROC curves as described by Hanley JA[193], the various scoring systems were assessed individually and in combination with each other in an attempt to improve their predictive power.

The analysis did not show any significantly improved predictive power as a result of combining scoring systems apart from APACHE II in combination with MAX-SOFA being better than APACHE II alone. The combination was not however, significantly better than MAX-SOFA alone and this may, reflect more on the poor predictive value of APACHE II in isolation.

There seemed no rationale for the combination of the APACHE II score with any other scoring system, as it exerted no discriminatory ability at all. It was not possible to combine the SAPS with Day 0-SOFA or Max-SOFA as it was collinear with them.

Table 9: Pairwise ROC curve comparisons

APACHE II vs. SAPS	
Difference between areas	0.191
Standard Error	0.0983
95% Confidence Interval	-0.00110 to 0.384
z statistic	1.949
Significance level	P = 0.0513
APACHEII vs. APACHE+SAPS	
Difference between areas	0.128
Standard Error	0.0688
95% Confidence Interval	-0.00716 to 0.262
z statistic	1.856
Significance level	P = 0.0635
SAPS vs. APACHE+SAPS	
Difference between areas	0.0638
Standard Error	0.0438
95% Confidence Interval	-0.0221 to 0.150
z statistic	1.456
Significance level	P = 0.1455

APACHE II vs. APACHE+DAY 0 SOFA	
Difference between areas	0.0696
Standard Error	0.0424
95% Confidence Interval	-0.0135 to 0.153
z statistic	1.642
Significance level	P = 0.1006
APACHE II vs. APACHE II +DELTA-SOFA	
Difference between areas	0.0115
Standard Error	0.0291
95% Confidence Interval	-0.0455 to 0.0685
z statistic	0.394
Significance level	P = 0.6936
APACHE II vs. APACHE II+MAX-SOFA	
Difference between areas	0.0998
Standard Error	0.0464
95% Confidence Interval	0.00886 to 0.191
z statistic	2.151
Significance level	P = 0.0315

SAPS vs. SAPS+DAY 0 SOFA	
Difference between areas	0.0172
Standard Error	0.0321
95% Confidence Interval	-0.0458 to 0.0802
z statistic	0.535
Significance level	P = 0.5928
SAPS vs. SAPS+DELTA-SOFA	
Difference between areas	0.00818
Standard Error	0.0215
95% Confidence Interval	-0.0339 to 0.0503
z statistic	0.381
Significance level	P = 0.7033
SAPS vs. SAPS+MAX-SOFA	
Difference between areas	0.0155
Standard Error	0.0362
95% Confidence Interval	-0.0555 to 0.0866
z statistic	0.429
Significance level	P = 0.6679

DAY 0 SOFA vs. APACHE II +DAY 0 SOFA	
Difference between areas	0.117
Standard Error	0.0791
95% Confidence Interval	-0.0380 to 0.272
z statistic	1.480
Significance level	P = 0.1390
DAY 0 SOFA vs. SAPS+DAY 0 SOFA	
Difference between areas	0.0221
Standard Error	0.0727
95% Confidence Interval	-0.120 to 0.165
z statistic	0.304
Significance level	P = 0.7612

MAX-SOFA vs. APACHE II+MAX-SOFA	
Difference between areas	0.0908
Standard Error	0.0758
95% Confidence Interval	-0.0577 to 0.239
z statistic	1.198
Significance level	P = 0.2308
MAXSOFA vs. SAPS+MAX-SOFA	
Difference between areas	0.0164
Standard Error	0.0660
95% Confidence Interval	-0.113 to 0.146
z statistic	0.248
Significance level	P = 0.8042

DELTA-SOFA vs. APACHE II +DELTA-SOFA	
Difference between areas	0.137
Standard Error	0.107
95% Confidence Interval	-0.0716 to 0.347
z statistic	1.289
Significance level	P = 0.1974
DELTA-SOFA vs. SAPS+DELTA-SOFA	
Difference between areas	0.0507
Standard Error	0.112
95% Confidence Interval	-0.170 to 0.271
z statistic	0.451
Significance level	P = 0.6518

3.3 Results II: Results dependant on fish oil administration

3.3.1 Primary outcome measures

There was a significant difference in the primary outcome which was worsening organ dysfunction. The fish oil treated cohort demonstrated a significant abrogation in the degree of organ dysfunction deterioration from baseline during their ITU stay assessed using the delta-SOFA and max-SOFA scores (2.2±2.2 vs. 1.0±1.5, p=0.005 and 10.1±4.2 vs. 8.1±3.2, p=0.041 respectively). A box plot comparing the delta-SOFA and maximum-SOFA scores of the treatment cohort to the control cohort is shown in Figure 18 and Figure 19 respectively.

Figure 17: Box plot comparing delta-SOFA scores

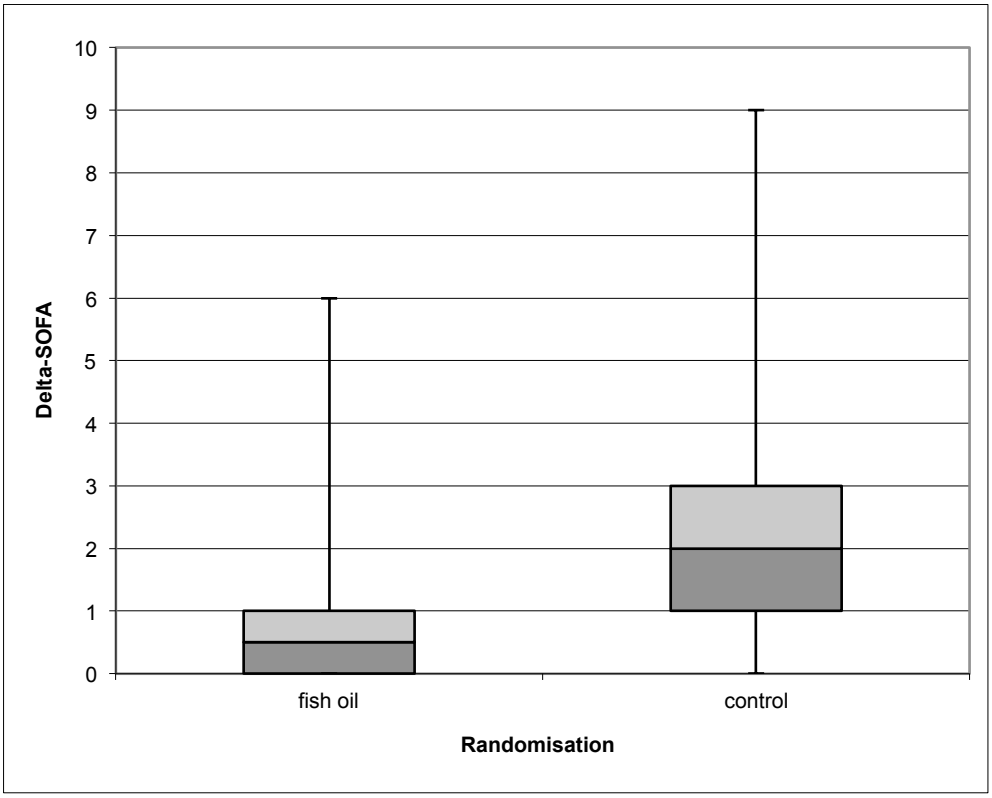
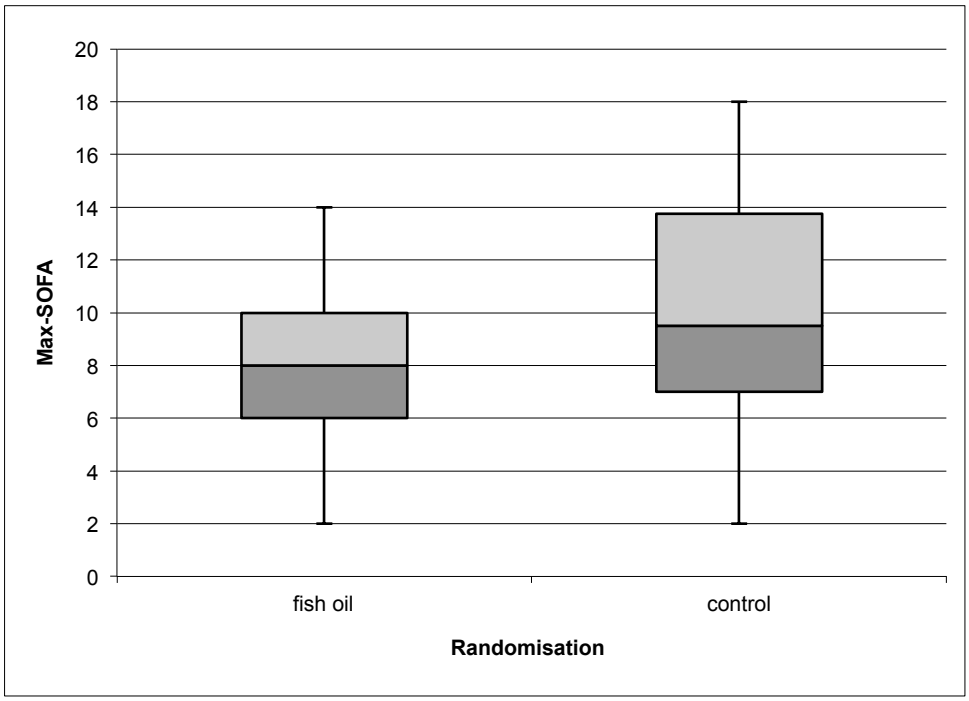


Figure 18: Box plot comparing maximum-SOFA scores



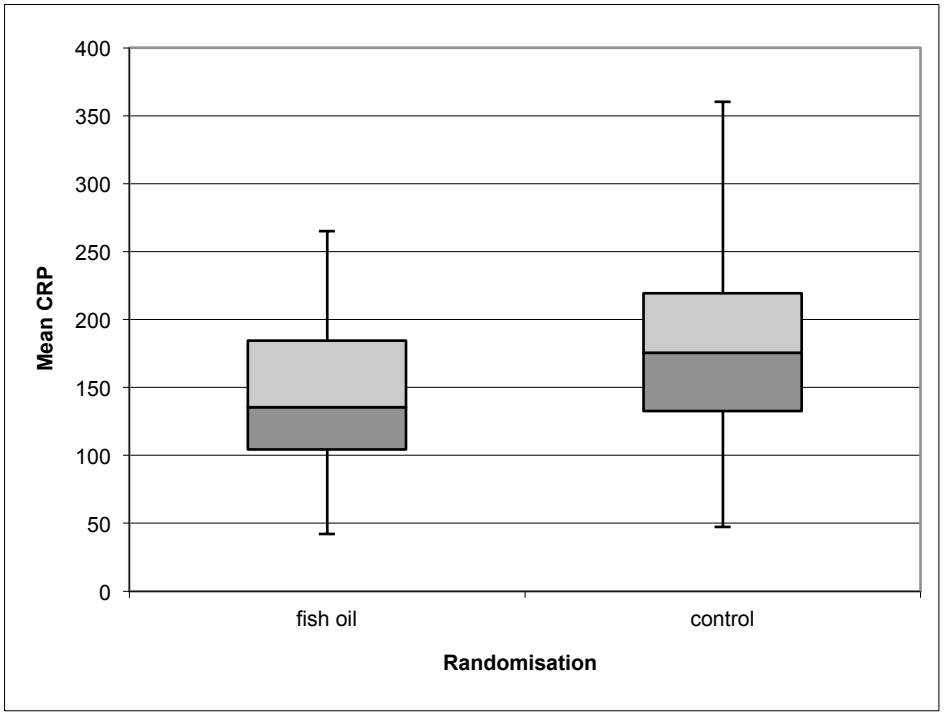
The delta-SOFA scores for days 1,3 and 5 were also significantly reduced in the fish oil treated cohort (p=0.030, p=0.038 and p=0.014). There was also a non-significant trend towards reduced organ dysfunction in the fish oil treated group for day 13 (p=0.173).

3.3.2 Results: Secondary outcome measures

There was no associated reduction in 28-day or inpatient mortality (p=0.197 and p=0.117 respectively). Kaplan-Meier survival curve for in-patient mortality in the entire study cohort is shown in Figure 21.

The mean CRP was also significantly reduced in the fish oil treated cohort (186.7±78 vs. 141.5±62.6, p=0.019). A box plot comparing the mean CRP of the treatment cohort to the control cohort is shown in Figure 20.

Figure 19: Box plot comparing mean CRP



There was no significant reduction in the length of ITU and total hospital stay between the treatment and control cohorts (p=0.858 and p=0.796 respectively). Box plots comparing the length of ITU and total hospital stay are presented in the Appendix.

Table 10: Results

Variable	Control Group (n=30)	Fish Oil Group (n=30)	p Value
SOFA score			
Delta-SOFA	2.2 ± 2.2	1.0 ± 1.5	0.005
Max-SOFA	10.1 ± 4.2	8.1 ± 3.2	0.041
Day 1 delta-SOFA	0.9 ± 1.3	0.5 ± 1.2	0.030
Day 3 delta-SOFA	1.1 ± 1.9	0.2 ± 0.6	0.038
Day 7 delta-SOFA	0.7 ± 0.9	0 ± 0	0.014
Day 13 delta-SOFA	0.4 ± 0.8	0 ± 0	0.173
Inflammatory markers			
Mean CRP	186.7 ± 78	141.5 ± 62.6	0.019
Days free of organ dysfunction			
Cardiovascular	8.7 ± 4.5	10.2 ± 3.8	0.174
Respiratory	7.2 ± 5.2	7.8 ± 4.7	0.888
Renal	8.4 ± 4.8	11.2 ± 2.7	0.052
Hepatic	10.8 ± 4.7	13.0 ± 2.0	0.117
Haematological	11.3 ± 3.8	12.7 ± 2.4	0.058
Days free of organ support			
Vasopressors	9.5 ± 4.4	11.4 ± 3.4	0.091
Ventilation	11.3 ± 4.3	10.0 ± 5.4	0.348
Renal replacement therapy	12.3 ± 3.4	12.9 ± 2.9	0.471
Mortality			
28-day mortality	8 (26.7)	4 (13.3)	0.197
Total inpatient mortality	9 (30)	4 (13.3)	0.117
Length of stay (days)			
In ITU	12.3 ± 12.4	8.8 ± 7.7	0.858
In hospital	33.5 ± 30.4	26.7 ± 18.2	0.796
New arrhythmia – no. (%)	6 (20)	1 (3.3)	0.103
Occurrence of secondary infection - no. (%)	5 (16.7)	3 (10)	0.706

Fish oil treated survivors also demonstrated a trend towards having a greater number of days free from any organ dysfunction, particularly renal and haematological dysfunction (p=0.052 and p=0.058 respectively). Similarly there was a trend towards

fewer new cardiac arrhythmias in the fish oil treated group when compared to the controls (20% vs. 3.3%, p=0.103). There was no significant difference between the groups in requirements for organ support with regards to ventilation, renal replacement therapy and inotropic support.

Figure 20: Kaplan-Meier survival curve for the whole study population (n=60)

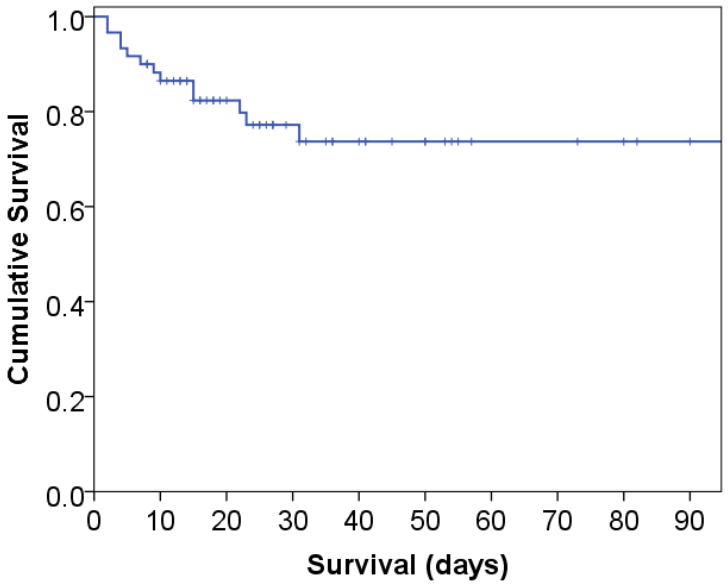
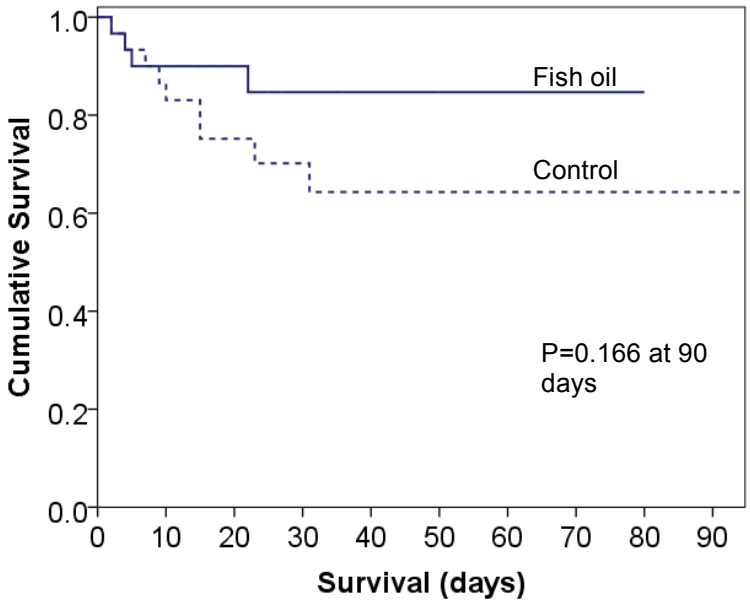


Figure 21: Kaplan-Meier curves showing survival according to fish oil use



P values were calculated with the use of log-rank test

3.3.3 Results of univariate and multivariate analysis

Univariable and multivariable survival analysis were performed to determine factors associated with in-patient mortality (Tables 11 and 12). When significant variables from univariate analysis were entered into multivariate analysis the only variables independently associated with mortality were hepatic failure, haematological failure and haematological dysfunction (Table 12: Multivariable analysis for independent factors for in-patient mortality). The variables day 0 SOFA, fish oil, day 0 albumin, elective surgery, any surgery, gram – pathogen and cardiovascular failure, although significant on univariate analysis, did not reach statistical significance on multivariable analysis.

Table 11: Baseline factors: Univariable logistic regression analysis for in-patient mortality

Variable	p	Relative Risk (95% CI)
Fish Oil	0.126	0.359 (0.097 to 1.33)
Age	0.23	0.463 (0.13 to 1.63)
Gender	0.25	0.463 (0.58 to 7.99)
APACHE II	0.902	1.006 (0.91 to 1.11)
Day 0 SOFA	0.025	1.284 (1.03 to 1.60)
Day 0 CRP	0.322	0.995 (0.99 to 1.01)
Day 0 Albumin	0.191	0.932 (0.84 to 1.04)
Day 0 BM	0.229	0.846 (0.644 to 1.11)
Surgery		
Elective Surgery	0.162	0.218 (0.03 to 1.85)
Emergency Surgery	0.741	0.785 (0.19 to 3.31)
Any Surgery	0.126	0.359 (0.097 to 1.33)
Co-morbidities		
Hypertension	0.61	0.724 (0.21 to 2.5)
Ischaemic heart disease	0.364	2.05 (0.44 to 9.65)
Congestive heart failure	0.999	0
COPD	0.553	1.473 (0.41 to 5.3)
Chronic renal failure	0.538	0.595 (0.11 to 3.10)
Diabetes	0.619	1.46 (0.33 to 6.54)
Alcohol abuse	0.999	0
Cancer	0.826	1.162 (0.30 to 4.44)
Immunocompromised	0.999	0

Transplant	0.999	0
Steroid use	0.999	0
IVDU	1.0	0
Baseline physiology		
MAP (mmHg)	0.322	0.965 (0.90 to 1.04)
Arterial pH	0.904	1.457 (0.003 to 655.14)
Serum lactate	0.92	0.985 (0.74 to 1.314)
PaO_2/FiO_2 ratio	0.763	1.0 (0.995 to 1.01)
Ventilated	0.859	0.894 (0.26 to 3.06)
Source of sepsis		
Abdomen	0.593	1.41 (0.40 to 4.94)
Chest	0.636	0.71 (0.17 to 2.97)
Urinary tract	0.999	1.242 (0.22 to 7.03)
Skin	0.999	0
Pathogen		
Gram + alone	0.889	0.886 (0.16 to 4.79)
Gram - alone	0.162	0.218 (0.026 to 1.85)
Mixed	0.589	1.455 (0.37 to 5.65)
Other	0.62	1.88 (0.156 to 22.47)
None	0.454	1.64 (0.45 to 5.92)
Baseline organ failure		
Cardiovascular	0.083	3.478 (0.85 to 14.27)
Respiratory	0.654	0.754 (0.22 to 2.59)
Renal	0.373	1.877 (0.47 to 7.49)
Hepatic	0.014	10 (1.59 to 63.1)
Haematological	0.094	8.364 (0.70 to 100.77)
Baseline organ dysfunction		
Cardiovascular	0.309	0.429 (0.084 to 2.19)
Respiratory	0.817	1.157 (0.34 to 3.97)
Renal	0.445	0.6 (0.162 to 2.23)
Hepatic	0.455	0.581 (0.14 to 2.42)
Haematological	0.027	4.317 (1.18 to 15.76)

Abbreviation: CI confidence interval; CRP C reactive protein; MAP mean arterial pressure; BM blood sugar; COPD chronic obstructive pulmonary disease; IVDU intravenous drug user

Table 12: Multivariable analysis for independent factors for in-patient mortality

Variable	p	Relative Risk (95% CI)
Hepatic failure	0.035	8.527 (1.16 to 62.83)
Haematological failure	0.021	22.262 (1.58 to 313.08)
Haematological dysfunction	0.044	4.714 (1.04 to 21.37)

Abbreviation: CI confidence interval

3.3.3.1 Results of univariate and multivariate analysis by sepsis severity strata

3.3.3.1.1 Less severe sepsis strata

Baseline demographics are presented in the appendix (Table 41). Results are presented in Table 13. Among the patients with less severe sepsis (n=35, predicted mortality of ≤ 40% based on the admission APACHE II score) there was a statistically significant reduction in mortality (p=0.041) and max-SOFA (p=0.009) with the group treated with fish oil. Kaplan-Meier survival curves were constructed for the less severe sepsis strata (Figure 23).

When significant variables from univariate analysis (Table 14) were entered into multivariate analysis the only variables independently associated with mortality were cardiovascular failure and haematological dysfunction (Table 15). The variables day 0 SOFA, diabetes as a co-morbidity, renal failure and cardiovascular dysfunction, although significant on univariate analysis, did not reach statistical significance on multivariable analysis.

3.3.3.1.2 More severe sepsis strata

In contrast, there was no significant difference between treatment groups in mortality in the stratum of more severe sepsis. Kaplan-Meier survival curves were constructed for the more severe sepsis strata and shown in the appendix (Figure 48). On multivariable analysis no independently significant variables were associated with in-patient mortality (data not included).

