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Manuscript Draft

Manuscript Number: YCLNU-D-17-00739R1

Title: Intravenous omega-3 fatty acids are associated with better clinical outcome and less inflammation in patients with predicted severe acute pancreatitis:A randomised double blind controlled trial

Article Type: Randomized Control Trials

Keywords: C-reactive protein; organ failure; systemic inflammatory response syndrome; omega-3; fish oil; severe acute pancreatitis

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Abstract: Background and Aims
Omega-3 fatty acids (FA) can ameliorate the hyper-inflammatory response that occurs in conditions such as severe acute pancreatitis (SAP) and this may improve clinical outcome. We tested the hypothesis that parenteral omega-3 FA from a lipid emulsion that includes fish oil could be beneficial in patients with predicted SAP by reducing C-reactive protein (CRP) concentration (primary outcome), and modulating the inflammatory response and improving clinical outcome (secondary outcomes).

Methods
In a phase II randomized double-blind single-centre controlled trial, patients with predicted SAP were randomised to receive a daily infusion of fish oil containing lipid emulsion (Lipidem® 20%, BBraun) for 7 days (n=23) or a daily infusion of a lipid emulsion without fish oil (Lipofundin® MCT 20%, BBraun) (n=22).

Results
On admission, both groups had comparable pancreatitis predicted severity and APACHE II scores. Administration of fish oil resulted in lower total blood leukocyte number (P=0.04), CRP (P=0.013), interleukin-8 (P=0.05) and intercellular adhesion molecule 1 (P=0.01) concentrations, multiple organ dysfunction score, sequential organ failure assessment score (P=0.004), early warning score (P=0.01), and systemic inflammatory response syndrome (P=0.03) compared to the control group. The fish oil group had fewer new organ failures (P=0.07), lower critical care admission rate (P=0.06)., shorter critical care stay (P=0.03) and shorter total hospital stay (P=0.04).

Conclusions
It is concluded that intravenous administration of a fish oil containing lipid emulsion, a source of omega-3 FA, improves clinical outcomes in patients with predicted SAP, benefits that may be linked to reduced inflammation

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5 & 6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	7 & 8
Participants	4a	Eligibility criteria for participants	8
	4b	Settings and locations where the data were collected	9 & 10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	7 & 8
Sample size	7a	How sample size was determined	10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	8 (no interim analysis)
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	9
	11b	If relevant, description of the similarity of interventions	8 & 9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10 & 11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10 & 11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11
	13b	For each group, losses and exclusions after randomisation, together with reasons	11
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12-14
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	12-14
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12-14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	19
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	20
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	20
Other information			
Registration	23	Registration number and name of trial registry	8
Protocol	24	Where the full trial protocol can be accessed, if available	Yes
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	20

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Figure 1 (Trial consort)

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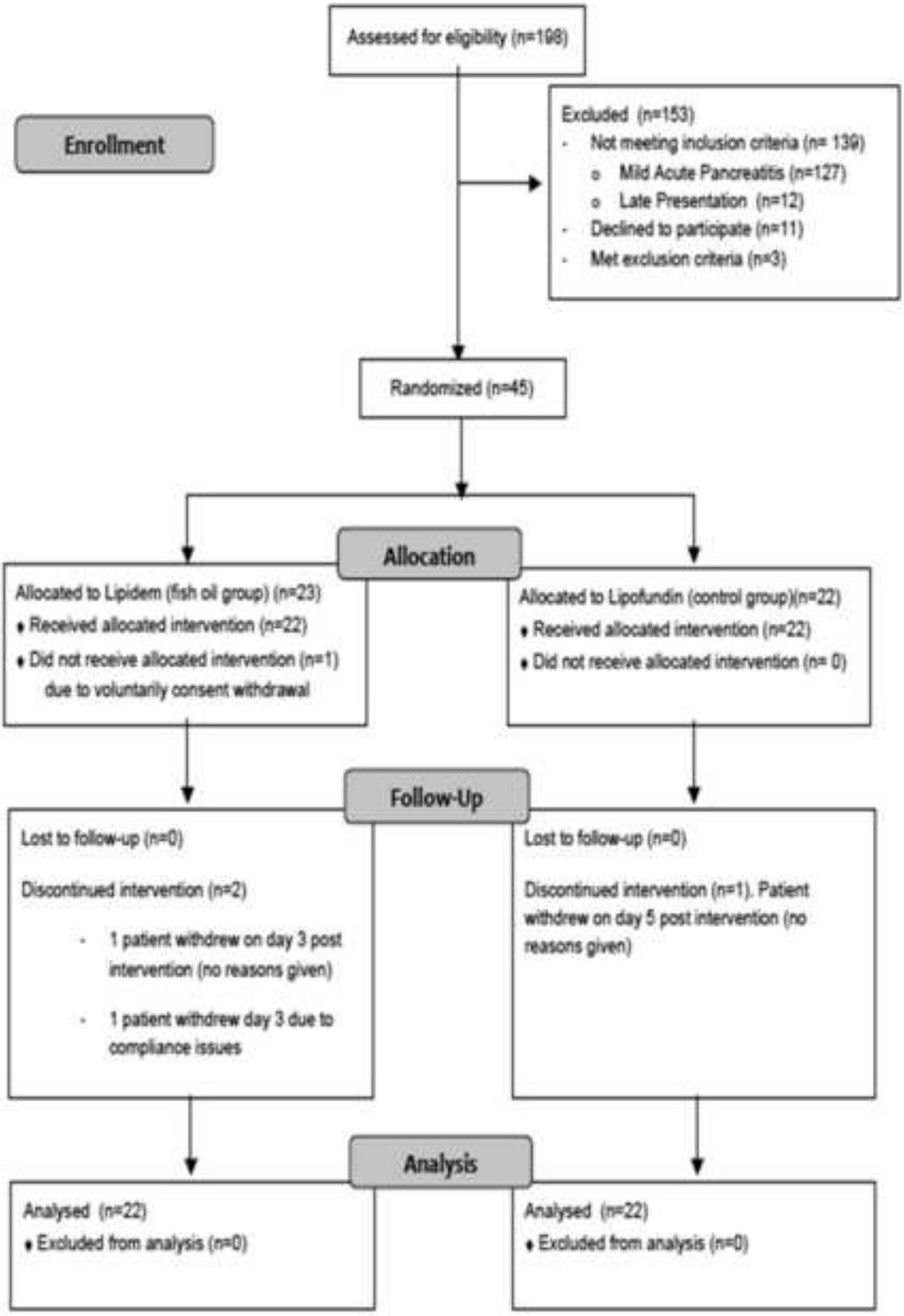