Table 13: Results for the strata of less severe sepsis

Variable	Control Group (n=18)	Fish Oil Group (n=17)	p Value
SOFA score			
Delta-SOFA	2 ± 2.19	0.94 ± 1.48	0.055
Max-SOFA	9.28 ± 3.80	6.18 ± 2.65	0.009
Day 1 delta-SOFA	0.44 ± 0.78	0.41 ± 1.22	0.341
Day 3 delta-SOFA	0.75 ± 1.76	0.15 ± 0.55	0.250
Day 7 delta-SOFA	0.83 ± 0.98	0 ± 0	0.031
Day 13 delta-SOFA	0.40 ± 0.89	0 ± 0	0.317
Inflammatory markers			
Mean CRP	180.1 ± 71.1	156.6 ± 61.2	0.20
Days free of organ dysfunction			
Cardiovascular	9.36 ± 4.38	10.56 ± 3.76	0.271
Respiratory	7.72 ± 5.12	7.68 ± 5.21	0.920
Renal	9.27 ± 4.29	12.62 ± 1.99	0.017
Hepatic	11.0 ± 3.74	13.63 ± 0.89	0.025
Haematological	11.45 ± 3.01	13.37 ± 1.86	0.002
Days free of organ support			
Vasopressors	10 ± 3.97	12.37 ± 2.70	0.034
Ventilation	11.27 ± 4.76	9.25 ± 5.97	0.319
Renal replacement therapy	12.9 ± 2.21	14 ± 0	0.030
Mortality			
28-day mortality	6 (33.3)	1 (5.8)	0.088
Total inpatient mortality	7 (38.9)	1 (5.8)	0.041
Length of stay (days)			
In ITU	18.92 ± 13.0	10.94 ± 9.48	0.74
In hospital	30.44 ± 27.10	30.29 ± 19.52	0.52
New arrhythmia – no. (%)	2 (10.1)	0 (0)	0.486
Occurrence of secondary infection - no. (%)	2 (10.1)	3 (17.6)	0.685

Table 14: Baseline factors for less severe sepsis- Univariable logistic regression analysis

Variable	p	Relative Risk (95% CI)
Fish Oil	0.042	0.098 (0.011 to 0.915)
Age	0.256	0.359 (0.061 to 2.11)
Gender	0.728	1.33 (0.26 to 6.74)
APACHE II	0.886	1.02 (0.83 to 1.24)
Day 0 SOFA	0.03	1.48 (1.04 to 2.10)
Day 0 CRP	0.658	0.998 (0.99 to 1.01)
Day 0 Albumin	0.218	0.917 (0.80 to 1.05)
Day 0 BM	0.733	0.943 (0.68 to 1.32)
Surgery		
Elective Surgery	0.438	0.408 (0.04 to 3.93)
Emergency Surgery	0.827	1.20 (0.23 to 6.19)

Any Surgery	0.643	0.688 (0.141 to 3.35)
Co-morbidities		
Hypertension	0.513	0.588 (0.12 to 2.89)
Ischaemic heart disease	0.914	1.14 (0.10 to 12.78)
Congestive heart failure	0.999	0
COPD	0.293	2.38 (0.47 to 11.92)
Chronic renal failure	0.999	0
Diabetes	0.194	4.17 (0.48 to 35.88)
Alcohol abuse	0.999	0
Cancer	0.53	1.714 (0.32 to 9.11)
Immunocompromised	0.999	0
Transplant	0.999	0
Steroid use	0.999	0
IVDU	1.0	0
Baseline physiology		
MAP (mmHg)	0.297	0.957 (0.88 to 1.04)
Arterial pH	0.397	0.019 (0.00 to 186.57)
Serum lactate	0.565	1.10 (0.80 to 1.52)
PaO_2/FiO_2 ratio	0.674	0.998 (0.991 to 1.01)
Ventilated	0.479	1.80 (0.36 to 9.05)
Source of sepsis		
Abdomen	0.643	0.688 (0.14 to 3.35)
Chest	0.958	0.952 (0.16 to 5.86)
Urinary tract	0.337	2.67 (0.36 to 19.71)
Skin	1.0	0
Pathogen		
Gram + alone	0.870	0.886 (0.16 to 4.79)
Gram - alone	0.999	0
Mixed	0.207	2.857 (0.56 to 14.6)
Other	1.0	0
None	0.391	2.1 (0.39 to 11.43)
Baseline organ failure		
Cardiovascular	0.05	6.0 (1.00 to 35.91)
Respiratory	0.927	1.08 (0.22 to 5.22)
Renal	0.194	4.167 (0.48 to 35.87)
Hepatic	1.0	0
Haematological	0.999	0
Baseline organ dysfunction		
Cardiovascular	0.13	0.179 (0.02 to 1.66)
Respiratory	0.927	1.077 (0.22 to 5.22)
Renal	0.532	0.567 (0.10 to 3.36)
Hepatic	0.958	0.952 (0.16 to 5.86)
Haematological	0.041	5.83 (1.07 to 31.76)

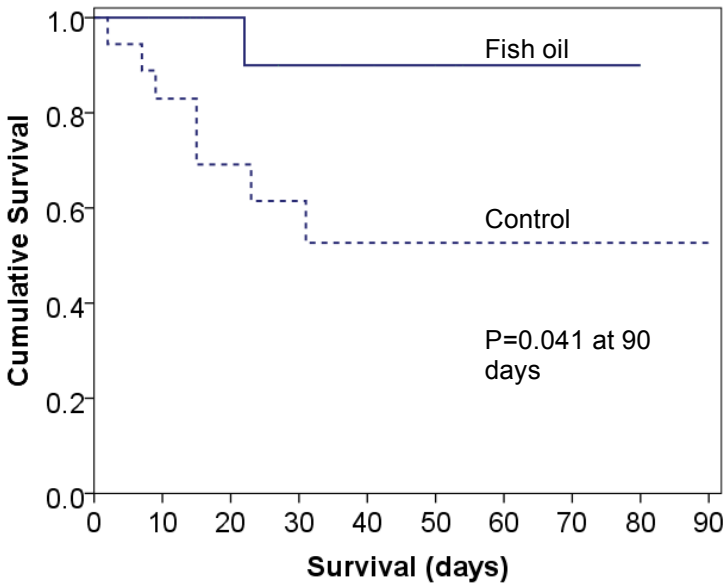
Abbreviation: CI confidence interval; CRP C reactive protein; BM blood sugar; MAP mean arterial pressure; COPD chronic obstructive pulmonary disease; IVDU intravenous drug user

Table 15: Multivariable analysis for independent factors for in-patient mortality in less severe sepsis

Variable	p	Relative Risk (95% CI)
Cardiovascular failure	0.034	14.94 (1.23 to 181.48)
Haematological dysfunction	0.05	7.73 (1.0 to 59.72)

Abbreviation: CI confidence interval

Figure 22: Kaplan-Meier curves showing survival according to fish oil use in the less severe sepsis strata



P values were calculated with the use of log-rank test

3.3.4 Subgroup analysis of the fish oil cohort

3.3.4.1 Factors associated with survival

On univariate analysis only ischaemic heart disease and baseline BM were associated with mortality, with a $p<0.10$, in the fish oil cohort. On multivariate logistical regression analysis ischaemic heart disease was the only factor found to be independently associated with mortality.

Table 16: Univariate analysis of factors associated with mortality in the fish oil cohort

Variable	p	Relative Risk (95% CI)
Age	0.156	1.077 (0.972-1.193)
Gender	0.518	2.200 (0.201-24.086)
APACHE II	0.110	1.176 (0.964-1.435)
SAPS	0.282	1.057 (0.995-1.170)
Day 0 SOFA	0.442	1.151 (0.804-1.649)
Day 0 CRP	0.839	0.998 (0.978-1.018)
Day 0 Albumin	0.624	1.049 (0.865-1.273)
Day 0 BM		
Surgery		
Elective Surgery	0.999	NA
Emergency Surgery	0.999	NA
Any Surgery	0.998	NA
Co-morbidities		
Hypertension	0.706	1.588 (0.144-17.561)
Ischaemic heart disease	0.028	16.5 (1.353-201.290)
Congestive heart failure	0.999	NA
COPD	0.935	0.905 (0.080-10.210)
Chronic renal failure	0.275	3.333 (0.384-28.959)
Diabetes	0.635	1.833 (0.150-22.366)
Alcohol abuse	0.999	NA
Cancer	0.935	0.905 (0.080-10.210)
Immunocompromised	0.999	NA
Transplant	0.999	NA
Steroid use	0.999	NA
IVDU	1.000	NA
Baseline physiology		
MAP (mmHg)	0.461	0.959 (0.857-1.073)
Arterial pH	0.702	9.879 (0.000-1238.739)
Serum lactate	0.327	0.572 (0.187-1.750)
PaO_2/FiO_2 ratio	0.479	1.003 (0.994-1.012)
Ventilated	0.663	0.625 (0.076-5.172)
Source of sepsis		
Abdomen	0.663	0.625 (0.076-5.172)
Chest	0.275	3.333 (0.384-28.959)
Urinary tract	0.999	NA
Skin	0.999	NA
Pathogen		
Gram + alone	0.199	4.200 (0.470-37.499)
Gram - alone	0.707	1.588 (1.44-17.561)
Fungal	0.999	NA
Developed sepsis on ward vs. on the ITU	0.999	NA
Days sepsis prior to trial recruitment	0.441	0.552 (0.122-2.503)
Baseline organ failure		

Cardiovascular	0.368	3.000 (0.275-32.746)
Respiratory	0.663	0.625 (0.076-5.172)
Renal	0.933	1.111 (0.097-12.750)
Hepatic	0.169	8.333 (0.407-170.666)
Haematological	1.000	NA
Baseline organ dysfunction		
Cardiovascular	0.933	1.111 (0.097-12.750)
Respiratory	0.706	0.630 (0.057-5.862)
Renal	0.607	0.533 (0.049-5.862)
Hepatic	0.275	3.333 (0.384-28.959)
Haematological	0.999	NA

Table 17: Multivariate analysis

Variable	p	Relative Risk (95% CI)
IHD	0.049	64.215 (1.025-4023.652)
BM	0.066	0.331 (0.102-1.075)

3.3.4.2 Factors associated with above median delta-SOFA score

The median delta-SOFA score in the cohort of patients who were administered fish oil was 2. Abdominal infection, respiratory infection, any surgery, IHD, CRF, DM, and Gram negative infection were all associated with a delta-SOFA score of >2 on univariate analysis. The association with an infective focus of Gram-negative bacteria was inverse meaning that gram negative infection was associated with a below median delta-SOFA score (RR=0.111 (0.016-0.755); p=0.025). On multivariate analysis however, there were no independent factors associated with this.

Table 18: Univariate analysis: factors associated with above median delta-SOFA score

Variable	p	Relative Risk (95% CI)
Age	0.863	0.994 (0.9223-1.070)
Gender	0.485	1.923 (0.307-12.053)
APACHE II	0.176	1.106 (0.959-1.280)
SAPS	0.667	1.018 (0.939-1.103)
Day 0 SOFA	0.904	1.018 (0.766-1.351)
Day 0 CRP	0.423	0.995 (0.984-1.007)
Day 0 Albumin	0.260	1.101 (0.931-1.303)
Day 0 BM	0.989	1.002 (0.716-1.403)
Surgery		

Elective Surgery	0.318	0.313 (0.32-3.068)
Emergency Surgery	0.999	NA
Any Surgery	0.016	0.059 (0.006-0.594)
Co-morbidities		
Hypertension	0.761	1.333 (0.209-8.486)
Ischaemic heart disease	0.026	8.889 (1.294-61.058)
Congestive heart failure	0.999	NA
COPD	0.278	2.700 (0.448-16.255)
Chronic renal failure	0.050	6.33 (1.001-40.071)
Diabetes	0.052	7.875 (0.980-63.310)
Alcohol abuse	0.382	3.667 (0.199-67.652)
Cancer	0.896	1.133 (0.172-7.469)
Immunocompromised	0.346	2.667 (0.347-20.508)
Transplant	0.933	1.111 (0.097-12.750)
Steroid use	0.709	1.44 (0.212-9.782)
IVDU	1.000	NA
Baseline physiology		
MAP (mmHg)	0.110	0.910 (0.810-1.022)
Arterial pH	0.769	0.300 (0.000-932.768)
Serum lactate	0.891	1.031 (0.671-1.584)
PaO_2/FiO_2 ratio	0.922	1.000 (0.993-1.008)
Ventilated	0.485	1.923 (0.307-12.053)
Source of sepsis		
Abdomen	0.067	0.175 (0.27-1.130)
Chest	0.007	16.667 (2.167-128.176)
Urinary tract	0.999	NA
Skin	0.999	NA
Pathogen		
Gram + alone	0.278	2.700 (0.448-16.255)
Gram - alone	0.025	0.111 (0.016-0.755)
Fungal	0.999	NA
Developed sepsis on ward vs. on the ITU	0.892	1.038 (0.604-1.784)
Days sepsis prior to trial recruitment	0.892	0.792 (0.074-8.518)
Baseline organ failure		
Cardiovascular	0.818	1.22 (0.222-6.730)
Respiratory	0.860	0.857 (0.154-4.764)
Renal	0.999	NA
Hepatic	0.999	NA
Haematological	1.000	NA
Baseline organ dysfunction		
Cardiovascular	0.177	3.562 (0.563-22.540)
Respiratory	0.761	0.750 (0.118-4.773)
Renal	0.699	1.406 (0.250-7.896)
Hepatic	0.410	0.381 (0.038-3.784)
Haematological	0.999	NA

Table 19: Multivariate analysis

Variable	p	Relative Risk (95% CI)
IHD	0.998	NA
DM	0.998	NA
Gram negative infection	0.998	NA
CRF	0.995	NA

3.3.4.3 Gram positive infective focus

Univariate (UVA) and multivariate (MVA) logistical regression analysis was carried out on all patients with Gram-positive sepsis to investigate if there were any factors that were independently associated with outcome. For both mortality and a delta-SOFA score greater than 2 there were no variables that were independently associated with gram positive sepsis on either UVA or MVA (data not shown).

3.3.4.4 Gram negative infective focus

Univariate and multivariate logistical regression analysis was carried out on all patients with Gram-negative sepsis to investigate if there were any factors that were independently associated with outcome.

3.3.4.4.1 Factors associated with mortality in Gram negative sepsis

On univariate analysis SAPS, day 0 SOFA score, hepatic failure and haematological dysfunction was associated with mortality and were entered into MVA. However on MVA, there were no factors independently associated with mortality.

Table 20: Univariate analysis – factors associated with mortality in gram negative sepsis

Variable	p	Relative Risk (95% CI)
Fish oil	0.105	0.277 (0.059-1.307)
Age	0.636	1.014 (0.958-1.073)
Gender	0.371	2.022 (0.432-9.461)
APACHE II	0.939	1.005 (0.892-1.132)
SAPS	0.069	1.078 (0.994-1.169)
Day 0 SOFA	0.053	1.270 (0.997-1.618)
Day 0 CRP	0.648	0.998 (0.988-1.008)
Day 0 Albumin	0.348	0.939 (0.824-1.034)
Day 0 BM	0.467	0.895 (0.663-1.235)
Surgery		
Elective Surgery	0.999	NA
Emergency Surgery	0.478	0.571 (0.122-2.682)
Any Surgery	0.105	0.277 (0.059-1.307)
Co-morbidities		
Hypertension	0.726	1.300 (0.300-5.637)
Ischaemic heart disease	0.672	1.500 (0.230-9.987)
Congestive heart failure	0.999	NA
COPD	0.900	0.905 (0.189-4.340)
Chronic renal failure	0.732	0.667 (0.065-9.796)
Diabetes	0.672	1.5 (0.230-9.796)
Alcohol abuse	1.000	NA
Cancer	0.744	0.771 (0.162-3.663)
Immunocompromised	0.999	NA
Transplant	0.999	NA
Steroid use	0.999	NA
IVDU	1.000	NA
Baseline physiology		
MAP (mmHg)	0.232	0.952 (0.679-1.032)
Arterial pH	0.778	0.228 (0.000-6637.049)
Serum lactate	0.677	1.075 (0.766-1.509)
PaO_2/FiO_2 ratio	0.949	1.000 (0.994-1.000)
Ventilated	0.875	0.889 (0.204-3.864)
Source of sepsis		
Abdomen	0.586	0.636 (0.125-3.235)
Chest	1.000	NA
Urinary tract	0.881	1.150 (0.185-7.144)
Skin	1.000	NA
Developed sepsis on ward vs. on the ITU	0.775	1.44 (0.117-17.904)
Days sepsis prior to trial recruitment	0.698	0.860 (0.400-1.846)
Baseline organ failure		
Cardiovascular	0.208	2.692 (-.572-12.596)
Respiratory	0.968	1.030 (0.236-4.504)
Renal	0.168	3.067 (0.624-15.075)
Hepatic	0.047	11.571 (1.038-128.967)
Haematological	0.454	3.000 (1.006-5.187)

Baseline organ dysfunction		
Cardiovascular	0.199	0.235 (0.026-2.145)
Respiratory	0.846	0.867 (0.204-3.676)
Renal	0.478	0.571 (0.122-1.661)
Hepatic	0.603	0.662 (0.140-3.123)
Haematological	0.054	4.500 (0.977-20.724)

Table 21: Multivariate analysis

Variable	p	Relative Risk (95% CI)
Hepatic failure	0.095	8.293 (0.694-99.054)

3.3.4.4.2 Factors associated with above median delta-SOFA score in gram negative sepsis

On UVA, the only independent variables associated with an above median delta-SOFA score were hypertension and fish oil use (which was inversely related). On MVA, fish oil administration was the only independent variable that was significantly related to a lower than median delta-SOFA score in gram-negative sepsis.

Table 22: Univariate analysis – factors associated with above median delta-SOFA score in gram-negative sepsis

Variable	p	Relative Risk (95% CI)
Fish oil	0.001	0.056 (0.010-0.325)
Age	0.517	0.983 (0.933-1.035)
Gender	0.543	1.523 (0.392-5.913)
APACHE II	0.808	1.014 (0.909-1.130)
SAPS	0.608	1.018 (0.952-1.088)
Day 0 SOFA	0.329	1.108 (0.902-1.360)
Day 0 CRP	0.288	1.005 (0.996-1.014)
Day 0 Albumin	0.776	1.017 (0.905-1.144)
Day 0 BM		
Surgery		
Elective Surgery	0.415	0.385 (0.039-3.836)
Emergency Surgery	0.298	0.473 (0.115-1.937)
Any Surgery	0.116	0.333 (0.085-1.312)
Co-morbidities		
Hypertension	0.070	0.278 (0.070-1.109)
Ischaemic heart disease	0.471	1.909 (0.329-11.082)
Congestive heart failure	0.896	0.846 (0.070-10.272)

COPD	0.256	2.250 (0.552-9.170)
Chronic renal failure	0.999	NA
Diabetes	0.286	0.292 (0.031-2.801)
Alcohol abuse	1.000	NA
Cancer	0.881	1.111 (0.278-4.434)
Immunocompromised	0.999	NA
Transplant	0.999	NA
Steroid use	0.999	NA
IVDU	1.000	NA
Baseline physiology		
MAP (mmHg)	0.156	0.950 (0.884-1.020)
Arterial pH	0.894	0.532 (0.000-6020.991)
Serum lactate	0.942	1.012 (0.730-1.238)
PaO_2/FiO_2 ratio	0.066	1.006 (1.000-1.013)
Ventilated	0.396	0.692 (0.169-2.549)
Source of sepsis		
Abdomen	0.590	0.658 (0.144-3.013)
Chest	1.00	NA
Urinary tract	0.716	1.364 (0.257-7.229)
Skin	1.000	NA
Developed sepsis on ward vs. on the ITU	0.896	0.846 (0.070-10.272)
Days sepsis prior to trial recruitment	0.894	0.959 (0.517-1.780)
Baseline organ failure		
Cardiovascular	0.275	2.127 (0548-8.259)
Respiratory	0.298	0.473 (0.115-1.937)
Renal	0.803	0.818 (0.169-3.956)
Hepatic	0.999	NA
Haematological	0.695	1.769 (0.102-30.709)
Baseline organ dysfunction		
Cardiovascular	0.810	1.200 (0.272-5.285)
Respiratory	0.671	1.333 (0.354-5.023)
Renal	0.718	0.778 (0.199-5.825)
Hepatic	0.558	1.500 (0.386-5.825)
Haematological	0.393	1.821 (0.460-7.234)

Table 23: Multivariate analysis

Variable	p	Relative Risk (95% CI)
Fish oil	0.003	0.064 (0.010-0.405)

3.4 Results III: Fatty Acid analysis

Blood samples from 41 patients (23 controls and 18 receiving FO) were available for analysis of PC, NEFA and PMN lipid fractions. In addition two patients had 4 hourly blood samples from the time at which the first FO infusion was commenced for a 24-hour period for pharmacodynamic analysis. Nineteen patients withdrew or did not permit consent for blood sampling.

3.4.1 Baseline Fatty Acid Levels

The mean FA levels for each of the 3 lipid fractions at baseline are shown in Table 24. The most abundant FA differed depending on the fraction analysed. In PC and NEFA fractions the most abundant FA was 16:00 (palmitic acid) and 18:1n-9 (oleic acid). In PMN fractions the most abundant FA was palmitic acid and stearic acid (18:00). EPA and DHA were present in small quantities only in all three lipid fractions. The AA/(DHA+EPA) ratio was 2.51, 1.14 and 5.45 in the PC, NEFA and PMN fractions respectively.

Table 24**: Baseline fatty acids concentrations as per lipid pool**

Fatty Acid (%)	PC	NEFA	PMN
14:00	0.348	1.357	0.953
16:00	32.869	24.769	25.130
16:1n-7	1.156	2.793	1.277
18:00	12.616	15.993	25.245
18:1n-9	17.072	36.169	24.333
18:1n-7	2.330	2.294	2.072
18:2n-6	19.637	9.216	7.242
18:3n-6	0.098	0.313	0.425
18:3n-3	0.312	1.299	0.7724
20:00	0.168	0.994	0.902
20:1n-9	0.197	0.579	0.948
20:2n-6	0.274	0.340	0.426
20:3n-6	1.984	0.611	0.932
20:4n-6 (AA)	7.127	1.348	8.048

22:00	0.078	0.145	0.162
20:4n-3	0.220	0.181	0.195
20:5n-3 (EPA)	0.744	0.276	0.504
22:4n-6	0.026	0.063	0.042
22:5n-3	0.643	0.357	0.944
22:6n-3 (DHA)	2.101	0.903	0.974

In concentration terms in the PC fraction the baseline levels for AA, EPA and DHA were 54.51±6.78 micrograms/ml, 5.40±1.22 micrograms/ml and 14.98±2.34 micrograms/ml respectively.

3.4.2 Pharmacodynamic FA analysis

Two patients were chosen to have 4 hourly blood sampling to allow for pharmacodynamic analysis following the start of the first FO infusion until the beginning of the next 24 hours later.

3.4.2.1 N-3 FA uptake

There was rapid incorporation of the n-3 FAs into all 3 lipid fractions.

3.4.2.1.1 PMN fraction

EPA and DHA was rapidly incorporated into the PMN membrane. EPA levels trebled within 8 hours whilst DHA levels increased by 50% in the same time (Figure 24). The levels appeared to plateau at around 16 hours from the beginning of the infusion. AA levels by contrast showed only minor variation in between infusions (Figure 25).

Figure 23: PMN fraction pharmacodynamics – DHA and EPA

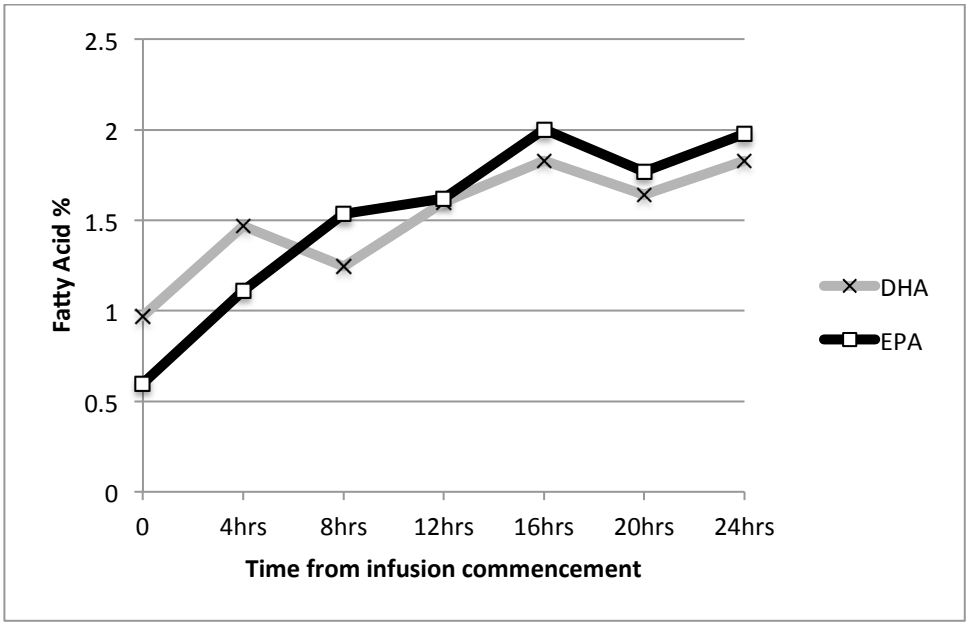
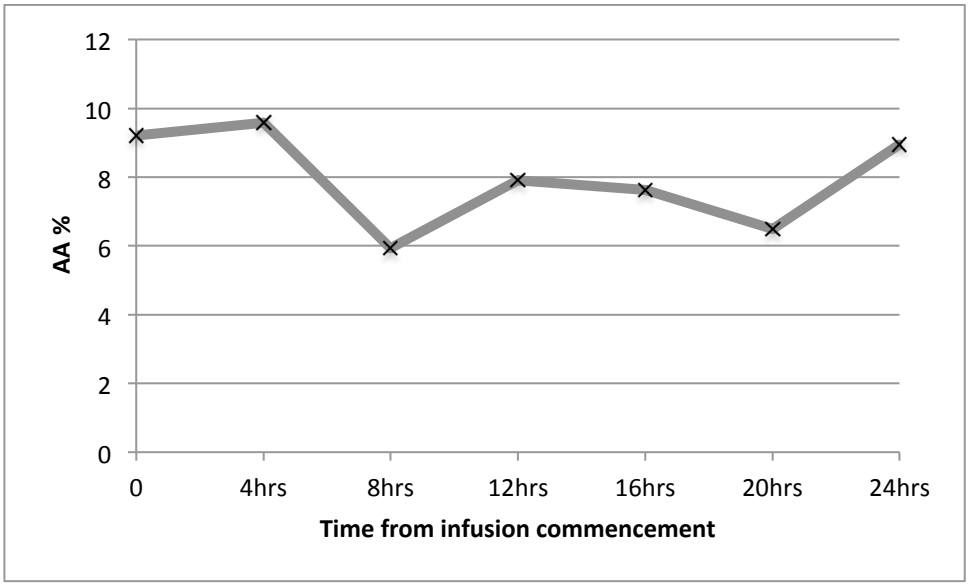


Figure 24: PMN fraction pharmacodynamics – AA



3.4.2.1.2 PC fraction

In the PC fraction EPA was rapidly incorporated into the lipid pool whilst DHA levels remained largely unchanged (Figure 26). At the end of the 24-hour period the EPA

levels had trebled from baseline. There was also only very minor variation in the AA levels (Figure 27).

Figure 25: PC fraction pharmacodynamics – DHA and EPA

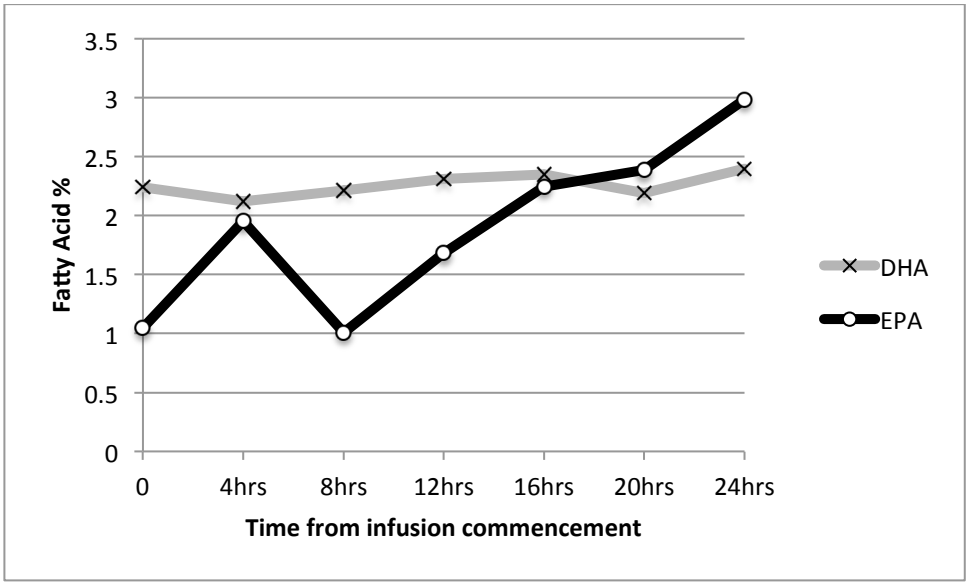
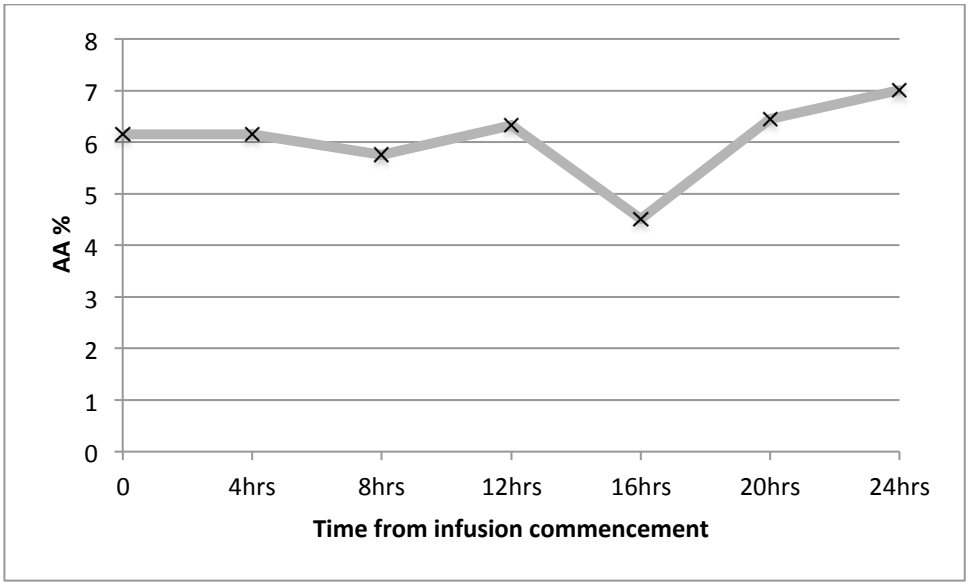


Figure 26: PC fraction pharmacodynamics – AA



3.4.2.1.3 NEFA fraction

In the NEFA fraction, both EPA and DHA levels peaked at 8 hours from baseline and then steadily dropped (Figure 28). Both FAs trebled in % from baseline levels. AA

appeared to increase until 16 hours, where it doubled in %, and then steadily declined (Figure 29).

Figure 27: NEFA fraction pharmacodynamics – DHA and EPA

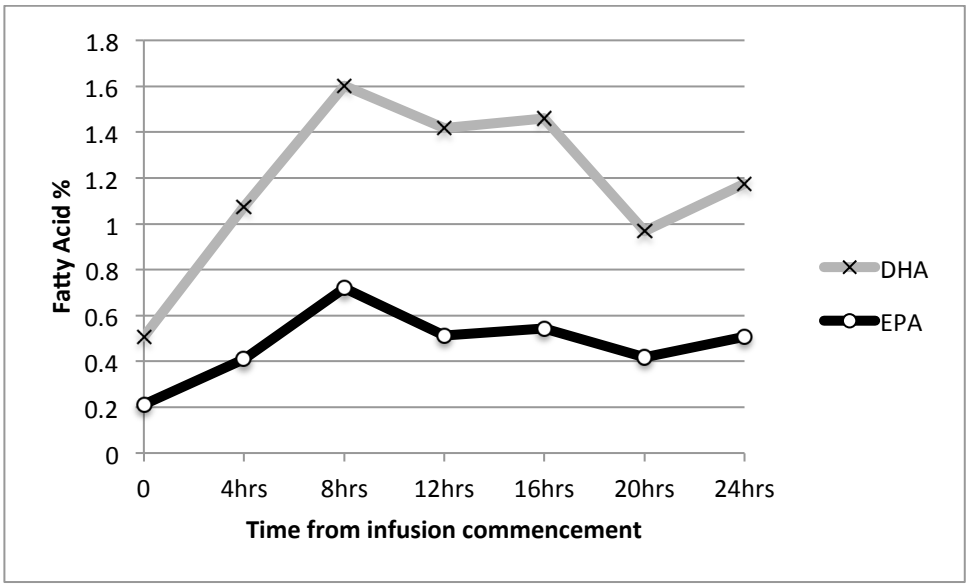
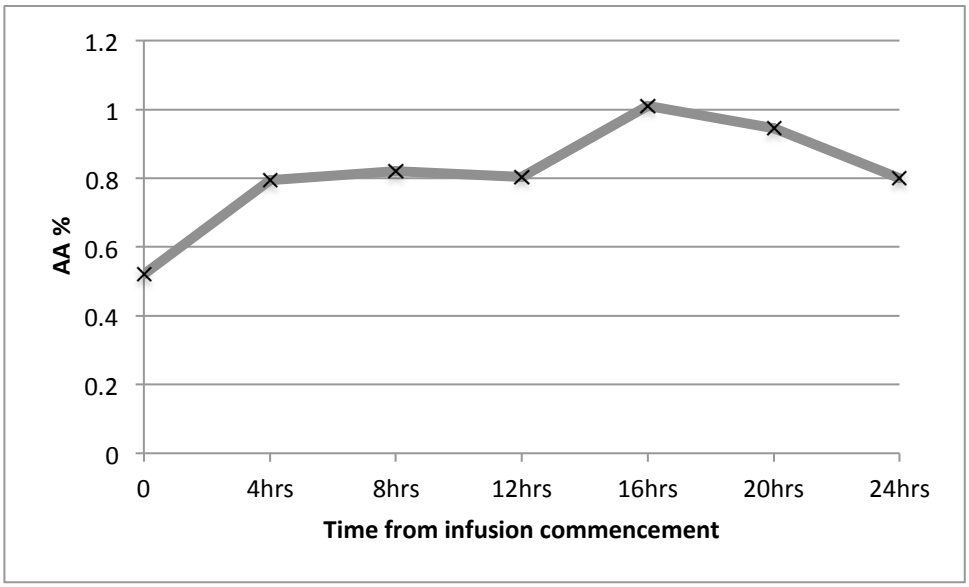


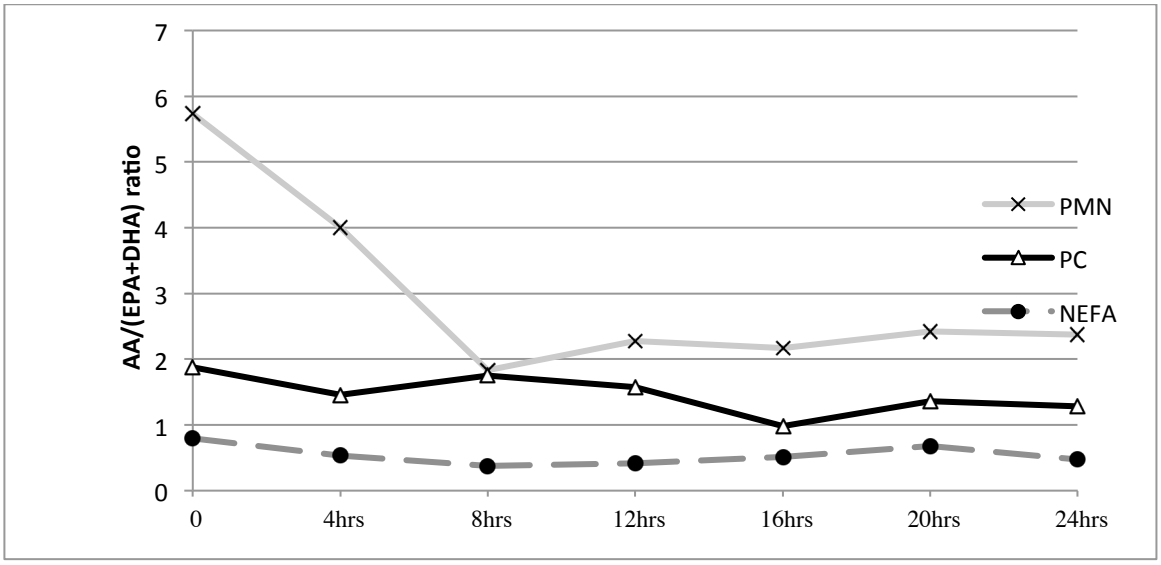
Figure 28: NEFA fraction pharmacodynamics – AA



3.4.2.1.4 AA/(EPA+DHA) ratio

There was rapid reduction in the AA/(EPA+DHA) ratio on commencement of the infusion in all lipid pools analysed (Figure 30). In the PMN fraction this reached the lowest and then plateaued at 8 hours. There was no 'trough' in n-3 levels between the infusions on day 0 and day 1.

Figure 29: Pharmacodynamics- AA/(EPA+DHA)



3.4.3 Fatty acid levels over study period

The tables below display the results for all twenty measured fatty acids in the PMN lipid fraction during the study period. Fatty acid levels for the NEFA and PC lipid fractions are shown in the Appendix. In the NEFA and PC lipid fractions apart from the AA, DHA and EPA fatty acids there are no other fatty acids demonstrating a consistent significant difference between the two cohorts. At baseline there are no significant differences in lipid levels between the two cohorts.

There are also significant differences seen with other fatty acids however these are less consistent. On day 5 the control group has lower 20:00 (arachidic acid) than the fish oil cohort but this is not seen on any other days. More consistent is the significantly greater level of 22:5,n-3 (docosapentaenoic acid, DPA) in the FO cohort on days 1,3 and 7. A significantly larger level of 18:3,n-3 (alpha-linolenic acid, ALA) is seen in the control cohort on days 2 and 7.

Table 25: PMN fraction fatty acids during study period (%)

	Day 0			Day 1		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.90±0.54	1.02±0.67	0.40	0.97±0.63	1.03±0.40	0.36
16:00	24.50±4.99	25.94±5.22	0.43	25.69±6.22	25.37±4.32	0.72
16:1n-7	1.27±0.68	1.28±0.66	1.00	1.25±0.67	1.49±0.63	0.26
18:00	24.13±7.85	26.68±7.07	0.46	29.38±7.92	25.20±5.68	0.12
18:1n9	24.07±5.66	21.18±5.23	0.15	20.89±6.63	23.69±5.45	0.12
18:1n-7	2.05±0.43	2.10±0.71	0.61	1.94±0.67	2.11±0.66	0.41
18:2n-6	8.22±4.40	5.98±2.61	0.12	6.32±2.96	5.66±2.99	0.38
18:3n-6	0.41±0.26	0.45±0.35	0.91	0.42±0.27	0.39±0.32	0.36
18:3n-3	0.76±0.50	0.79±0.58	0.98	0.88±0.60	0.79±0.55	0.62
20:00	0.83±0.44	0.99±0.82	0.80	1.01±0.51	0.76±0.26	0.06
20:1n-9	0.87±0.45	1.05±0.57	0.35	1.03±0.41	0.97±0.65	0.25
20:2n-6	0.43±0.23	0.42±0.19	0.40	0.35±0.18	0.40±0.21	0.36
20:3n-6	0.85±0.30	1.04±0.55	0.71	0.74±0.40	0.82±0.31	0.33
20:4n-6	7.86±4.19	8.29±4.56	0.40	6.41±4.20	7.67±3.34	0.25
22:00	0.15±0.10	0.18±0.13	0.52	0.14±0.11	0.18±0.15	0.60
20:4n-3	0.21±0.18	0.17±0.14	1.00	0.04±0.18	0.23±0.15	0.98
20:5n-3	0.50±0.27	0.51±0.29	0.44	0.60±0.59	0.93±0.67	0.03
22:4n-6	0.04±0.03	0.05±0.34	0.44	0.05±0.02	0.04±0.02	0.38
22:5n-3	0.95±0.48	0.94±0.45	0.93	0.77±0.45	1.09±0.42	0.03
22:6n-3	1.00±0.51	0.94±0.37	0.91	0.92±0.59	1.17±0.47	0.15

	Day 2			Day 3		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	1.04±0.55	1.03±0.67	0.63	1.27±1.13	0.91±0.31	0.77
16:00	24.62±4.70	26.68±6.77	0.63	25.61±6.92	25.09±5.84	0.52
16:1n-7	1.33±0.55	1.37±0.75	0.95	1.19±0.60	1.36±0.75	0.68
18:00	25.31±6.88	25.36±7.39	0.74	25.16±9.32	22.07±7.47	0.38
18:1n9	23.15±4.95	20.86±5.55	0.48	22.92±7.40	23.87±6.30	0.68
18:1n-7	2.14±0.57	1.90±0.60	0.26	2.00±0.67	2.12±0.64	0.77
18:2n-6	6.88±2.49	6.45±3.19	0.92	7.27±3.66	7.10±2.42	1.00
18:3n-6	0.51±0.28	0.37±0.25	0.07	0.39±0.19	0.37±0.34	0.41
18:3n-3	1.10±0.73	0.59±0.50	0.02	1.02±0.97	0.78±0.55	0.91
20:00	0.96±0.42	0.70±0.46	0.07	1.06±0.73	0.96±0.62	0.86
20:1n-9	1.02±0.56	1.03±0.56	0.77	0.97±0.65	0.94±0.59	0.91
20:2n-6	0.49±0.22	0.37±0.23	0.10	0.42±0.21	0.34±0.16	0.41
20:3n-6	0.83±0.27	0.81±0.47	0.55	0.81±0.42	0.88±0.34	0.48
20:4n-6	7.76±4.07	11.43±5.43	0.95	5.97±4.17	8.66±4.17	0.07
22:00	0.17±0.13	0.20±0.13	0.43	0.16±0.12	0.07±0.03	0.03
20:4n-3	0.21±0.18	0.47±0.43	0.04	0.24±0.12	0.26±0.28	0.48
20:5n-3	0.56±0.39	1.25±0.70	0.01	0.68±0.36	1.28±0.59	0.00
22:4n-6	0.04±0.03	0.04±0.02	0.60	0.05±0.03	0.04±0.03	0.18
22:5n-3	0.84±0.43	1.15±0.70	0.23	0.81±0.49	1.37±0.71	0.04
22:6n-3	1.04±0.47	1.23±0.76	0.60	1.00±0.49	1.52±0.64	0.05

	Day 5			Day 7		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.86±0.58	1.08±0.69	0.45	0.66±0.44	0.71±0.30	0.56
16:00	22.64±2.68	26.32±6.98	0.23	22.88±3.86	22.33±3.82	1.00
16:1n-7	0.94±0.37	1.39±0.56	0.06	1.26±0.47	1.15±0.54	0.56
18:00	30.59±7.98	22.73±7.17	0.02	26.16±3.73	23.36±3.87	0.20
18:1n9	20.50±3.86	24.24±3.98	0.06	22.56±2.84	23.42±3.63	0.82
18:1n-7	1.76±0.53	2.17±0.63	0.17	1.89±0.31	2.16±0.42	0.42
18:2n-6	6.65±2.36	7.34±3.07	0.69	7.96±2.84	7.98±2.77	0.91
18:3n-6	0.28±0.22	0.31±0.23	0.63	0.45±0.20	0.23±0.08	0.02
18:3n-3	1.26±1.01	0.56±0.42	0.07	1.11±0.59	0.49±0.31	0.03
20:00	1.19±0.50	0.66±0.35	0.02	0.96±0.40	0.78±0.25	0.36
20:1n-9	1.17±0.30	0.59±0.26	0.00	1.12±0.74	0.65±0.28	0.25
20:2n-6	0.35±0.14	0.34±0.14	0.97	0.59±0.18	0.44±0.28	0.20
20:3n-6	0.81±0.29	0.88±0.31	0.83	0.96±0.41	0.89±0.23	0.91
20:4n-6	7.63±3.54	7.17±3.78	0.83	7.89±4.21	10.13±3.73	0.25
22:00	0.14±0.12	0.11±0.10	0.63	0.17±0.11	0.08±0.04	0.13
20:4n-3	0.21±0.09	0.16±0.06	0.27	0.43±0.58	0.17±0.09	0.36
20:5n-3	0.77±0.69	1.26±0.64	0.20	1.18±1.49	1.42±0.81	0.42
22:4n-6	0.07±0.04	0.03±0.02	0.02	0.04±0.03	0.05±0.03	0.82
22:5n-3	1.06±0.55	1.16±0.61	0.76	0.81±0.38	1.59±0.58	0.02
22:6n-3	1.13±0.60	1.59±0.83	0.23	0.92±0.37	1.95±0.70	0.01

	Day 10			Day 13		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.73±0.36	0.64±0.31	0.44	0.55±0.34	1.24±0.88	0.09
16:00	28.45±9.83	30.43±11.54	0.80	28.40±10.45	24.53±2.84	0.83
16:1n-7	0.95±0.48	0.84±0.48	0.80	0.79±0.69	1.30±0.29	0.14
18:00	30.18±12.00	29.24±9.58	1.00	27.04±8.07	27.97±5.18	0.83
18:1n9	18.30±0.08	18.47±6.82	0.44	19.06±6.19	20.24±2.02	0.67
18:1n-7	1.77±0.59	1.88±0.70	0.61	1.99±0.57	1.85±0.22	0.67
18:2n-6	6.28±4.37	4.51±3.83	0.44	6.56±5.56	5.03±1.42	0.52
18:3n-6	0.23±0.11	0.19±0.13	0.44	0.24±0.09	0.39±0.24	0.39
18:3n-3	0.80±0.66	0.41±0.38	0.20	0.32±0.22	0.81±0.42	0.03
20:00	0.93±0.42	0.47±0.37	0.20	0.78±0.34	0.96±0.59	0.67
20:1n-9	0.75±0.21	0.57±0.32	0.30	0.87±0.42	1.08±0.39	0.29
20:2n-6	0.28±0.09	0.29±0.23	0.61	0.45±0.20	0.34±0.15	0.39
20:3n-6	0.62±0.31	0.74±0.64	0.61	1.02±0.62	0.84±0.17	0.83
20:4n-6	7.35±4.21	6.95±5.04	1.00	9.07±4.74	9.48±6.66	1.00
22:00	0.07±0.03	0.11±0.04	0.12	0.10±0.03	0.11±0.08	0.83
20:4n-3	0.16±0.09	0.11±0.05	0.80	0.16±0.10	0.19±0.24	0.52
20:5n-3	0.39±0.17	1.41±1.30	0.44	0.47±0.31	0.87±0.98	0.52
22:4n-6	0.05±0.03	0.02±0.01	0.12	0.05±0.03	0.02±0.01	0.14
22:5n-3	0.87±0.32	1.19±0.83	0.44	0.99±0.43	1.36±0.59	0.20
22:6n-3	0.84±0.38	1.56±1.18	0.44	1.10±0.61	1.38±0.61	0.67

3.4.3.1 AA, DHA and EPA over study period

There was no significant difference in the AA concentration between the control and the FO cohort during the study period. The cohort treated with FO had a significantly larger percentage of EPA and DHA in all lipid fractions on several days. A significant increase in DHA and EPA was found from day 1. The figures below show the levels of the EPA, DHA and AA FAs evaluated over the study period for each of the lipid fraction.