Figure 2

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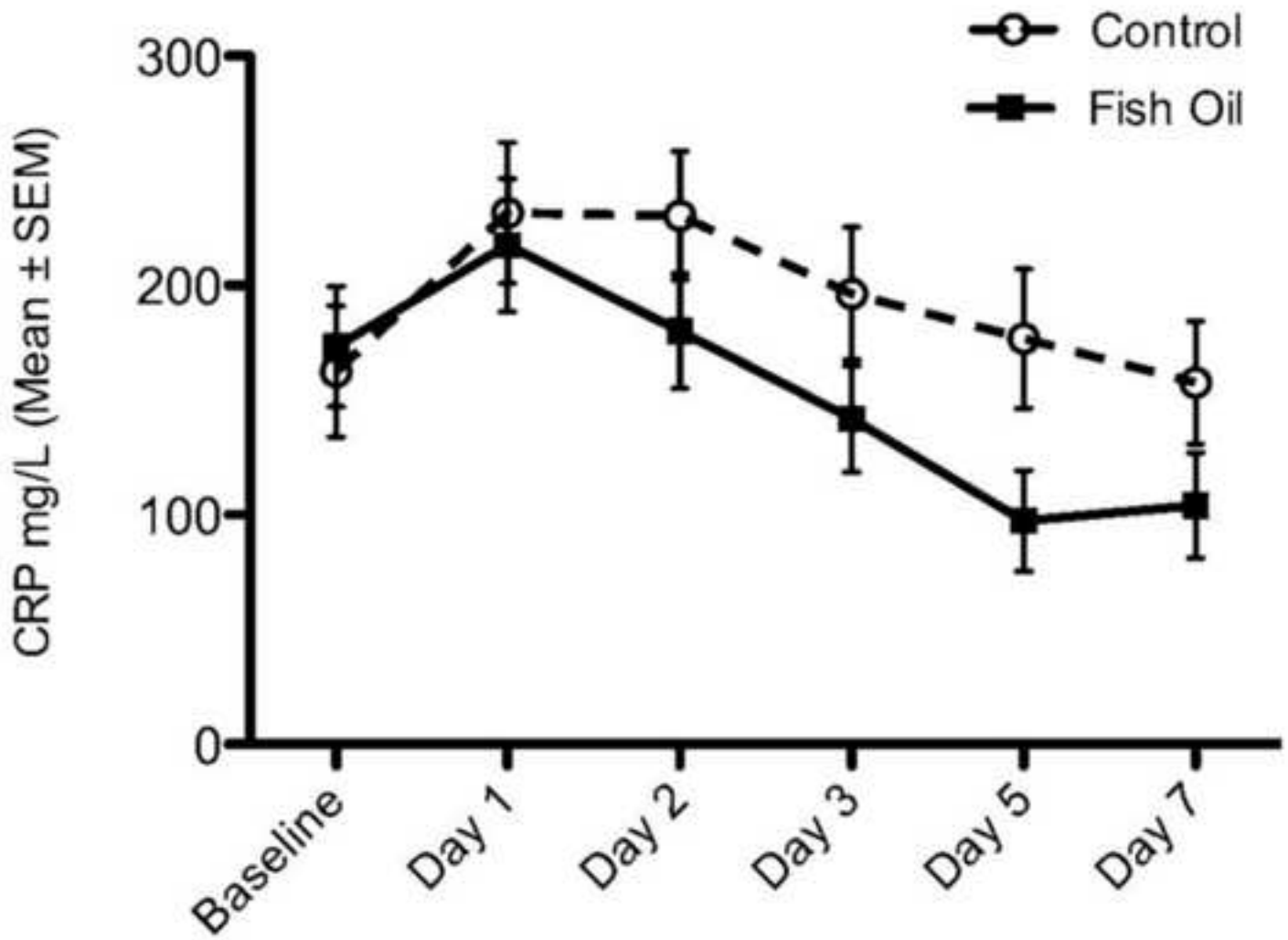