3.4.3.2 PMN fraction

There was no significant difference in the percentage of AA in the PMN lipid fraction throughout the study period. Both EPA and DHA increased in the FO treated cohort within the first day. EPA appeared to almost double in concentration within the first day whilst the concentration of DHA did not significantly increase until day 3

(p=0.046). Both EPA and DHA concentrations peaked at day 7. Compared to baseline levels, EPA and DHA had increased 2.5 and 2 fold respectively.

Table 26: PMN fraction EPA, DHA and AA concentrations

	FA								
	AA			EPA			DHA		
Day	FO	C	p	FO	C	p	FO	C	p
0	8.29±4.56	7.86±4.19	NS	0.51±0.27	0.50±0.29	NS	0.94±0.51	1.00± 0.37	NS
1	7.67±3.33	6.41±4.20	NS	0.93±0.67	0.60 ±0.59	0.027	1.17±0.47	0.92± 0.59	NS
2	11.42±5.43	7.76±4.07	NS	1.25±0.70	0.56±0.39	0.005	1.23±0.75	1.04± 0.47	NS
3	8.66±4.16	5.97±4.17	NS	1.28±0.59	0.68±0.36	0.003	1.52±0.64	1.00±0.48	0.046
5	7.17±3.78	7.63±3.54	NS	1.26±0.63	0.77±0.68	NS	1.59±0.82	1.13±0.59	NS
7	10.13±3.72	7.89±4.21	NS	1.42±0.81	1.18±1.49	NS	1.95±0.70	0.92±0.37	0.008
10	6.95±5.04	7.35±4.21	NS	1.41±1.30	0.39±0.17	NS	1.56±1.18	0.84±0.38	NS
13	9.48±6.66	9.07±4.74	NS	0.87±0.99	0.47±0.31	NS	1.38±0.61	1.10±0.61	NS

Figure 30: PMN fraction EPA concentration in patients receiving fish oil vs. control

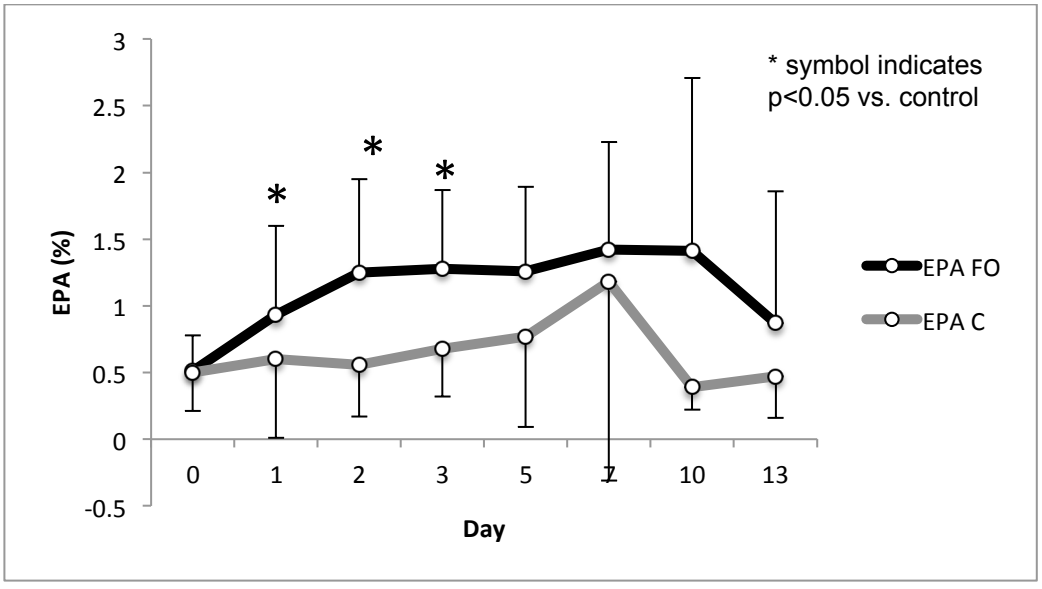


Figure 31: PMN fraction DHA concentration in patients receiving fish oil vs. control

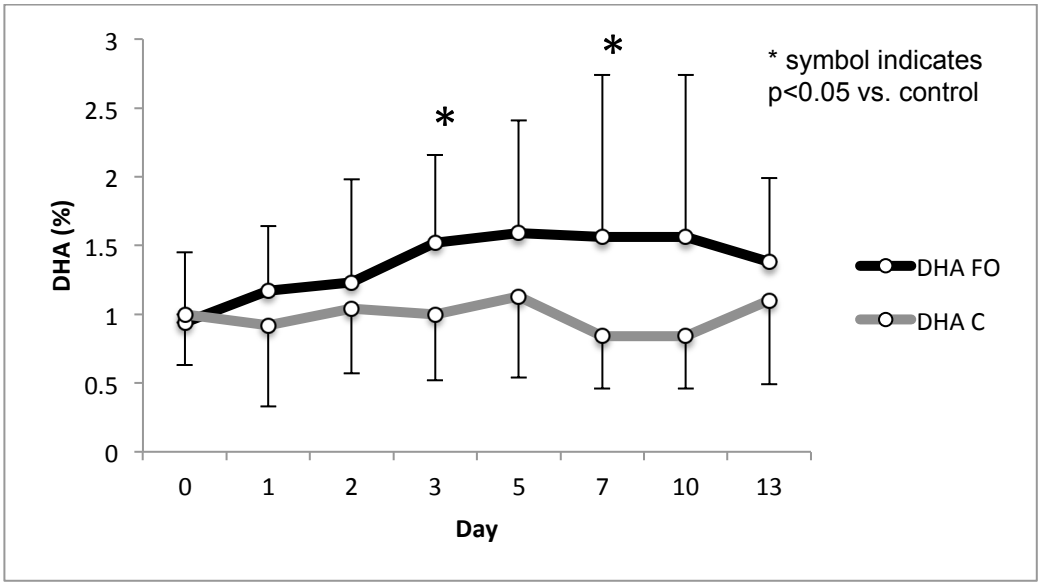
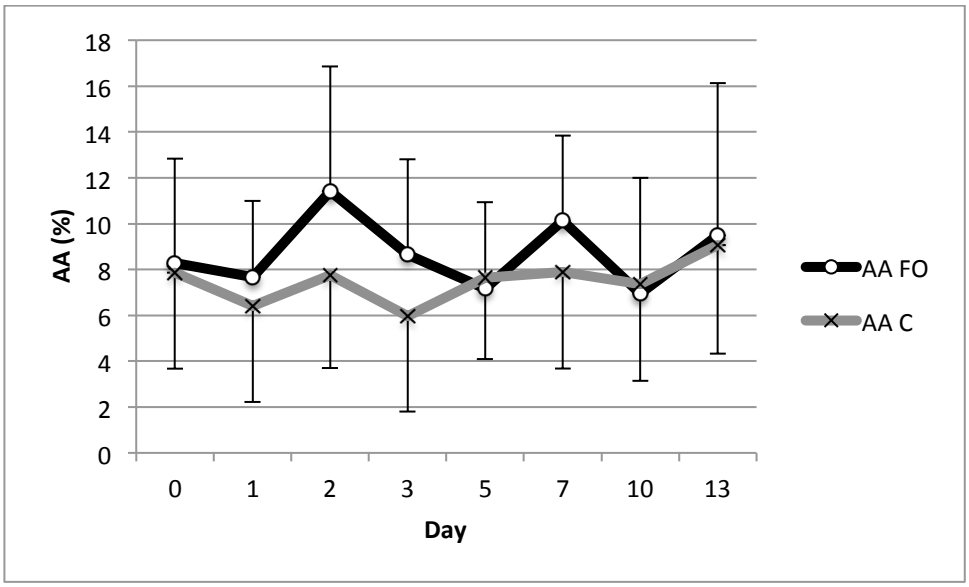


Figure 32: PMN fraction AA concentration in patients receiving fish oil vs. control



3.4.3.3 PC fraction

Again, there was no significant difference in AA concentration throughout the study period between the cohorts in the PC fraction. Both EPA and DHA concentrations were significantly higher in the FO treated cohort within 1 day ($p \leq 0.001$). The levels peaked for EPA and DHA on day 10. The concentration increase from baseline for EPA and DHA was 4.5 and 2 fold respectively on day 10.

Table 27: PC fraction EPA, DHA and AA concentrations

	FA								
	AA			EPA			DHA		
Day	FO	C	p	FO	C	p	FO	C	p
0	7.37±2.06	6.94±1.71	NS	0.79±0.23	0.70±0.29	NS	2.24±0.63	1.99±0.84	NS
1	8.12±2.57	6.47±1.81	NS	2.28±0.58	0.02±0.01	<0.001	2.77±0.73	1.95±0.99	0.001
2	8.07±2.17	6.69±1.63	NS	2.68±1.16	0.7±0.354	<0.001	2.77±0.61	1.82±0.78	<0.001
3	7.46±2.13	5.92±1.90	NS	3.12±1.05	0.57±0.27	<0.001	3.1±1.0	1.68±0.85	0.001
5	6.15±1.14	6.43±1.82	NS	2.57±1.08	0.59±0.23	0.008	3.17±1.44	1.74±0.66	0.021
7	5.67±1.06	6.52±1.74	NS	2.85±1.36	0.78±0.45	0.005	3.33±1.12	1.67±0.445	0.005
10	6.37±1.39	5.98±0.39	NS	3.75±1.89	0.45±0.11	0.014	4.31±1.47	1.54±0.38	0.014
13	6.82±2.31	6.22±0.95	NS	2.77±1.93	0.51±0.17	0.016	3.93±2.00	1.6±0.284	NS

Figure 33: PC fraction EPA concentration in patients receiving fish oil vs. control

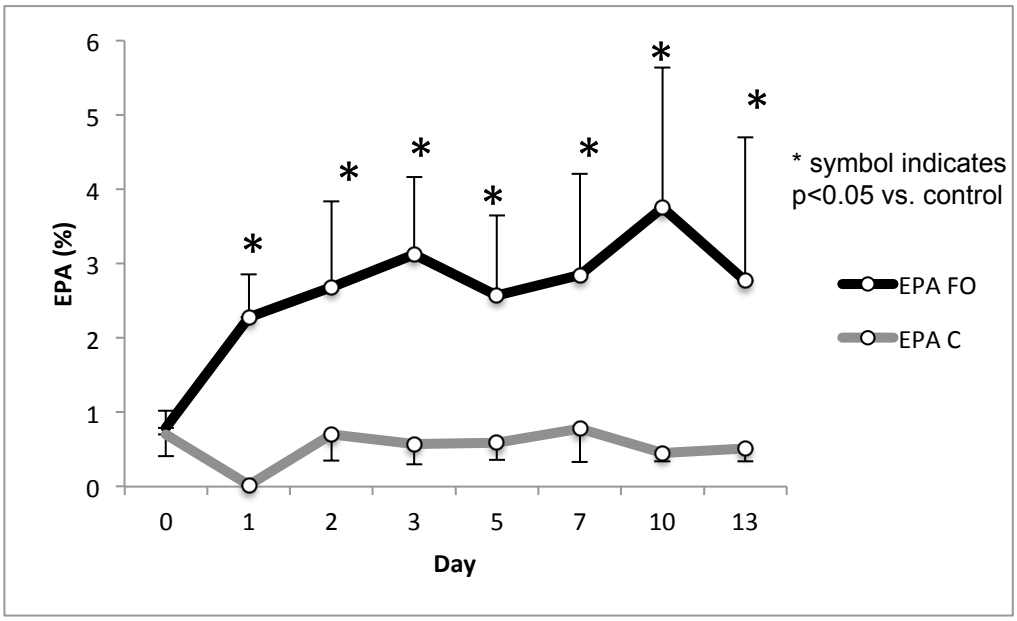


Figure 34: PC fraction DHA concentration in patients receiving fish oil vs. control

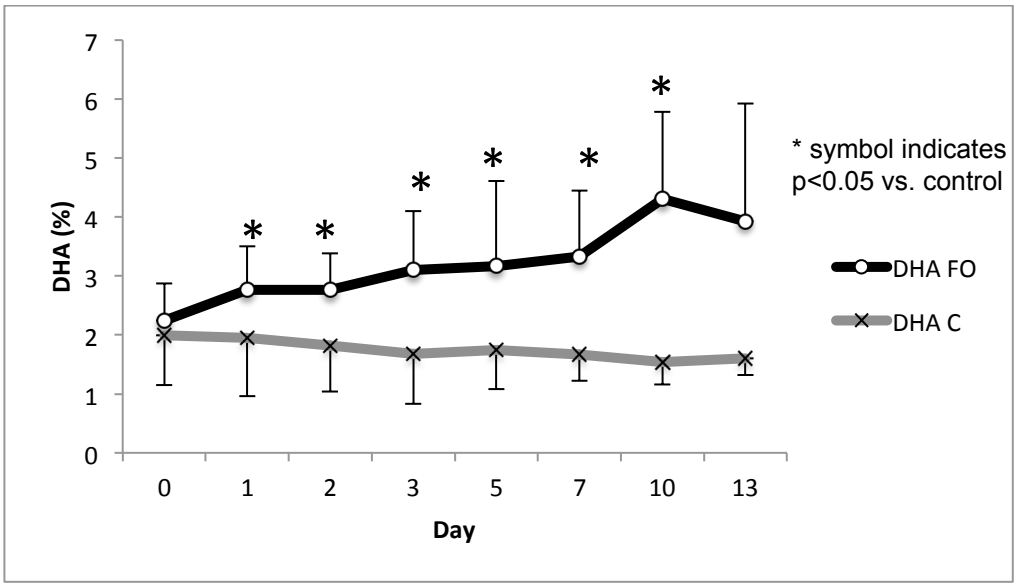
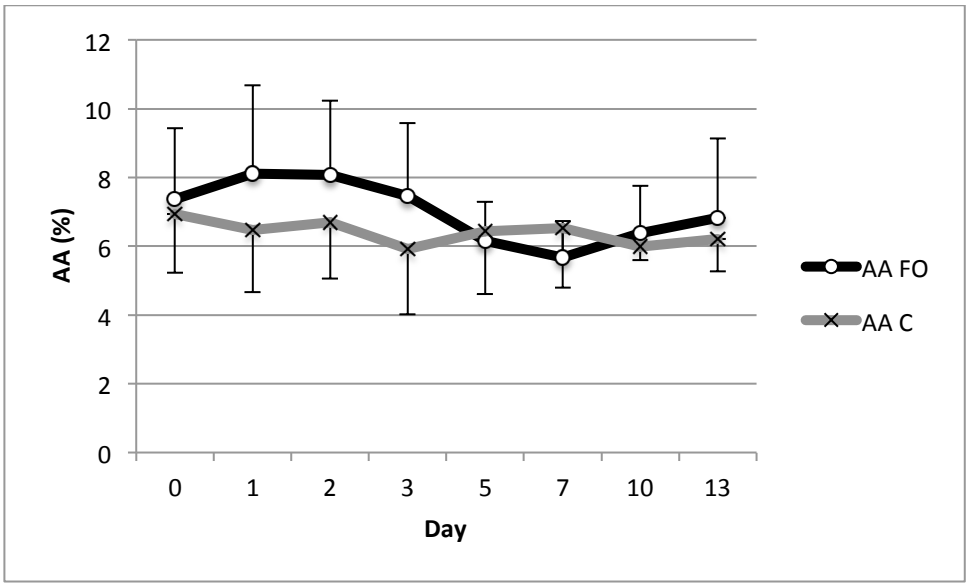


Figure 35: PC fraction AA concentration in patients receiving fish oil vs. control



3.4.3.4 NEFA fraction

There was no significant difference in the concentration of AA between the cohorts throughout the study period. Both EPA and DHA levels were greater in the FO treated cohort. EPA levels increased significantly within 1 day (p=0.023) and DHA within 2 days (p=0.008). EPA levels peaked on day 2 whilst DHA peaked on day 7.

Table 28: NEFA fraction EPA, DHA and AA concentrations

	Fatty Acid								
	AA			EPA			DHA		
Day	FO	C	p	FO	C	p	FO	C	p
0	1.44±0.79	1.28±0.67	NS	0.34±0.19	0.23±0.12	NS	0.96±0.50	0.87±0.31	NS
1	1.26±0.72	1.49±0.07	NS	0.39±0.25	0.24±0.12	0.023	1.20±0.57	0.93±0.34	NS
2	1.33±0.53	1.58±0.87	NS	0.57±0.25	0.23±0.008	<0.001	1.55±0.53	1.03±0.62	0.008
3	1.46±0.65	1.57±0.81	NS	0.57±0.14	0.27±0.04	<0.001	1.50±0.45	0.86±0.34	0.001
5	1.33±0.42	1.45±0.70	NS	0.49±0.24	0.31±0.25	NS	1.47±0.84	0.87±0.43	NS
7	1.54±0.84	1.11±0.42	NS	0.49±0.19	0.23±0.04	0.001	1.91±0.90	0.64±0.29	0.005
10	1.24±0.42	1.55±0.45	NS	0.51±0.20	0.17±0.007	0.028	1.85±0.71	0.86±0.19	0.009
13	1.58±0.50	1.33±0.43	NS	0.54±0.32	0.22±0.09	NS	1.82±0.75	0.78±0.20	0.027

Figure 36: NEFA fraction EPA concentration in patients receiving fish oil vs. control

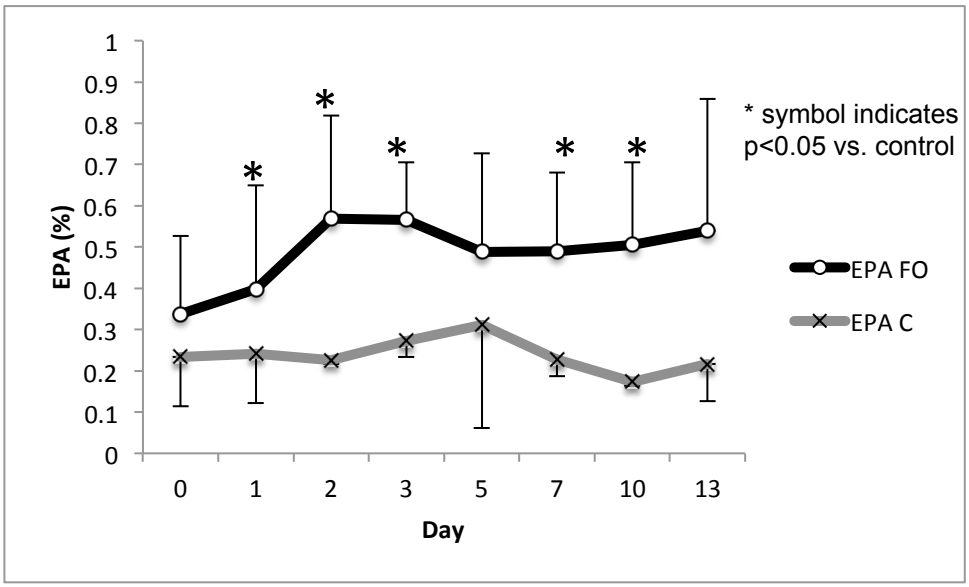


Figure 37: NEFA fraction DHA concentration in patients receiving fish oil vs. control

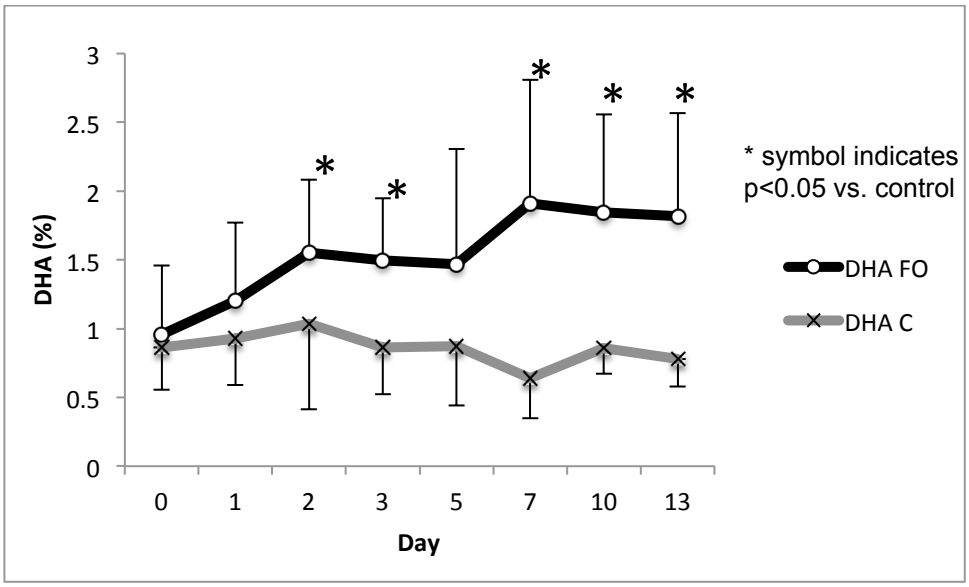
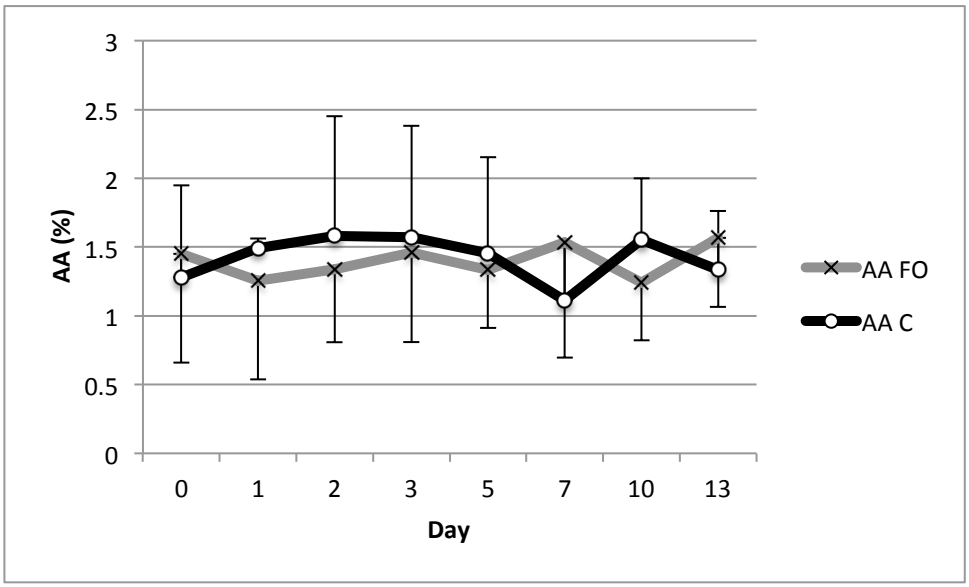


Figure 38: NEFA fraction AA concentration in patients receiving fish oil vs. control



3.4.4 AA/(DHA+EPA) ratio over study period

During the study period the ratio reduced in the cohort treated with FO and increased in the control cohort. There was a significant difference between the control and treatment group on several days.

Table 29: AA/(DHA+EPA) ratio over study period

		Day							
		0	1	2	3	5	7	10	13
PMN	Control	5.2945	4.7221	5.7131	3.3859	4.5038	5.0665	5.5683	6.052
	FO	5.7481	3.7552	3.8265	3.0235	2.7529	3.2847	2.6108	4.6136
	p	0.59	0.156	0.042	1	0.058	0.203	0.121	0.394
PC	Control	2.7291	2.7045	2.8216	2.7874	2.8472	2.7072	3.078	2.9906
	FO	2.4857	1.6548	1.6026	1.241	1.3661	1.0362	0.9671	1.4253
	p	0.478	0	0	0	0.008	0.001	0.014	0.028
NEFA	Control	1.2215	1.296	1.2841	1.4814	1.2587	1.3638	1.5402	1.3427
	FO	1.1787	0.7697	0.6683	0.7025	0.7906	0.6412	0.5554	0.8869
	p	0.926	0.001	0	0.001	0.007	0.001	0.016	0.221

Figure 39: AA/(DHA+EPA) ratio over study period in PMN fraction

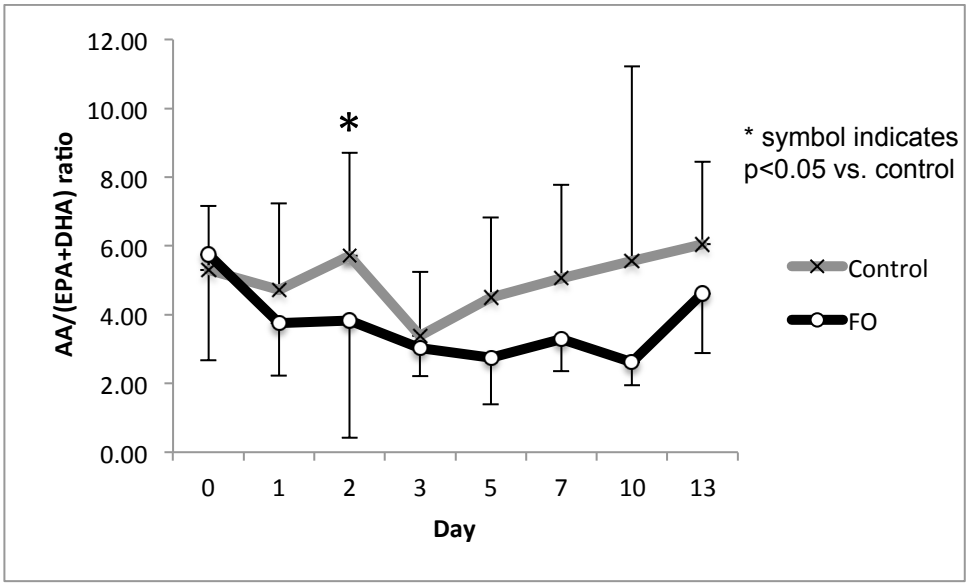


Figure 40: AA/(DHA+EPA) ratio over study period in PC fraction

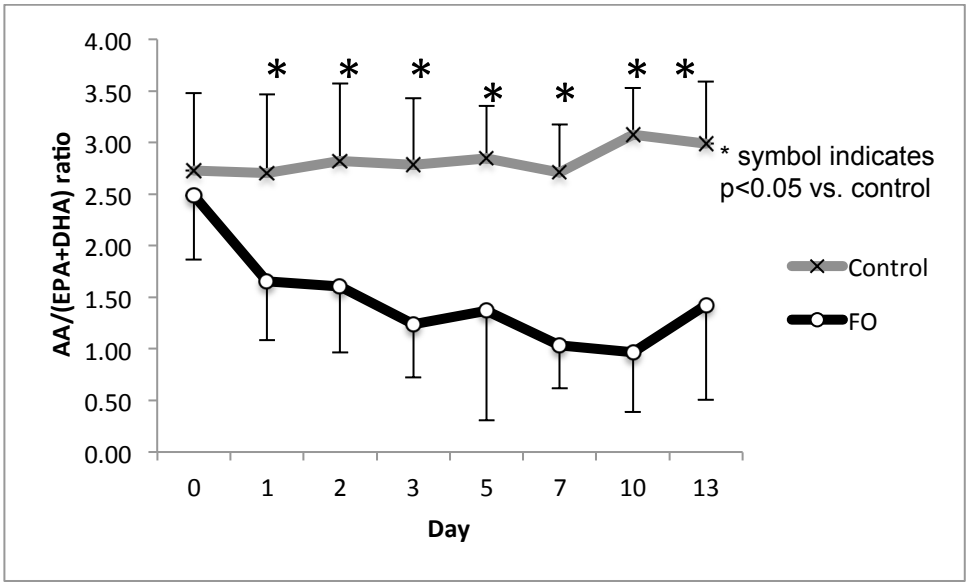
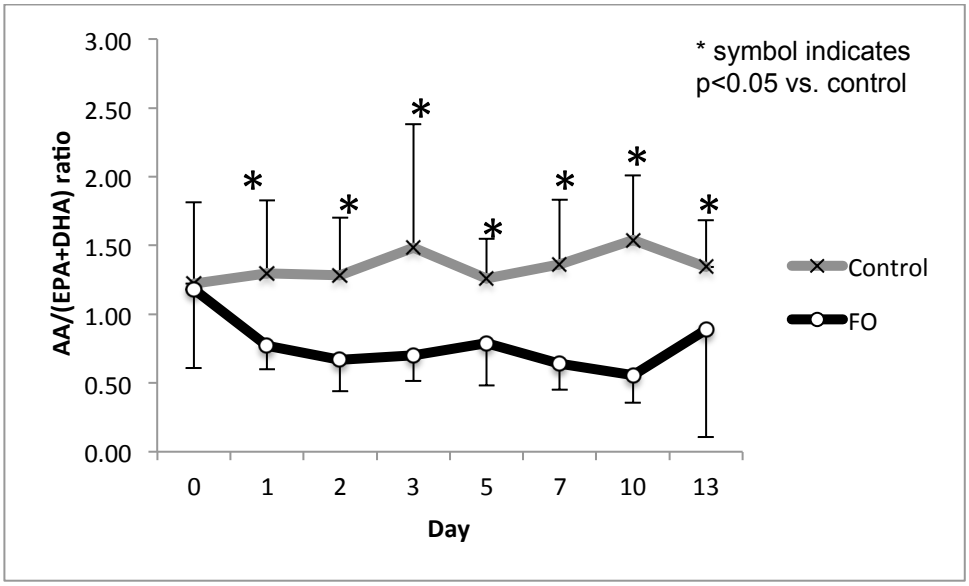


Figure 41: AA/(DHA+EPA) ratio over study period in NEFA fraction



3.4.5 AA/(EPA+DHA) ratio and mortality

There was a trend towards survivors having a lower AA/(EPA+DHA) ratio when compared to non-survivors. On several days, and in various lipid fractions, this was significant. Despite the trend in all lipid fractions, there was no significant reduction in the ratio in the survivors in the NEFA fraction.

Table 30: AA/(EPA+DHA) ratio and mortality

		Day							
		0	1	2	3	5	7	10	13
PMN	Survivors	5.71	4.19	4.71	3.06	3.19	3.91	3.66	4.61
	Non-survivors	4.51	4.79	5.84	3.75	5.81	4.95	8.09	8.95
	p	0.16	0.67	0.23	0.23	0.38	0.56	0.08	0.04
PC	Survivors	2.58	2.13	2.13	1.83	1.95	1.51	1.97	2.04
	Non-survivors	2.85	2.90	2.95	2.83	3.03	2.50	2.67	2.86
	p	0.44	0.02	0.02	0.06	0.07	0.11	0.25	0.19
NEFA	Survivors	1.24	1.09	0.97	1.03	0.98	0.88	0.96	1.70
	Non-survivors	1.07	1.09	1.15	1.57	1.16	1.12	1.34	2.12
	p	0.13	0.97	0.45	1.00	0.28	0.11	0.22	0.70

Figure 42: AA/(EPA+DHA) ratio and mortality in PMN fraction

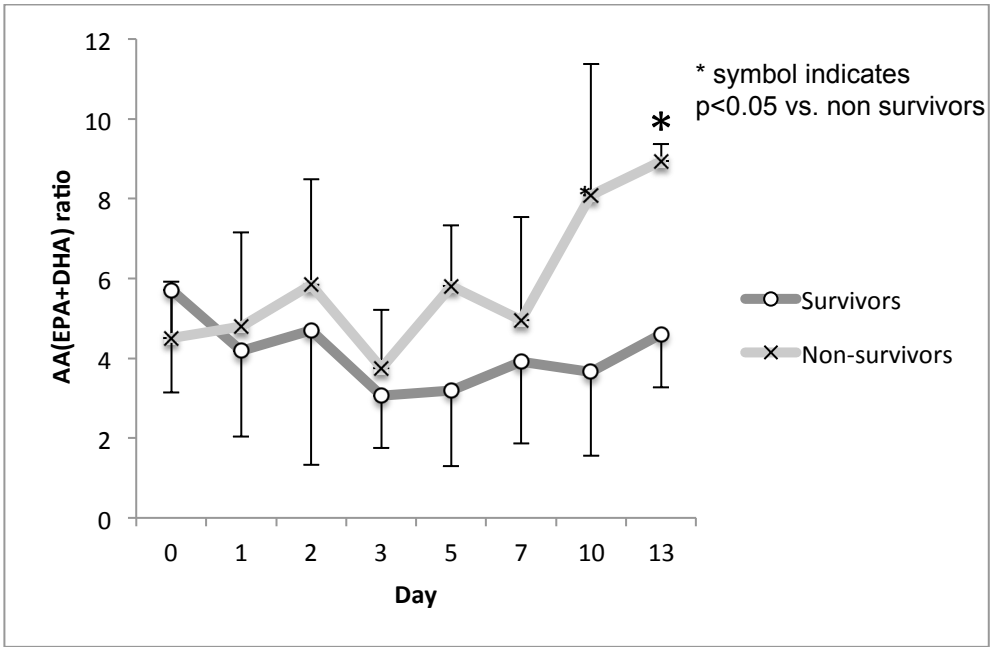


Figure 43: AA/(EPA+DHA) ratio and mortality in PC fraction

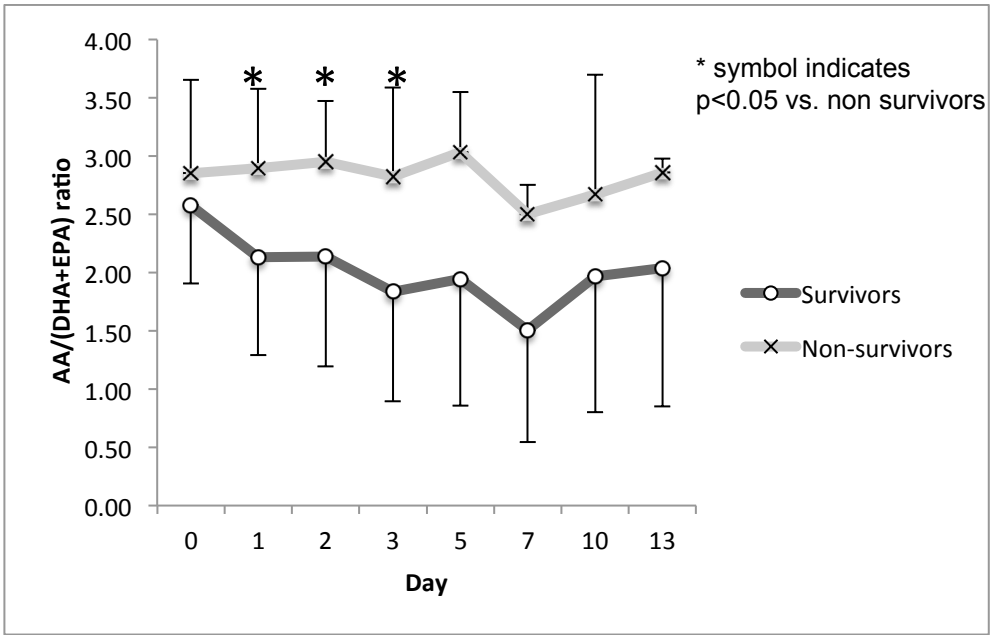
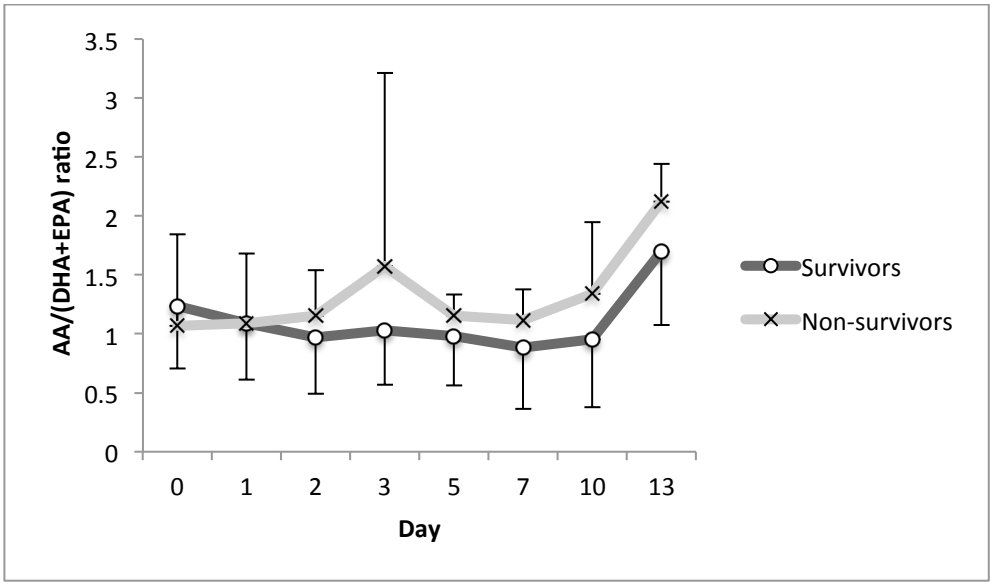


Figure 44: AA/(EPA+DHA) ratio and mortality in NEFA fraction



3.4.6 Univariate analysis of day 2 delta-FA and mortality

The clinical data suggested a significant difference in organ dysfunction on day 2 between survivors and non-survivors. Day 2 'delta-FA' scores were therefore calculated for AA, DHA and EPA to attempt to assess any relation to the change in FA levels from baseline to day 2 and correlate this to mortality.

The data did not show any significant correlation between the day 2 delta-FA levels and mortality. Delta n-3 relates to the day 2 (EPA+DHA) – day 0 (EPA+DHA).

Table 31: Univariate analysis of day 2 delta-FA and mortality

Lipid pool	FA score	p	Relative risk (95% CI)
PMN	Delta AA	0.336	1.213 (0.818-1.800)
	Delta EPA	0.187	0.298 (0.049-1.798)
	Delta DHA	0.688	0.741 (0.172-3.201)
	Delta n-3	0.287	0.556 (0.189-1.637)
PC	Delta AA	0.128	0.448 (0.159-1.261)
	Delta EPA	0.283	0.426 (0.090-2.023)
	Delta DHA	0.299	0.234 (0.015-3.622)
	Delta n-3	0.234	0.508 (0.166-1.550)
NEFA	Delta AA	0.608	1.815 (0.187-17.660)
	Delta EPA	0.961	0.853 (0.001-507.072)
	Delta DHA	0.546	0.492 (0.049-4.921)
	Delta n-3	0.609	0.618 (0.098-3.903)

3.4.7 Demographic relevance to fatty acid concentrations

3.4.7.1 Correlation of gender to fatty acid concentration

3.4.7.1.1 Baseline fatty acids

At baseline (day 0) there was no significant difference between AA, DHA and EPA levels between males and females in any lipid fraction.

Table 32: Baseline FAs per gender

		Female	Male	p
NEFA	AA	1.34	1.36	0.93
	EPA	0.30	0.25	0.40
	DHA	0.99	0.83	0.25
PC	AA	7.54	6.79	0.22
	EPA	0.80	0.69	0.25
	DHA	2.18	2.03	0.58
PMN	AA	9.13	7.33	0.18
	EPA	0.53	0.47	0.86
	DHA	1.10	0.86	0.13

3.4.7.1.2 Gender response to Omegaven™ infusion

There was no significant gender difference in the cohort treated with Omegaven™ in fatty acid levels from baseline to day 3 in all lipid fractions.

Table 33: FA uptake per gender

		Female		Male		
		Day 0	Day 3	Day 0	Day 3	p
NEFA	AA	9.42	9.32	7.7	8.00	0.68
	EPA	0.55	1.16	0.47	1.40	0.35
	DHA	0.47	1.57	0.87	1.47	0.84
PC	AA	8.31	7.57	6.82	7.32	0.31
	EPA	0.71	3.03	0.85	3.23	0.35
	DHA	2.27	2.93	2.23	3.34	0.23
PMN	AA	1.78	1.54	1.23	1.32	0.84
	EPA	0.41	0.61	0.29	0.49	0.88
	DHA	1.22	1.54	0.78	1.42	0.68

3.4.7.2 Correlation of age and fatty acid levels

The median age of the entire study population was 65.5 years. The cohort was therefore divided into those aged >65.5 and those aged <65.5 years of age for the purpose of analysis.

3.4.7.2.1 Baseline fatty acids

At baseline there was no significant difference in the fatty acid levels between those aged above and below 65.5 years in any of the lipid fractions.

Table 34: Baseline FAs per age

		<65.5 years	>65.5 years	p
NEFA	AA	1.56	0.29	0.17
	EPA	0.29	0.27	0.65
	DHA	1.02	0.65	0.18
PC	AA	7.71	5.21	0.11
	EPA	0.71	0.27	0.57
	DHA	2.26	0.82	0.31
PMN	AA	8.99	7.32	0.24
	EPA	0.53	0.48	0.60
	DHA	0.95	0.99	0.77

3.4.7.2.2 Age response to Omegaven™ infusion

In the PMN lipid fraction the cohort aged >65.5 years had significantly greater responses to the Omegaven™ infusion than those aged <65.5 years. The EPA and AA levels were significantly greater the older subgroup. No other significant differences were found in any other lipid fraction.

Table 35: FA uptake and age

		<65.5 years		>65.5 years		
		Day 0	Day 3	Day 0	Day 3	p
NEFA	AA	10.21	10.12	6.33	6.00	0.93
	EPA	0.48	1.39	0.56	1.21	0.52
	DHA	1.06	1.61	0.85	1.34	0.91
PC	AA	8.44	8.56	6.86	7.11	0.91
	EPA	0.70	2.49	0.87	3.38	0.24
	DHA	2.62	3.68	2.09	2.30	0.13
PMN	AA	2.05	1.69	0.88	1.33	0.02
	EPA	0.49	0.58	0.26	0.54	0.04
	DHA	1.55	1.69	0.83	1.29	0.25

4 Discussion

4.1 Introduction

This study aimed to investigate the effects of omega-3 in critically ill septic patients. A vast amount of physiological data was collected allowing for trends in altered pathophysiology in response to sepsis to be analysed. The study demonstrated that amongst critically ill septic patients, measuring organ dysfunction daily during the ITU stay provided additional prognostic information over baseline measures (such as the APACHE score).

In addition, the mechanisms by which omega-3 FAs exert their effects were examined by means of measuring the fatty acid levels in several different lipid pools of the critically ill patient allowing for comparison to healthy individuals, survivors and non-survivors and those who received parenteral fish oil versus a control.

4.2 Trends in the pathophysiology of critically ill patients with sepsis

Changes in pathophysiological variables are frequently and clearly related to the outcomes in the critically ill patient and allow a record of patient physiology that may help explain the outcomes. When baseline and serial scores for all organ systems were considered in aggregate, dysfunction of the respiratory, renal, hepatic, haematological and cardiac systems were significantly associated with mortality.

This study confirmed that mortality is closely related to organ dysfunction/failure and that the SOFA score, in particular, is good at predicting patients unlikely to survive. This was reflected in the max-SOFA score being significantly higher in the non-

surviving cohort (p=0.028). Other studies have also demonstrated the superior discriminative power of the aggregated score (max-SOFA) over any of its individual components. Moreno and colleagues reported an area under the ROC curve of 0.847 (SE 0.012) which was significantly higher (cardiovascular score p=0.005, and all other organ systems p<0.001) than any of its individual components[81].

The degree of organ dysfunction/failure from baseline, represented by the delta-SOFA score, also showed good correlation with mortality, although it wasn't significant (p=0.082). This stresses not only the importance of physiological derangement on admission the ITU, but also the impact of subsequent cumulative organ dysfunction on patient outcomes. The study supports the notion that it is the magnitude of the organ dysfunction abnormalities that ultimately influences survival of the critically ill patient[75].

The difference in time points at which the organ systems reach maximum dysfunction during sepsis also highlights the complex pathophysiological processes involved in sepsis[81]. It also supports the concept that mortality due to multi-organ dysfunction depends on the number of failing organ systems, the severity and duration of dysfunction/failure and the specific combination of organ failure [76,77]. The overwhelming cause of death in this study was secondary to multi-organ failure (MOF). MOF is a term originally reported by Tilney and colleagues describing the postoperative course of ruptured aortic aneurysms who noted that shock could lead to postoperative failure of hitherto uninvolved organ systems[194]. Thus, the cause of death in ITU in this trial was rarely the result of evolution of the septic insult that precipitated the admission but the development of a progressive physiological dysfunction in organ systems remote from the site of the primary septic focus.

In respect to the sepsis severity scores on admission, the SOFA score was a better predictor of mortality in univariate analysis than the APACHE-II score (p=0.025 vs. 0.902). This corroborates the rationale that the number of organs which "fail" is proportional to the risk of death. The SOFA score actually goes much further than this and allows for a spectrum of altered physiology from normal, through dysfunction, to failure which is closely related to mortality.

One criticism of the use of the SOFA score calculations concerns the use of the most aberrant physiologic data. It is possible that such data includes some physiological variables with an ephemeral and iatrogenic cause. Other studies have incorporated a '10-minute time limit' on altered physiology in an attempt to avoid the extreme alterations that could be regarded as 'false alarms'[84,195,196]. Furthermore, while three organ systems are described by variables that are likely to change little over a 24-hour period in the ITU (namely creatinine concentration, platelet count and bilirubin concentration), the remaining two systems are described by volatile variables that may change significantly from one hour to the next (MAP and pO_2/FiO_2).

This study has shown that in survivors organ dysfunction/failure improves rapidly, often within 24 hours subsequent to the diagnosis of sepsis. The non-surviving cohort demonstrated a very different trend. In the haematological, hepatic and cardiovascular systems, there was little change from the baseline dysfunction until day 7 when an improvement was seen, except with the hepatic dysfunction, which worsened until day 7. Respiratory function gradually deteriorated throughout the study period in the non-survivors. This finding is corroborated by another study, which showed that persistent respiratory (and hepatic) dysfunction was not significantly associated with

mortality until week two[197]. The renal system dysfunction remained static until day 3, when an improvement was seen.

This pattern of failing organ systems is supported by other study's, which report a predictable course of the MOF syndrome[73] beginning with the pulmonary system and followed by the hepatic and renal system, with haematological and cardiac failure being the later manifestations of MOF. CNS dysfunction, although not measured in this study, can occur either late or early. Marshall and colleagues demonstrated that the development of the maximal degree of organ dysfunction occurred at different time intervals from a mean of 1.8±4.7 days for respiratory dysfunction to 4.7±5.5 days for hepatic dysfunction. They concluded that clinically important organ dysfunction develops early during the ITU stay rather than as a late event. This is consistent with this studies finding in that non-survivors had more persistent and severe organ dysfunction as opposed to the survivors who had a rapid improvement from baseline dysfunction.

This study demonstrated on multivariate logistical regression analysis that the significant predictors for mortality were hepatic and haematological failure and haematological dysfunction. This finding is not largely supported by the literature. Other studies have demonstrated that the impact on outcome of organ dysfunction/failure was higher for cardiovascular and renal scores [81,82]. Moreno and colleagues demonstrated, using a non-stepwise logistical regression equation, that the highest relative contribution to outcome was cardiovascular (odds ratio 1.68, 95% CI 1.49-1.91), followed by renal (odds ratio 1.46, 95% CI 1.29-1.64), haematological (odds ratio 1.22, 95% CI 1.06-1.40) and the respiratory system (odds ratio 1.18, 95%

CI 1.01-1.38) [81]. Different study populations may explain the difference in this finding, since the hospital where this study took place was a specialist center for renal patients who not only have renal system failure at baseline, but also the heamatological manifestations of renal disease. The classical sequential pattern of organ failure and the systems exerting the highest predictor of mortality may, however, be modified by the presence of pre-existing disease and/or by the nature of the precipitating sepsis. In patients with intrinsic renal disease, renal failure may precede hepatic and even respiratory failure in patients. Likewise, cardiac failure may be an early feature in patients with pre-existing myocardial damage[73]. This is an important biological principle, stressing the heterogeneous nature of critically ill septic patients, that although the SIRS responses to infection are similar among patients developing MOF, the exact pattern of organ failure is influenced by the patient's physiological reserve and comorbidity.

4.3 Clinical outcomes dependant on fish oil

This single centre, randomised, controlled trial investigated the effects on inflammatory markers, organ dysfunction and other clinical parameters of parenteral omega-3 in critically ill adult patients with sepsis. The control group received no added intervention above standard care directed by the intensivists. This is the largest study investigating the effects of this lipid emulsion (Omegaven^{TM}) as monotherapy for attenuating the effects of excess inflammation.

Omegaven^{TM} has been used in other disease processes, notably in post-surgical patients, where improved clinical outcomes [171,186], immune function [171] and reduced inflammatory mediators [128,171] were found. In a critically ill heterogeneous group of patients Heller *et al* reported a dose-dependant reduction in mortality [177]. Five studies

have investigated Omegaven™ in septic patients but with mixed results [123,136,185,198,199]. Two of these studies found no difference in clinical or biochemical outcomes [185,198].

This study was able to demonstrate a significant reduction in the development of morbidity with respect to organ dysfunction (delta-SOFA, 2.2±2.2 vs. 1.0±1.5, p=0.005 and maximum-SOFA, 10.1±4.2 vs. 8.1±3.2, p=0.041). The study by Moreno *et al*, which compared mortality in critically ill ITU patients, reported that the reduction of delta-SOFA from 2 to 1 correlated with a reduction of mortality of 15.2 to 8.5% [81]. Another study by Ferreira *et al*, in a different population of critically ill patients, reported that a reduction in maximum-SOFA from 10 to 8 represented a reduction in mortality from 46% to 27% [58]. It should however be borne in mind, however, that it is not the intention of the SOFA score to relate to mortality but to describe a sequence of complications in the critically ill [78].

This study was also able to demonstrate a significant reduction in mean CRP. Whilst there was a trend towards more organ dysfunction free days and fewer developments of new cardiac arrhythmias, this was non-significant. The study was unable to demonstrate any significant difference in mortality or length of ITU or acute hospital stay. For reasons already discussed, much larger numbers would be required to demonstrate a difference in mortality. For patients in the strata of less severe sepsis (indicated by an APACHE-II score predicting a mortality of ≤40%), treatment with fish oil was associated with a significant reduction in mortality (p=0.041) on univariate analysis. It did not remain significant on multivariate analysis however.

The study was powered to detect a 50% reduction in new organ dysfunction. Whilst the power calculation initially suggested that 140 patients would need to be recruited to the study, significant results were found with much smaller numbers. This could be due to population and methodology differences between this study and the study published by Pontes and colleagues [139], which was used in the power calculation. The use of the SOFA score for a power calculation (because it is an ordinal variable) is, in addition, difficult to use for sample size calculations.

4.3.1 Safety of parenteral fish oil

The study has demonstrated that parenteral Omegaven™ can be given safely and early to critically ill septic patients. This finding has been supported by the literature [103,181]. No side effects were experienced apart from one report of a 'fish-like' taste in a patient's mouth. In one patient the infusion was stopped prematurely due to the development of a coagulopathy. Whilst this was not thought to be attributable to the fish oil it was ceased, as per manufacturer's guidance, as it has been suggested that it may increase bleeding time [200]. The relationship between increased bleeding and high concentrations of n -3 is yet to be proven however, with several studies reporting no evidence of this phenomenon [201,202].

The manufacturer of Omegaven™ (Fresenius Kabi) does not recommend fish oil based emulsions as a nutrition monotherapy due to theoretical concerns that fish oils may cause oxidative stress. The Omegaven™ is, however, enriched with the antioxidant α-tocopherol to counteract any oxidative risk. The other risk of using fish oil as monotherapy is the development of essential fatty acid deficiency, which

typically occurs when <1%–2% of total calories are provided from essential fatty acids. Studies have not, however, supported this notion [203]. All patients in our study received nutrition (enteral or parenteral) as directed by the dieticians and intensivists, depending on their condition and gut function. Omegaven™ was given as monotherapy for attenuating the effects of inflammation rather than providing nutrition. Its calorific content is, in fact, negligible (112 kcal/100ml).

4.3.2 Comparison of clinical results to the literature

4.3.2.1 Recommended dose of fish oil

There are no definitive recommendations in the literature pertaining to daily dose of n -3 required to ameliorate the pro-inflammatory effects of omega-6 FAs in critically ill septic patients [204]. The varying daily dose of n-3 supplementation reported in the literature may also be significant in explaining the different results. In patients suffering from hyperlipidaemia and those with chronic renal failure, a daily dose of at least 0.5-1g/day [205] and 1.5-2.4g/day [206,207] respectively has been recommended. Differences also exist in the literature as to the nomenclature used to describe dosing with the terms 'fish oil', 'n -3' and 'DHA and EPA' being used interchangeably creating difficulties in interpreting results. In addition, most studies use different formulations, administrative routes, duration and rates of fish oil emulsions. The quantities of the active n-3 FAs DHA and EPA differ as a consequence and these are major confounding factors explaining the considerable inter-study variation in findings.

Table 36: Current studies investigating the effects of fish oil in sepsis

Author	Year	n	Study subjects	Initial severity	Intervention						Outcome
					Name	Weight adjusted dosing (y/n)	Route	Dose (DHA and EPA)*	Duration (days)	Daily regimen	
Pontes-Arruda *et al*	2011	106	Sepsis	APACHE II median 19.5, SOFA median 5.5	Oxepa™	Y	Enteral	Median 0.11 g/kg/d* (6.65g FO/d)	≤7	Continuous feed	Significantly reduce new organ failures
Khor *et al*	2011	28	Sepsis	APACHE II median 16.3-19.3	Omegaven™	N	IV	Mean 0.05-0.11g/kg/d* (0.18±0.04g FO/kg/d)	5	Infused over 6 hours every day	Significantly reduce procalcitonin, APACHE II on day 3,5,7.
Grau-Carmona *et al*	2011	132	Sepsis and ALI or ARDS	APACHE II median 19, SOFA median 9	Oxepa™	Y	Enteral	0.09 g/kg/d* (6.65g fish oil/d)	As indicated. Median 11	Continuous feed	Non significant trend towards reduced SOI
Wohlmuth *et al*	2010	71	Abdominal sepsis	SAPS II median 37-40	Omegaven™	N	IV	0.12 g/kg/d	≤7	Infused over 30-60 minutes as bolus	No differences
Sungurtekin *et al*	2011	40	SIRS and sepsis	APACHE II median 19.5–20.5	Omegaven™	Y	IV	0.16-0.35 g/kg/d* (0.6g fish oil/ kg/d)	7	Continuous feed	Significant reduction day 7 TNF-α, IL-1 a IL-6. Increase in IL-
Friesecke *et al*	2008	160	SIRS and sepsis	SAPS II mean 49-54	Omegaven™	Y	IV	0.1 g/kg/d	7	Continuous feed	No difference in inflammatory or clin outcomes
Barbosa *et al*	2010	23	SIRS and sepsis	SOFA mean 8.9-9.5	Lipoplus™	Y	IV	0.09 +/-0.02 g/kg/d	5	Continuous infusion	Significant reduction IL-6, IL-10 and improved respiratory function
Pontes-Arruda *et al*	2006	103	Severe sepsis or shock	SOFA mean 8.6-8.8	Oxepa™	Y	Enteral	0.11 g/kg/d* (6.65g fish oil/d)	As indicated	Continuous feed	Improved oxygenati reduced ventilator fr days and new organ dysfunctions
Mayer *et al*	2003	21	Sepsis	APACHE II 15.2- 19.6	Omegaven™	N	IV	0.13-0.3 g/kg/d* (9.4-20.7 g fish oil/d)	5	3x 6 hour infusions per day	Enhanced proinflammatory cytokine profile in control group

* Based on a calculation (where indicated) presuming an average weight of 70kg unless weight stated in article

4.3.2.2 Fish oil's effects on organ dysfunction in sepsis

The study demonstrated a significant reduction in the development of morbidity in respect to organ dysfunction (delta-SOFA, 2.2±2.2 vs. 1.0±1.5, p=0.005). Sepsis represents an important financial burden on the healthcare system, and any reduction in terms of reduced organ dysfunction and subsequent support [208] must be considered to have a potential economic impact regarding reductions in the overall cost of care.

Nine studies have investigated the effects of fish oil on critically ill septic adult patients (Table 36) [110,123,139,183,185,187,188,198,199]. The studies are heterogeneous in methodology and fish oil dosing and regime and demonstrate conflicting outcomes on organ dysfunction. Three studies did not give a weight adjusted dose leading to over or more frequently under-dosing of therapy [110,123,198]. Three studies, in addition to EPA, DHA and GLA, used a treatment comprising elevated levels of antioxidant vitamins (Oxepa™; Abbott Nutrition, Ohio USA) as an enteral feed meaning the effects of fish oil alone could not be determined [139,183,188].

Other studies have investigated the effects of fish oil enriched enteral feed in combination with addition nutrients believed to modulate immune function. Most notably arginine-containing formulas have been investigated together with fish oil [182]. These results need to be interpreted with caution, however, as a recent meta-analysis has suggested that arginine (whilst beneficial to elective surgical patients) may be

detrimental to the critically ill patient with a trend towards increased mortality [99]. Therefore, the failure of fish oil emulsions containing arginine may not be due to the lack of efficacy of fish oil but the negative effects of arginine.

The large phase III RCT (OMEGA) study was conducted to investigate a twice daily enteral supplement containing EPA, DHA, GLA, and antioxidants in patients with acute lung injury (ALI) [209]. The trial demonstrated a lack of efficacy and was stopped prematurely after recruitment of 272 of the planned 1,000 patients. The study showed no improvement in the outcomes of death at 60 days, ventilator-free days at day 28, or ICU-free days at day 28.

Dose of daily EPA/DHA in the literature varied between 0.05-0.3 g/kg/day, provided by either enteral or parenteral routes (**Error! Reference source not found.**). In this study, Omegaven™ provided parenteral DHA/EPA at 0.054-0.12 g/kg/day (equating to 0.2 g/kg/day of fish oil). A study by Heller and colleagues demonstrated that doses of 0.1-0.2 g/kg/day were needed to significantly improve rates of survival, >0.05g/kg/day were associated with reduced lengths of ITU and hospital stay and 0.15-0.2 g/kg/day was associated with a reduced demand for antibiotics [177].

The study by Wohlmuth and colleagues retrospectively reviewed 42 patients with abdominal sepsis treated with a single bolus of 10g intravenous fish oil, compared to historical controls [198]. The study failed to demonstrate any clinical effect of the fish oil on outcomes. It has, however, been criticised for significant pharmacological and statistical errors [210]. Specifically, there were concerns of fish oil under dosing (0.12g/kg/d), too fast an infusion rate causing fat overload, selection bias, inadequate

sample size, unclear timing, and questionable propensity adjustment. Studies have shown that lipid infusion at a rate of 0.21 or 0.22 g/kg/h actually aggravated lung injury with respect to oxygenation index, shunt fraction, pulmonary vascular resistance and compliance [211,212]. Omegaven™, in this study, was given at 0.5ml/kg/hour and did not cause these potential problems.

4.3.2.3 Effects of fish oil on inflammatory markers

The study demonstrated a significant reduction in mean CRP. A systematic review of RCTs investigating the effects on inflammatory markers associated with omega-3 supplementation supported the finding of inflammatory marker suppression [134]. It identified three studies specifically investigating the effects in septic patients. Omega-3 dose and duration of therapy ranged from 6·4- 23·6 g/day and 5-10 days respectively across the studies. The three studies showed a reduction in IL-6 [187], suppression of pro-inflammatory mediators by mononuclear leukocytes [136] and improved neutrophil function [110]. One further study investigating MCT/LCT emulsion versus parenteral Omegaven™ (given at 0.6g/kg) in septic patients showed no significant difference in CRP or white cell count [199]. A similar finding of no change in inflammatory markers was found in another randomised trial of septic ITU patients [185]. A study investigating the effects of parenteral fish oil emulsion in patients with acute pancreatitis also showed a significant reduction in CRP and white blood cell levels after 5 days of treatment [176].

4.3.2.4 Effects of fish oil on new cardiac arrhythmias

The study demonstrated a trend for patients receiving parenteral fish oil to develop fewer cardiac arrhythmias (20% vs. 3.3%, p=0.103). This finding has been supported

by other studies investigating the anti-arrhythmic properties of omega-3 [213-216]. A systematic review and meta-analysis, combining five studies and 10,097 patients, however failed to support this hypothesis [217]. There was evidence of moderate heterogeneity across the included trials (I^2=53.5%; P=0.07). The mechanisms to explain these anti-arrhythmic effects include the modulation of ion channels and the autonomic nervous system [218,219].

4.4 Fatty acid analysis

The fatty acids in different locations (lipid pools or fractions) are considered to represent different roles. The PC and NEFA fractions, therefore, represent the transport roles whilst PMNs represent the functional role with particular relevance in critical illness and sepsis. Plasma NEFA concentration represents the net contributions of the release of fatty acids from adipose tissue by the action of hormone-sensitive lipase, incomplete entrapment of fatty acids released from lipoproteins by lipoprotein lipase activity and uptake into tissues[220]. In addition there is the storage pool, which was not measured in this study, represented by adipose tissue triglycerides.

4.4.1 Baseline fatty acid levels

The baseline levels of all fatty acids in the plasma free fatty acid (PC) lipid fraction are higher in this study of septic patients when compared to healthy controls[110]. The percentages of EPA and DHA in the study's septic cohort were reduced when compared to healthy controls[221]. This may reflect the state of critical illness and active inflammation in the study population.

Table 36: FA profile in sepsis vs. healthy adults (%)

Fatty Acid (%)	NEFA in this study	NEFA in healthy adults[221]	PC in this study	PC in healthy adults
20:4n-6 (AA)	1.348	-	7.127	-
20:5n-3 (EPA)	0.276	0.4±0.2	0.744	1.1±0.5
22:6n-3 (DHA)	0.903	1.6±0.8	2.101	3.2±1.2

Table 37: FA concentration profile in the PC fraction in sepsis and in health

Fatty Acid (micogram/ml)	PC in this study	PC in healthy adults[110]
20:4n-6 (AA)	54.51±6.78	10.9±1.2
20:5n-3 (EPA)	5.40±1.22	1.3±0.3
22:6n-3 (DHA)	14.98±2.34	9.3±1.8

As reported in other studies, the levels of plasma free fatty acids in septic patients were markedly raised when compared to the healthy population[222,223]. This was the case at baseline before any lipid emulsion infusions had commenced. This may reflect the magnitude of the metabolic response to severe sepsis resulting from shock in the study patients.

There are several factors that may contribute to this metabolic response:

i. Plasma free fatty acids, in a general metabolic response to systemic stress, are raised[108].

ii. Sepsis increases lipolysis from adipocytes and hepatic *de novo* lipogenesis[224].

iii. Elevated levels of secretory phospholipase A_2.[225]

iv. Vasopressors activate lipoprotein lipase and the hormone sensitive lipoprotein lipase of adipose tissue to preferentially increase free plasma fatty acids[226].

v. Heparin (given to all our study patients without contraindication) also activates lipoprotein lipase[107].

The most common fatty acids, as a percentage of total fatty acid, were 16:00, 18:1,n-9 and 18:00 and this is supported by a previous study[221]. The fatty acid profile of patients with critical illness differs from that of healthy adults. This was also a finding in the study by Barros and colleagues who found lower levels of myristic acid (14:00), di-homo-gamma-linolenic acid (20:3n-6) and EPA in the critically ill cohort[227]. This study also found a reduced di-homo-gamma-linolenic acid but baseline myristic acid and EPA were similar to that of healthy adults.

Table 38: Fatty acid profile of the PC fraction in sepsis and in health

Fatty Acid (%)	PC in this study	PC in healthy adults[227]
14:00	0.348	0.3±0.02
16:00	32.869	30.49±0.51
16:1n-7	1.156	-
18:00	12.616	15.4±0.52
18:1n-9	17.072	8.31±0.36
18:1n-7	2.330	0.5±0.04
18:2n-6	19.637	22.63±0.9
18:3n-6	0.098	4.08±0.3
18:3n-3	0.312	0.19±0.01
20:00	0.168	-
20:1n-9	0.197	-
20:2n-6	0.274	-
20:3n-6	1.984	4.08±0.3
20:4n-6 (AA)	7.127	11.43±0.6
22:00	0.078	-
20:4n-3	0.220	-
20:5n-3 (EPA)	0.744	0.69±0.08
22:4n-6	0.026	-
22:5n-3	0.643	-
22:6n-3 (DHA)	2.101	2.77±0.17

4.4.2 Pharmacodynamics analysis

After commencing the fish oil infusion, a rapid increase in EPA and DHA was noted. This corresponded to a reduction in the AA/(EPA+DHA) ratio by nearly one third within 8 hours of the fish oil infusion. The levels of EPA increased more than DHA and were most pronounced in the NEFA lipid fraction; where the concentration was treble that at baseline. This is in line with other study findings[228] and adds further evidence that the body handles EPA and DHA differently[187,229]. The levels of DHA in the PC lipid fraction were almost unchanged from baseline, a finding that is supported by another study[229].

The rapid appearance of EPA and DHA with parenteral FO infusion is an advantage over the slower appearance of these fatty acids when FO is given orally[230]. The high turnover of the lipid pool in critically ill patients may also explain the rapid appearance of these FAs. The parenteral administration of FO provides the fatty acids directly into the bloodstream, introducing them to lipid fractions like PC and directly exposing circulating cells, such as PMNs, very quickly.

There were also differences in the rate of EPA and DHA incorporation between the lipid pools. The EPA and DHA were slower to be incorporated into PMNs than the PC and NEFA lipid pools; 16 hours versus 8 hours for maximum incorporation respectively. This finding suggest the slower turnover of these fatty acids in cells and is described by other authors too[229].

The rates of incorporation of EPA and DHA into the lipid pools are significantly faster than compared to enteral fish oil preparations in all three fractions. In a study by Browning and colleagues[230], EPA reaches maximum levels after 18, 38 and 249 days in PC, NEFA and mononuclear cells (MNC) respectively after 3.27g/day oral EPA+DHA. This was associated with a maximum % of EPA of 3.5, 1.1 and 2.3 in PC, NEFA and MNC respectively. In this study after a single dose of fish oil the maximum % of EPA was 3.0, 0.7 and 2.0 in the PC, NEFA and PMN fractions respectively but in just a few hours. The maximum % of DHA was 5.9, 3.1 and 3.3 in PC, NEFA and MNC respectively at these same time points. In this study the maximum % of DHA was 2.4, 1.6 and 1.8 PC, NEFA and PMN fractions respectively but again in just a few hours and after a single fish oil infusion.

Its immediate appearance indicates rapid hydrolysis of the EPA- and DHA-containing triglycerides in critically ill septic patients. It is thought that synthetic lipid aggregates activate endothelial lipoprotein lipases, including the translocation of this enzyme from its cellular binding site into the intravascular compartment. Due to its activation, and the escape from local cellular uptake mechanisms, free plasma fatty acids increase[106].

Interestingly there was also a rise of AA in the NEFA lipid fraction in patients treated with fish oil. This rise was not however, as impressive as that seem with EPA or DHA and was not evident in any other lipid pool. It may be that this increase is secondary due to cleaved endogenous AA-containing lipid pools due to the substitution by EPA and/or DHA. Mayer and colleagues described the same finding in the NEFA fraction in their study that also investigated the effects of Omegaven[110].

At baseline, the PC lipid fraction had the greatest percentage of DHA/EPA, followed by PMN and lastly NEFA. It was also the PC fraction that had the lowest increase in response to the fish oil infusion; the DHA concentration remained unchanged after a single fish oil infusion. The concentrations at baseline were much lower in the PMN fraction, however, in this pool the greatest maximum concentration increase was seen (by a factor of 4) after the infusion. This result may reflect the mobilisation of fatty acids during critical illness and sepsis and the importance and potential enrichment of fish oil in this functional pool.

4.4.3 Fatty acid change over the study period

This study examined a number of different lipid pools in which EPA and DHA are measured in recent omega-3 related interventional studies. The three pools examined all demonstrated the significant and rapid appearance of DHA and EPA within them. The time responses of the incorporation of these FAs were different and reflected the varying turn over of the differing lipid fractions. The rapid appearance of EPA and DHA, and the relative FA concentrations, in the PC fraction after a single infusion is in line with the findings by Barros and colleagues in their study that also investigated Omegaven™ in septic patients[227].

The study also demonstrated the different handling of DHA and EPA by the body. Despite Omegaven™ containing similar volumes of the two n-3 FAs, it was EPA that was incorporated into all three lipid fractions the most rapidly and by a greater

percentage compared to baseline. This is supported by the results of other studies[227,229,230].

The incorporation of DHA was the least in the cellular (PMN) lipid fraction. It is this lipid pool, which represents that which is most closely linked to sepsis and is therefore most relevant to outcome. 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. 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 resistance of immune cells to change in DHA content is also supported by other reports[231-233]. The fatty acid composition of immune cell phospholipids may not alter as much as other lipid fractions because these cells exert a significant level of control over their plasma membrane composition although, when the n-3 FAs are provided in the PC and NEFA lipid pools in great abundance, this situation does not prevail[234]. This adds to the growing evidence that EPA and DHA are handled differently.

There is some evidence to suggest that EPA and DHA act differently. One study has made an indirect finding that the anti-chemotactic effects of fish oil might be due to EPA rather than DHA[235], although no study has yet attempted to discriminate between the chemotactic effects of the two n-3 FAs. Other studies have suggested that EPA, but not DHA, increased the attachment of bacteria to monocytes[236] and decreased the activity of the natural killer cell[237]. Evidence also suggests that EPA in particular may have a more suppressive effect on the T cell with the rationale that its incorporation

into the membranes disrupts the membrane rafts and interferes with signalling platforms, leading to impaired activation and function of the T cells.

Not all studies suggest the superiority of EPA over DHA however. It has also been shown that DHA may have a stronger affinity for raft regions and therefore a more influential effect on lipid rafts leading to an increase in the fluidity and reduced order[238,239]. This is thought to be likely to the difference in molecular orientations of the two structurally similar molecules since DHA possesses an additional double bond enabling it to interact with cholesterol, sphingolipids and phospholipids and therefore disrupt their organisation. It seems therefore, that EPA and DHA may act very differently. Sometimes this is complementary and at other times it may be in direct opposition.

4.4.4 The effect of age on the sensitivity to fish oil

The study demonstrated that older patients (aged over 65.5 years) had a significantly increased concentration of AA and EPA after fish oil infusion, but this only occurred in the PMN lipid fraction. It is well documented that immune function changes with age[240] but there are only scanty reports examining the roles of fatty acids at different ages. Several studies have identified differences in fatty acid profiles with more aged subjects however, most of these fail to take into account differences in dietary consumption of fatty acids and it has been shown that fish fat intake may increase with age[241].

This study's finding is in support of those by Meydani and colleagues who found larger increase in plasma EPA and DHA in older women compared to young women

after oral fatty acid supplementation[242]. The reasons for this finding were not clear although it was postulated that older people might have more efficient absorption of n-3 fatty acids and/or because of hormonal differences. This was, however, a postulation based on animal data and hence may not be transferrable to human subjects[243,244]. Crowe and colleagues also reported a positive association with age and plasma EPA and DHA in both men and women[241]. Other studies have demonstrated higher levels of palmitoleic acid (16:1,n-7) in more aged subjects[245], which they attributed to differences in insulin resistance and weak positive correlations for myristic acid (16:00) and weak inverse correlations for linoleic acid (18:2,n-6) with age[246].

In a review by Sijben and Calder, it was concluded that diseased individuals, including the critically ill and the elderly with co-mobidities were more sensitive to immunomodulation by n-3 FAs due to depletion of the natural buffering capacity seen in younger more healthy individuals[247]. This was due to a higher turnover rate of immunological cells in the diseased state as well as augmented production of pro-inflammatory eicosanoid synthesis. Thus, this study supports the notion that FAs are handled differently depending on the age and/or disease state of the individual.

4.4.5 The effect of gender on fatty acid profiles

This study found no significant difference between males and females in baseline lipid profiles in all 3 fractions and also after treatment with fish oil infusion. It has been suggested that females handle fatty acids differently to males and that they have a greater capacity to convert alpha-linolenic acid (ALA) to EPA and DHA[248]. One

possible explanation for this finding could be the ability of oestrogen to up-regulate delta-6 desaturase, which is the rate limiting step in this pathway[248,249].

Another study has shown that the ability of males to convert ALA to DHA was either very low or absent and that the uptake of pre-formed DHA in the diet would be critical for adequate membrane DHA concentration maintenance[249]. Pawlosky and colleagues who reported that the synthesis of EPA to DHA was threefold higher in women than in men consolidated this finding[250,251].

4.4.6 Ethnicity based differences

Although ethnicity was not specifically evaluated in this study, there are reports that a subject's genetic background influences the levels of circulating fatty acids. The baseline levels of FAs have been associated with genetic markers in known desaturation and elongation genes[252]. Among carriers of the minor allele of a representative SNP in FADS2 (rs1535) there has been an association with the reduced conversion ability of ALA to EPA. A common variation in the n-3 FA metabolic pathway genes was discovered in populations of European ancestry that can influence circulating levels of FAs.

4.5 Concerns regarding the detrimental effects of high EPA and DHA in sepsis

The study showed that there was a trend for improved survival outcomes with a low AA/(EPA+DHA) ratio, a finding that is supported by similar studies[187,227]. The mechanisms by which n-3 FAs modulate the immune function has been described extensively above and relates to both the reduced inflammatory and pro-resolving

response. However, whilst inflammation is commonly believed to be detrimental, it is also an essential response to survival after an infectious or traumatic insult. In the context of acute inflammation, therefore, any attenuation of response to a pathogen maybe seen as an impairment of immune function and may lead to secondary infections and delayed pathogen clearance.

4.5.1 Impaired immunological function

Some animal studies have demonstrated negative outcomes associated with the alteration of the innate immune response to bacterial, viral and fungal pathogens. Others have shown that dietary EPA and DHA can both improve and impair the host resistance depending on the pathogen[253]. The animal studies have shown delayed clearance of the influenza virus, *Mycobacterium tuberculosis* and *Salmonella enteritidis* and increased bacterial load of *Listeria monocytogenes* as well as reduced wound healing[254-258]. In other studies, supplementation with DHA and EPA has been associated with suppression of T cell activation but increased B cell activation, which, depending on the B cell lineage, could promote a pro-inflammatory response[259-261]. Virella and colleagues however, demonstrated that the consumption of n-3 FAs reduced the function of both T and B cells in humans[262]. More specifically n-3 was shown to reduce lymphocyte proliferation, impair IL-2 biosynthesis, and inhibit immune cell nitrous oxide production thereby impairing the host defence[263].

Fenton and colleagues suggested that immunomodulation by high n-3 FA administration could negatively affect the acute response to pathogens leading to pathogen persistence by altering the dynamics of inflammation driven pathogen

clearance[253]. However, the proposed impairment of immunity did not relate to any clinical effect in terms of more secondary infections or a prolonged stay in the ITU in this study that would support this notion.

4.5.2 Differences in Gram positive and negative sepsis

Much of the purported benefits of a diet high in omega-3 come from studies involving Greenland Eskimos and their association with low levels of ischaemic heart disease [264]. It was also noted, that the native population had a higher incidence of tuberculosis[265]. Concern was raised whether this could be attributed as a consequence of high omega-3 intake, although the contribution of poor social conditions, such as overcrowding, was difficult to separate[266]. Nonetheless, following this finding there has been a wealth of studies reporting the differences in actions of omega-3 on different pathogens.

This study suggested that the outcomes for gram-negative sepsis were improved in patients treated with omega-3. In the cohort treated with fish oil, gram-negative sepsis was associated with below median delta-SOFA score on UVA (RR=0.111 (0.016-0.755); p=0.025). In addition, in those patients with known gram-negative sepsis, fish oil was the only independent variable to be significantly associated with below median delta-SOFA score (RR=0.064 (0.01-0.405); p=0.003). No such association, either detrimental or positive, was found for the gram-positive sepsis.

4.5.2.1 Gram-negative sepsis

Numerous studies have investigated the effects of omega-3 on outcomes in gram-negative infection. In response to *Salmonella typhi*, studies have demonstrated a

reduced pyrogenic response[267] and reduced response to the pyrogenic cytokines[268]. Animal studies have shown that rats fed diets high in omega-3 had increased survival and diminished/prevented infection-induced changes in immune cell function including proliferation and PGE_2, IL-2 and IL-10 biosynthesis in response to bacterial peritonitis by *B. fragilis and E. coli*[115,269]. Another animal study investigating the translocation of *E. coli* in the gut of rats demonstrated that omega-3 was associated with a significant reduction in the number of viable bacteria in the mesenteric lymph nodes and liver, which the authors proposed, was due to improved killing of the bacteria[270].

Not all studies however, report a benefit on host response to omega-3 in gram-negative sepsis. Peck and colleagues reported , reduced survival in burns infected with *Pseudomonas aeruginosa* but did not find the same effect with an intraperitoneal challenge[271]. Chang and colleagues, demonstrated that mice fed a diet high in omega-3 showed poor survival and diminished bacterial clearance from the spleen after an oral challenge of *Salmonella typhimurium*[272]. A similar study by a different group however, found no such effect, although in this study, *Salmonella typhimurium* was given intraperitoneal[273].

Thus in summary, the literature suggests that omega-3 can have both positive and detrimental effects on the host response to gram-negative infection.

4.5.2.2 Gram-positive sepsis

A study by Barton and colleagues reported, using a murine model, improved survival after a challenge of *Staphlococcus aureus* given intra-abdominally. The authors noted

that, in vitro, LPS-stimulated PGE_2 production by isolated liver Kupffer cells was significantly lower in the omage-3 fed animals. Similarly, improved survival rates and reduced levels of PGE_2 in the lung homogenates were described in another study looking at rat pups, inoculated with group B streptococci.

However, more recent studies investigating the outcomes of omega-3 in *Listeria monocytogenes* have reported adverse effects. Fritsche and colleagues reported delayed bacterial clearance, lower levels of serum IL-2 and IFN and decreased survival[274,275]. The impairment of these cytokines may critically impair the host defence against the intracellular *Listeria monocytogenes* and reduce survival.

There are conflicting reports regarding the host response to gram-positive sepsis and omega-3. It appears to improve the host response to exotoxin-secreting bacteria, which has similar characteristics, in terms of tissue damage secondary to host-derived mediators, to gram-negative bacteria[276]. The ability of omega-3 to alter the outcomes of sepsis may more be related to the appropriate a balance of the necessary versus the inappropriately excessive production of pro-inflammatory mediators.

4.6 Current Study Strengths

4.6.1 Treatment delivery

The strength of the current study's methodology comes from the timing of the fish oil infusion from the onset of sepsis (within 12 hours of diagnosis). The timing is of particular importance since the immunological effects after a single infusion fade

within 24 hours [277-279]. In addition, Omegaven™ was used at a weight adjusted daily dose shown by previous studies to demonstrate a clinical effect [177]. The study used fish oil as monotherapy and not in combination with any confounding additives such as n -6 FAs or antioxidant vitamins, such as selenium.

4.6.2 Single-center trial

Using a single-center to recruit patients has minimised the heterogeneity of the test population for reasons discussed previously. Whilst single-center trials have certain advantages with regards to eliminating bias, the recruitment of large numbers within a reasonable amount of time becomes difficult and may introduce 'investigator fatigue'. Practices for the withdrawal of life support, which varies between centers, is also minimised by a single-center study and therefore reduces bias.

4.7 Study Limitations

Several limitations of the study should be mentioned. Firstly, the sample size was relatively small for a heterogeneous group of patients. This was due to the previously mentioned critical care reconfiguration at the study hospital. This study is, however, currently the largest prospective randomised control trial, investigating fish oil as monotherapy, reported. Despite the small size, a significant reduction in morbidity and the inflammatory marker CRP was discovered. The small sample size may also lead to type II statistical errors.

Another limitation concerns the fish oil dose. No current data exists as to the optimum dose of omega-3 needed to observe a clinical effect due the heterogeneity of studies

reported. In particular the precise daily dose of n -3 was uncertain as Omegaven™ contains a range of concentrations of EPA and DHA (EPA, 12.5–28.2 g/L; DHA, 14.4–30.9 g/L). It is likely however, given the high relative concentrations, the doses given represent saturation and the fatty acid level analysis suggests consistent increases in DHA and EPA in the cohort treated with parenteral fish oil.

The duration of the fish oil infusion may have been too short for an effect on the prolonged course of critical illness (23.3% of our total patients stayed in the ICU in excess of 14 days). The manufacturers recommend Omegaven™ is not given for more than 4 weeks duration. Despite this, incorporation of n -3-PUFAs into leukocyte membranes is detectable within 2 days of fish oil infusion [136,280] and is likely to be present in reasonable concentrations for some time after the cessation of the final fish oil infusion.

A further weakness of the study was that it was not blinded. Although all medical therapy was instigated and managed by the intensivists, there remains a chance of introducing performance bias. This occurs when patients in one group experience care or exposures not experienced by patients in the other group(s) and the differences in care affect the study outcome. It also introduces the risk of assessment bias. However, the outcome parameters used in this study are objective. Blinding in this particular study was far from straightforward with the greatest degree of difficulty encountered in deciding upon the most appropriate control formula to use. Visually similar and currently available 'white emulsions' that could act as a control, include omega-6 FA lipid emulsions and propofol (clearly an unsuitable control). The criticism of using formulas rich in LA stems from the theory that in critical illness the provision of LA,

which is metabolised to AA, further stimulates the production of pro-inflammatory mediators. Such theory appears unsubstantiated [103] and has caused conflicting opinion by those claiming that a diet rich in LA does not exacerbate a pre-existing inflammatory condition [181].

The reasons are that the enzymatic steps in LA metabolism, namely by Δ-6 and Δ-5 desaturase enzymes (Figure 1), is rate limiting and therefore an excess of AA does not occur [281]. The rate is further limited by catabolic hormone production as part of the inflammatory process, thus limiting the ability to produce AA. A study by Pontes-Arruda and colleagues, that incorporated an isocaloric control formula enriched with LA, did not find any deterioration in the inflammatory condition or clinical outcomes [139].

Finally, the blood sampling undertaken for fatty acid analysis was not always taken at a consistent time with regard to the absorptive state of the patient. In the post absorptive state, in patients fed enterally, dietary fatty acids 'escape' into the plasma via the action of lipoprotein lipase on dietary (chylomicron) triacylglycerol (TAG) and this may artificially raise the fatty acids measured.

4.8 Fish oil and sepsis – What next?

This study has found that parenteral FO can be given safely to critically ill patients with sepsis. The n-3 FAs are rapidly incorporated into the circulating lipid pools after a single infusion when given as a once daily, weight dependant dose. There is no 'trough effect' encountered as a result of a daily 'bolus' infusion as apposed to a slower infusion over the full 24 hours. The FO was not given to patients after 14 days

however, and the ‘washout’ period remains unknown. A future study should deliver the FO preparation over the entire ITU stay.

The study has demonstrated that the early (within 12 hours) administration of FO following the diagnosis of sepsis may improve outcomes. The study was not powered to detect a significant difference in mortality. The merits of using morbidity as a primary outcome has already been discussed, although mortality is frequently a preferred and more easily accepted outcome. Future studies should be powered to detect differences in mortality together with adequate subgroup analysis. This would likely require a multi-centre trial. The results of this study certainly suggest that a multi-centre trial is warranted.

The current trial was not blinded since there was no suitable placebo available. A future study should include an inert placebo and/or opaque containers and giving sets. The analysis of critically ill patients physiological variables corroborates evidence that the first 24 hours is crucial for survival. What is still unanswered is whether patients would benefit more from an infusion prior to the onset of critical illness and ITU admission. Further studies may investigate the role of providing early parenteral FO to ward based patients who develop adverse signs of sepsis, perhaps incorporating the early warning score (EWS), to investigate if that prevents disease progression, organ dysfunction and requirement for ITU admission. There would however, be logistical issues relating to costs and central line access in those patients without severely altered physiology.

This study suggested that the outcomes for gram-negative sepsis were superior to gram-positive sepsis for patients treated with omega-3. In the cohort treated with FO, gram-negative sepsis was associated with below median delta-SOFA score on UVA (RR=0.111 (0.016-0.755); p=0.025)., In those patients with known gram-negative sepsis, fish oil was the only independent variable to be significantly associated with below median delta-SOFA score (RR=0.064 (0.01-0.405); p=0.003). Whilst this was not significant for mortality, it suggests that, in future studies it will be of great importance to record the pathogen implicated in sepsis to determine definitely if it is gram-negative sepsis that benefits most from n-3 FAs.

Table 39: Summary of future work direction

Issue	Future direction
Fish oils effect on mortality	Adequately powered RCT Multi-centre trial
Duration and commencement of infusion	FO to be given during whole ITU stay until death/discharge Role of initiating FO infusion prior to critical illness on the ward
Demographic differences and FO	Adequately powered study to investigate the effects of age and gram- positive/negative sepsis on outcomes
Blinded trial	The need for a suitable placebo
Inclusion criteria	The clinical diagnosis of sepsis is currently the best however a biomarker may be more robust
Pharmacodynamics	Analysis of FA 'washout' time following FO cessation

4.9 Conclusion

The study has demonstrated that parenteral fish oil can be given safely and early to critically ill patients with sepsis. This is associated with a significant reduction in new organ dysfunction and inflammatory mediators (CRP). Fish oil may be most efficacious in patients with gram-negative sepsis with less severe sepsis (predicted mortality of ≤ 40% based on the APACHE II score) at presentation. The development of multiple organ dysfunctions from a systemic and uncontrolled inflammation forms the common pathway leading to mortality.

The results of this study suggest that a large multi-center trial is warranted to elucidate the true potential of parenteral fish oil in the attenuation of hyper-inflammation in the critically ill septic patient and in its potential to reduce not just morbidity but also mortality.

5 Appendix

Figure 45: Box plot comparing ITU length of stay

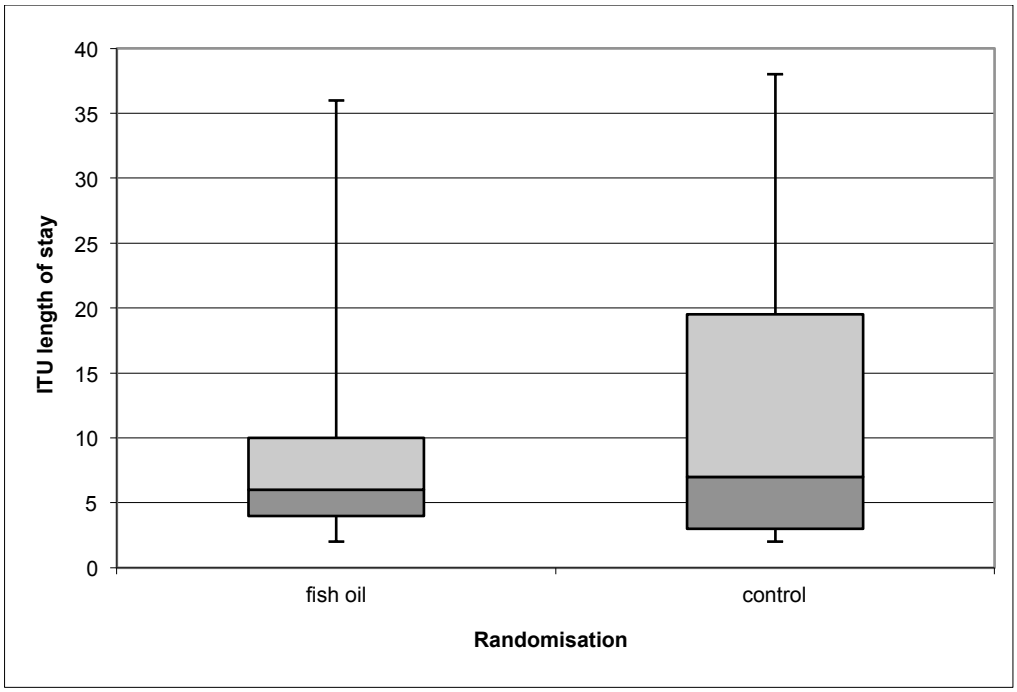


Figure 46: Box plot comparing total length of hospital stay

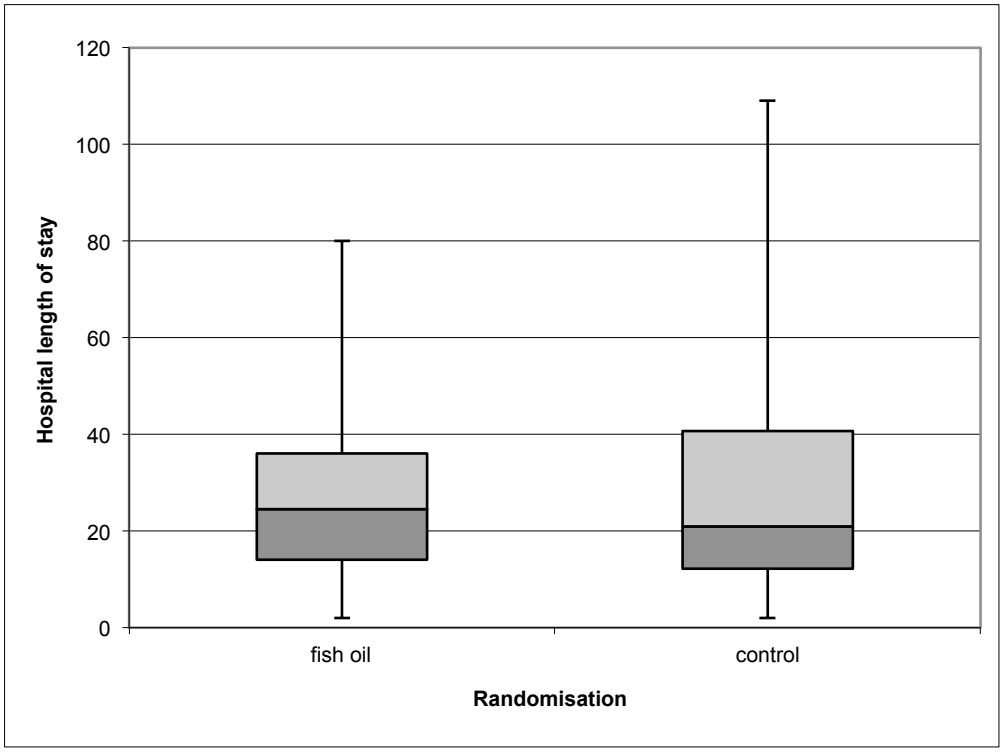
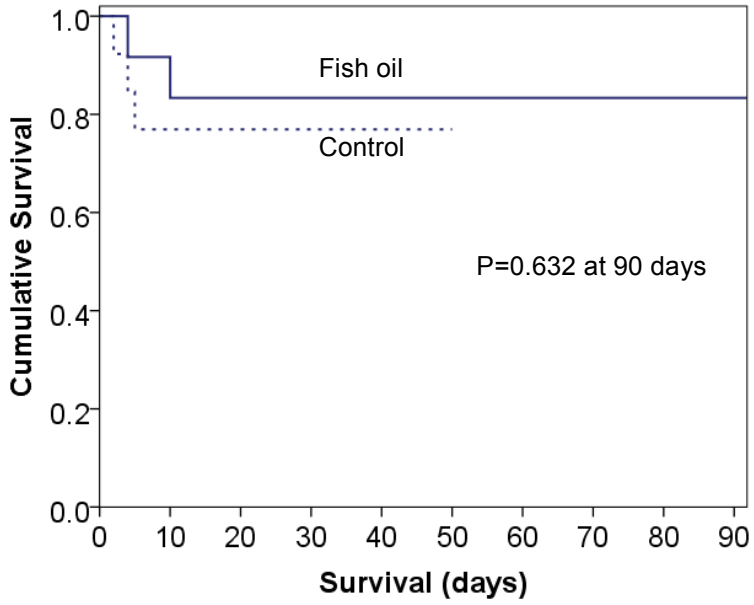


Table 40: Baseline physiology and demographics of patients in the less severe sepsis strata

Characteristic	Control Group (n=18)	Fish oil Group (n=17)	p value
Age – yr	59.9 ± 10.6	63.1 ± 13.2	0.441
Gender – female:male	8:10	7:10	0.845
Recent surgery – no. (%)	9 (50)	6 (35)	0.380
Elective	3 (16.7)	5 (29.4)	0.443
Emergency	6 (33.3)	6 (35.3)	1.00
APACHE II	13.8 ± 3.5	14.6 ± 4.5	0.336
Corresponding mortality risk (%)	19.61 ± 5.77	20.23 ± 6.98	0.654
SOFA score	7.22 ± 2.82	5.41 ± 2.06	0.038
Co-morbidities – no. (%)			
Hypertension	10 (55.6)	11 (64.7)	0.581
Ischaemic heart disease	1 (5.6)	3 (17.6)	0.338
Congestive heart failure	1 (5.6)	2 (11.8)	0.603
COPD	7 (38.9)	5 (29.4)	0.555
Chronic renal failure	2 (11.1)	3 (17.6)	0.658
Diabetes	3 (16.7)	1 (5.9)	0.603
Liver disease	0 (0)	0 (0)	1.00
Alcoholism	0 (0)	2 (11.8)	0.229
Cancer	6 (33.3)	4 (23.5)	0.711
Immunocompromised	0 (0)	3 (17.6)	0.104
Steroid use	0 (0)	5 (29.4)	0.019
Solid organ transplant	0 (0)	2 (11.8)	0.229
Intravenous drug abuse	0 (0)	1 (5.9)	0.486
Baseline biochemistry			
Albumin	23.3 ± 5.29	25.35 ± 6.65	0.326
CRP	222.18 ± 89.14	185.38 ± 108.20	0.379
Blood glucose	8.48 ± 2.58	8.53 ± 2.42	0.779
Haemodynamic variables			
Mean arterial pressure (mmHg)	63.3 ± 8.91	71.7 ± 17.26	0.044
Arterial pH	7.29 ± 0.10	7.33 ± 0.07	0.206
Serum lactate (mmol/litre)	2.79 ± 2.82	1.70 ± 1.19	0.228
Respiratory variables			
PaO_2/FiO_2	245.11 ± 10.78	187.89 ± 98.70	0.111
Ventilated – no. (%)	7 (38.9)	11 (64.7)	0.127
Focus of sepsis – no. (%)			
Chest	4 (22.2)	5 (29.4)	0.711
Abdomen	10 (55.6)	10 (58.8)	0.845
Urinary tract	4 (22.2)	1 (5.9)	0.338
Skin	0 (0)	1 (5.9)	0.486
Pathogen type cultured – no. (%)			
Gram + alone	2 (11.1)	3 (17.6)	0.658
Gram – alone	6 (33.3)	3 (17.6)	0.443
Mixed	5 (27.8)	6 (35.3)	0.632
Other	0 (0)	1 (5.9)	0.486
No pathogen	5 (27.8)	4 (23.5)	1.00

Characteristic	Control Group (n=18)	Fish oil Group (n=17)	p value
Baseline Organ Failure - no. (%)			
Cardiovascular	10 (55.6)	5 (29.4)	0.118
Respiratory	6 (33.3)	11 (64.7)	0.063
Renal	2 (11.1)	2 (11.8)	1.00
Hepatic	1 (5.6)	0 (0)	1.00
Haematological	2 (11.1)	0 (0)	0.486
Baseline Organ Dysfunction - no. (%)			
Cardiovascular	7 (38.9)	6 (35.3)	0.826
Respiratory	12 (66.7)	5 (29.4)	0.028
Renal	8 (44.4)	4 (23.5)	0.193
Hepatic	7 (38.9)	2 (11.8)	0.121
Haematological	10 (55.6)	1 (5.9)	0.002

Figure 47: Kaplan-Meier curves showing survival according to fish oil use in the more severe sepsis strata



P values were calculated with the use of log-rank test

Table 42: Baseline Demographics

							Organ Failures- Baseline				
Study ID	Age	Gender	Randomised	Source	APACHE-II	Death risk (%)	Cardiovascular	Respiratory	Renal	Hepatic	Haematological
1	57	M	C	R	27	55		1	1		
2	48	F	F	A	8	8		1			
3	62	M	F	A	19	25		1			
4	71	F	C	R	29	55	1	1			
5	80	M	F	A	28	55	1	1			
6	67	F	F	R	18	25		1			
7	52	F	F	A	14	15					
8	58	F	C	R	20	40	1	1			
9	82	M	F	A	17	25		1	1		
10	68	F	F	A	15	25	1	1			
11	62	M	C	A	19	25	1	1	1		
12	66	F	C	R	21	40		1	1		
13	56	M	F	A	16	25					
14	86	M	C	A	17	25					
15	81	F	C	U	12	15					
16	84	M	C	A	22	40	1			1	
17	46	M	C	R	28	55		1			
18	62	F	C	R	10	15	1	1			
19	63	M	F	R	22	40	1		1		
20	50	M	F	U	22	40	1				
21	72	M	C	A	22	40	1		1	1	
22	74	M	F	A	23	40	1	1			
23	49	M	C	A	13	15	1				1
24	73	M	C	R	11	15	1				
25	65	F	F	R	27	55	1	1			
26	79	F	C	U	20	40					
27	70	F	F	A	20	40	1		1		1
28	49	M	F	U	15	25			1		
29	70	M	F	A	23	40			1		
30	52	F	C	U	11	15	1	1			

M Male; F Female; C Control; F Fish oil; A Abdominal sepsis; U Urinary Sepsis; R Respiratory sepsis; S Skin sepsis

							Organ Failures- Baseline				
Study ID	Age	Gender	Randomised	Source	APACHE-II	Death risk (%)	Cardiovascular	Respiratory	Renal	Hepatic	Haematological
31	66	F	F	A	22	40	1	1			
32	39	F	C	U	15	25	1	1			
33	70	F	C	R	19	25	1				
34	68	M	F	A	5	8					
35	66	M	C	A	17	25					
36	78	M	C	A	15	25					
37	77	M	C	A	11	15					
38	86	F	C	U	22	40					
39	54	M	C	A	23	40			1		
40	43	M	F	A	5	8	1	1		1	
41	66	M	F	A	22	40		1			
42	68	F	C	A	15	25	1	1		1	
43	47	M	C	A	12	15					
44	59	M	F	R	19	25	1			1	
45	85	F	F	A	27	55		1			
46	49	M	C	A	7	8	1	1	1		
47	53	F	C	U	10	15					
48	68	F	F	A	18	25	1	1	1		
49	59	F	F	R	18	25	1	1			
50	69	M	F	A	16	25		1			
51	68	M	C	A	17	25	1				
52	50	M	F	R	26	55	1	1			
53	42	M	F	R	18	25	1				
54	63	M	F	R	14	15					
55	64	F	F	S	13	15	1	1			
56	78	F	C	A	30	75	1	1			
57	89	M	F	A	30	75	1	1			
58	67	F	F	S	33	75				1	
59	48	F	C	A	24	40	1	1	1		
60	56	F	C	R	18	25	1				1

M Male; F Female; C Control; F Fish oil; A Abdominal sepsis; U Urinary Sepsis; R Respiratory sepsis; S Skin sepsis

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Study ID	Organ Dysfunctions- Baseline					Baseline No. of failing organs	Pathogen type in cultures				
	Cardiovascular	Respiratory	Renal	Hepatic	Haematological		Gram + alone	Gram - alone	Mixed	Other	No pathogen
1	1					3				1	
2						1					1
3						1					1
4				1		3					1
5			1	1		4	1				
6	1					2				1	
7	1	1				2			1		
8			1			3			1		
9			1			3	1				
10						2			1		
11					1	4			1		
12						2					1
13		1	1		1	3		1			
14	1	1	1		1	4					1
15		1	1		1	3					1
16		1	1		1	5		1			
17				1		2		1			
18						2		1			
19		1			1	4					1
20		1	1			3	1				
21		1			1	5		1			
22			1	1		4			1		
23		1		1		4					1
24		1	1			3					1
25			1			3					1
26		1	1			2					1
27		1		1		5			1		
28	1					2		1			
29		1				2	1				
30				1	1	4		1			

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Study ID	Organ Dysfunctions- Baseline					Baseline No. of failing organs	Pathogen type in cultures				
	Cardiovascular	Respiratory	Renal	Hepatic	Haematological		Gram + alone	Gram - alone	Mixed	Other	No pathogen
31			1	1	1	5					1
32		1	1	1	1	5			1		
33	1	1	1		1	4		1			
34	1	1				2			1		
35	1	1	1			3		1			
36	1	1	1	1		4		1			
37	1	1		1	1	4					1
38	1					2		1			
39			1		1	5				1	
40						1		1			
41			1			4					1
42	1	1		1	1	4	1				
43		1			1	4			1		
44			1			2					1
45				1		4					1
46	1	1				2	1				
47					1	4			1		
48			1			3			1		
49	1					2					1
50		1		1		3			1		
51			1	1		4		1			
52	1	1				3	1				
53	1	1				4	1				
54				1		3	1				
55						2			1		
56			1	1	1	5	1				
57			1			2					1
58				1		4		1			
59		1		1		3			1		
60						3			1		

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Table 41: PC fraction FA profile during study period

	Day 0			Day 1			Day 2		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.32	0.38	0.24	0.36	0.30	0.16	0.32	0.34	0.34
16:00	33.16	32.47	0.41	32.54	33.12	0.42	32.58	32.43	0.85
16:1n-7	1.16	1.15	0.41	1.11	1.12	0.73	1.26	1.19	0.82
18:00	12.17	13.24	0.13	12.74	12.51	0.85	12.34	12.78	0.54
18:1n9	17.03	17.12	0.95	17.37	16.74	0.29	17.37	17.51	0.91
18:1n.7	2.29	2.38	0.41	2.38	2.45	0.52	2.23	2.37	0.27
18:2n-6	20.53	18.41	0.06	20.83	16.80	0.00	20.91	16.02	0.00
18:3n-6	0.10	0.10	0.64	0.09	0.10	0.19	0.11	0.09	0.11
18:3n-3	0.31	0.31	0.84	0.31	0.27	0.29	0.28	0.32	0.62
20:00	0.17	0.17	1.00	0.17	0.12	0.22	0.14	0.17	0.31
20:1n-9	0.20	0.19	0.88	0.24	0.23	0.34	0.19	0.18	0.85
20:2n-6	0.27	0.28	0.68	0.23	0.21	0.64	0.24	0.24	0.94
20:3n-6	1.76	2.29	0.04	1.72	1.90	0.48	2.00	1.84	0.54
20:4n-6	6.94	7.38	0.39	6.47	8.12	0.04	6.69	8.07	0.06
22:00	0.08	0.08	0.64	0.07	0.07	0.78	0.07	0.07	0.85
20:4n-3	0.02	0.25	0.24	0.18	0.23	0.24	0.19	0.23	0.09
20:5n-3	0.70	0.80	0.30	0.64	2.28	0.00	0.70	2.68	0.00
22:4n-6	0.03	0.03	0.79	0.02	0.02	0.83	0.02	0.02	0.31
22:5n-3	0.59	0.72	0.02	0.56	0.62	0.14	0.55	0.68	0.05
22:6n-3	2.00	2.25	0.07	1.95	2.77	0.00	1.82	2.77	0.00

	Day 3			Day 5			Day 7		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.38	0.32	0.49	0.40	0.31	0.29	0.47	0.36	0.06
16:00	32.98	33.01	0.91	32.26	32.71	1.00	31.86	33.10	0.35
16:1n-7	1.29	1.13	0.56	1.19	1.14	0.72	1.37	1.03	0.08
18:00	12.58	12.29	0.82	12.64	12.61	0.93	12.48	11.61	0.41
18:1n9	18.30	17.14	0.23	19.11	17.51	0.25	19.17	17.72	0.20
18:1n.7	2.29	2.69	0.06	2.47	2.53	0.59	2.91	2.63	0.56
18:2n-6	20.37	16.14	0.00	19.20	17.78	0.13	18.22	18.29	1.00
18:3n-6	0.12	0.10	0.36	0.13	0.09	0.13	0.16	0.06	0.01
18:3n-3	0.38	0.30	0.25	0.36	0.24	0.16	0.38	0.26	0.03
20:00	0.14	0.15	0.77	0.19	0.10	0.04	0.18	0.12	0.10
20:1n-9	0.22	0.14	0.07	0.27	0.21	0.21	0.22	0.17	0.10
20:2n-6	0.25	0.21	0.17	0.28	0.25	0.42	0.34	0.22	0.03
20:3n-6	1.76	1.70	0.73	1.84	1.70	1.00	2.12	1.62	0.13
20:4n-6	5.92	7.46	0.06	6.43	6.15	0.86	6.52	5.67	0.29
22:00	0.08	0.06	0.07	0.09	0.07	0.42	0.11	0.06	0.08
20:4n-3	0.19	0.24	0.18	0.24	0.13	0.06	0.37	0.19	0.01
20:5n-3	0.57	3.12	0.00	0.59	2.57	0.01	0.78	2.85	0.01
22:4n-6	0.02	0.02	0.86	0.03	0.02	0.33	0.03	0.02	0.35
22:5n-3	0.50	0.67	0.05	0.55	0.70	0.29	0.64	0.71	0.56
22:6n-3	1.68	3.10	0.00	1.74	3.17	0.02	1.67	3.33	0.01

	Day 10			Day 13		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	0.33	0.38	0.46	0.36	0.30	0.47
16:00	32.81	33.52	0.81	31.33	32.01	0.60
16:1n-7	1.27	1.00	0.22	1.18	1.02	0.47
18:00	11.05	12.13	0.46	11.64	12.85	0.35
18:1n9	20.06	14.87	0.01	18.49	15.21	0.03
18:1n.7	2.70	2.60	0.46	2.68	2.72	0.60
18:2n-6	20.14	17.23	0.22	21.40	18.27	0.35
18:3n-6	0.08	0.06	0.33	0.09	0.11	0.25
18:3n-3	0.24	0.28	0.62	0.29	0.28	0.75
20:00	0.09	0.31	0.33	0.10	0.14	0.60
20:1n-9	0.18	0.16	0.81	0.27	0.19	0.18
20:2n-6	0.25	0.21	0.33	0.32	0.22	0.12
20:3n-6	1.94	1.73	0.81	2.62	1.99	0.35
20:4n-6	5.98	6.37	0.81	6.22	6.83	0.92
22:00	0.06	0.06	0.81	0.09	0.06	0.35
20:4n-3	0.18	0.22	0.33	0.15	0.26	0.18
20:5n-3	0.45	3.75	0.01	0.51	2.77	0.02
22:4n-6	0.03	0.02	0.62	0.02	0.02	0.92
22:5n-3	0.62	0.95	0.01	0.65	0.80	0.08
22:6n-3	1.54	4.31	0.01	1.60	3.93	0.12

Table 42: NEFA fraction FA profile during study period

	Day 0			Day 1			Day 2		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	1.33	1.39	0.60	1.44	1.40	0.63	1.26	1.52	0.06
16:00	24.30	25.46	0.29	25.63	25.81	0.92	24.76	25.73	0.20
16:1n-7	2.85	2.71	0.56	2.53	2.69	0.87	2.68	2.90	0.55
18:00	15.20	17.15	0.22	18.95	17.68	0.30	17.47	17.68	0.91
18:1n9	37.13	34.77	0.23	32.77	34.87	0.26	34.18	33.22	0.72
18:1n.7	2.29	2.30	0.58	2.10	2.23	1.00	2.32	2.29	0.84
18:2n-6	9.97	8.10	0.09	9.20	8.57	0.44	9.67	8.61	0.23
18:3n-6	0.29	0.34	0.40	0.24	0.19	0.18	0.25	0.26	0.55
18:3n-3	1.27	1.34	0.46	1.16	1.03	0.50	1.29	1.12	0.91
20:00	0.85	1.21	0.08	0.90	0.86	0.58	0.83	0.95	0.72
20:1n-9	0.53	0.65	0.18	0.50	0.39	0.18	0.56	0.43	0.10
20:2n-6	0.33	0.35	0.95	0.41	0.24	0.01	0.44	0.31	0.07
20:3n-6	0.57	0.66	1.00	0.71	0.46	0.01	0.66	0.63	0.48
20:4n-6	1.28	1.45	0.58	1.49	1.26	0.26	1.58	1.34	0.63
22:00	0.14	0.16	0.71	0.18	0.13	0.03	0.18	0.15	0.36
20:4n-3	0.17	0.20	0.12	0.20	0.21	0.33	0.17	0.30	0.00
20:5n-3	0.23	0.34	0.06	0.24	0.40	0.02	0.23	0.57	0.00
22:4n-6	0.06	0.07	0.11	0.07	0.06	0.30	0.06	0.06	0.75
22:5n-3	0.33	0.48	0.14	0.34	0.33	0.72	0.36	0.38	0.46
22:6n-3	0.86	0.96	0.64	0.93	1.20	0.19	1.03	1.55	0.01

	Day 3			Day 5			Day 7		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	1.43	1.39	0.98	1.38	1.36	0.88	1.48	1.19	0.35
16:00	25.43	25.47	0.75	26.47	25.34	0.65	26.07	24.17	0.64
16:1n-7	2.84	2.54	0.31	2.03	2.42	0.41	2.85	2.19	0.16
18:00	17.52	19.09	0.51	21.40	17.80	0.17	17.82	17.24	0.64
18:1n9	33.21	32.47	0.84	30.09	33.45	0.33	34.50	34.34	0.91
18:1n.7	21.96	2.08	0.47	1.87	2.30	0.17	2.09	2.14	0.81
18:2n-6	9.27	8.76	0.75	8.98	9.09	0.82	8.01	10.28	0.08
18:3n-6	0.33	0.27	0.31	0.33	0.29	0.65	0.26	0.24	0.64
18:3n-3	1.47	1.20	0.13	1.35	1.26	0.94	1.37	1.24	0.56
20:00	1.06	0.78	0.19	0.93	0.98	0.82	1.06	0.74	0.35
20:1n-9	0.65	0.44	0.05	0.57	0.57	0.94	0.56	0.40	0.20
20:2n-6	0.41	0.29	0.07	0.43	0.37	0.55	0.41	0.36	0.64
20:3n-6	0.70	0.68	0.62	0.67	0.60	0.60	0.86	0.55	0.24
20:4n-6	1.57	1.46	0.98	1.46	1.33	0.82	1.11	1.54	0.29
22:00	0.19	0.20	0.93	0.24	0.19	0.10	0.19	0.19	1.00
20:4n-3	0.20	0.33	0.01	0.21	0.24	0.29	0.18	0.29	0.06
20:5n-3	0.27	0.57	0.00	0.31	0.49	0.07	0.23	0.49	0.01
22:4n-6	0.07	0.08	0.66	0.08	0.08	0.76	0.06	0.06	0.72
22:5n-3	0.32	0.41	0.08	0.33	0.37	0.36	0.26	0.44	0.03
22:6n-3	0.86	1.50	0.00	0.87	1.47	0.07	0.64	1.91	0.01

	Day 10			Day 13		
	Mean control	Mean Fish Oil	p	Mean control	Mean Fish Oil	p
14:00	1.50	1.34	0.75	1.10	1.11	0.81
16:00	24.94	24.26	0.60	22.57	24.76	0.33
16:1n-7	2.70	2.67	0.60	2.82	3.43	0.46
18:00	19.32	18.83	0.47	15.91	18.88	0.46
18:1n9	32.03	33.75	0.92	39.18	32.64	0.33
18:1n.7	1.87	2.42	0.18	2.10	2.64	0.46
18:2n-6	9.95	8.06	0.35	10.22	8.06	0.05
18:3n-6	0.32	0.15	0.35	0.18	0.30	0.09
18:3n-3	1.40	1.11	0.35	1.09	0.86	0.09
20:00	1.09	1.02	0.92	0.57	0.81	0.14
20:1n-9	0.49	0.59	0.60	0.48	0.40	0.81
20:2n-6	0.43	0.53	0.60	0.23	0.49	0.05
20:3n-6	0.70	0.83	0.92	0.42	0.74	0.09
20:4n-6	1.55	1.24	0.25	1.33	1.58	0.33
22:00	0.17	0.14	0.60	0.19	0.16	0.46
20:4n-3	0.13	0.28	0.02	0.16	0.30	0.09
20:5n-3	0.17	0.51	0.03	0.22	0.54	0.14
22:4n-6	0.05	0.05	0.92	0.06	0.08	1.00
22:5n-3	0.32	0.37	0.35	0.38	0.42	0.81
22:6n-3	0.86	1.85	0.01	0.78	1.82	0.03

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