Figure 3

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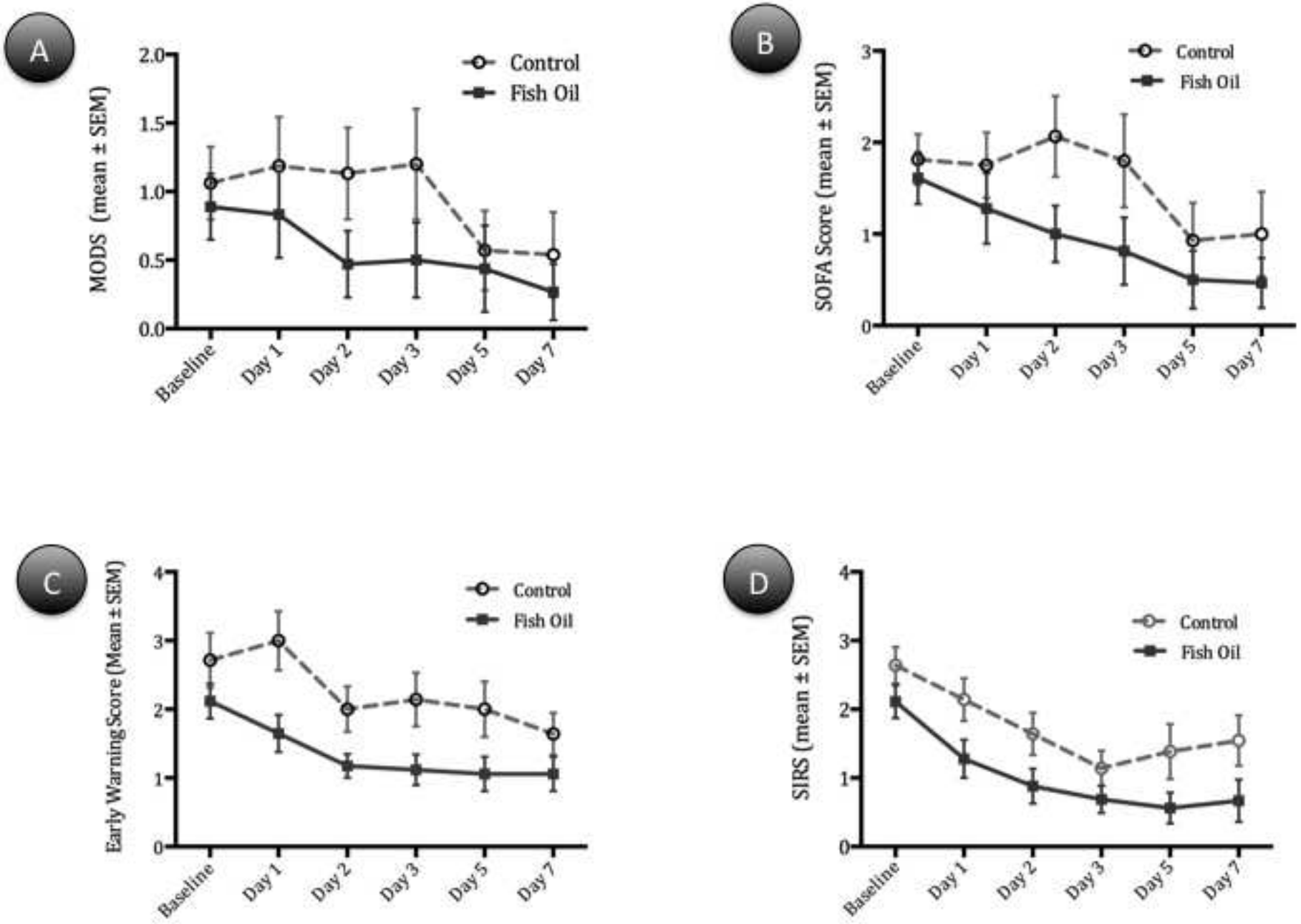

Figure 4

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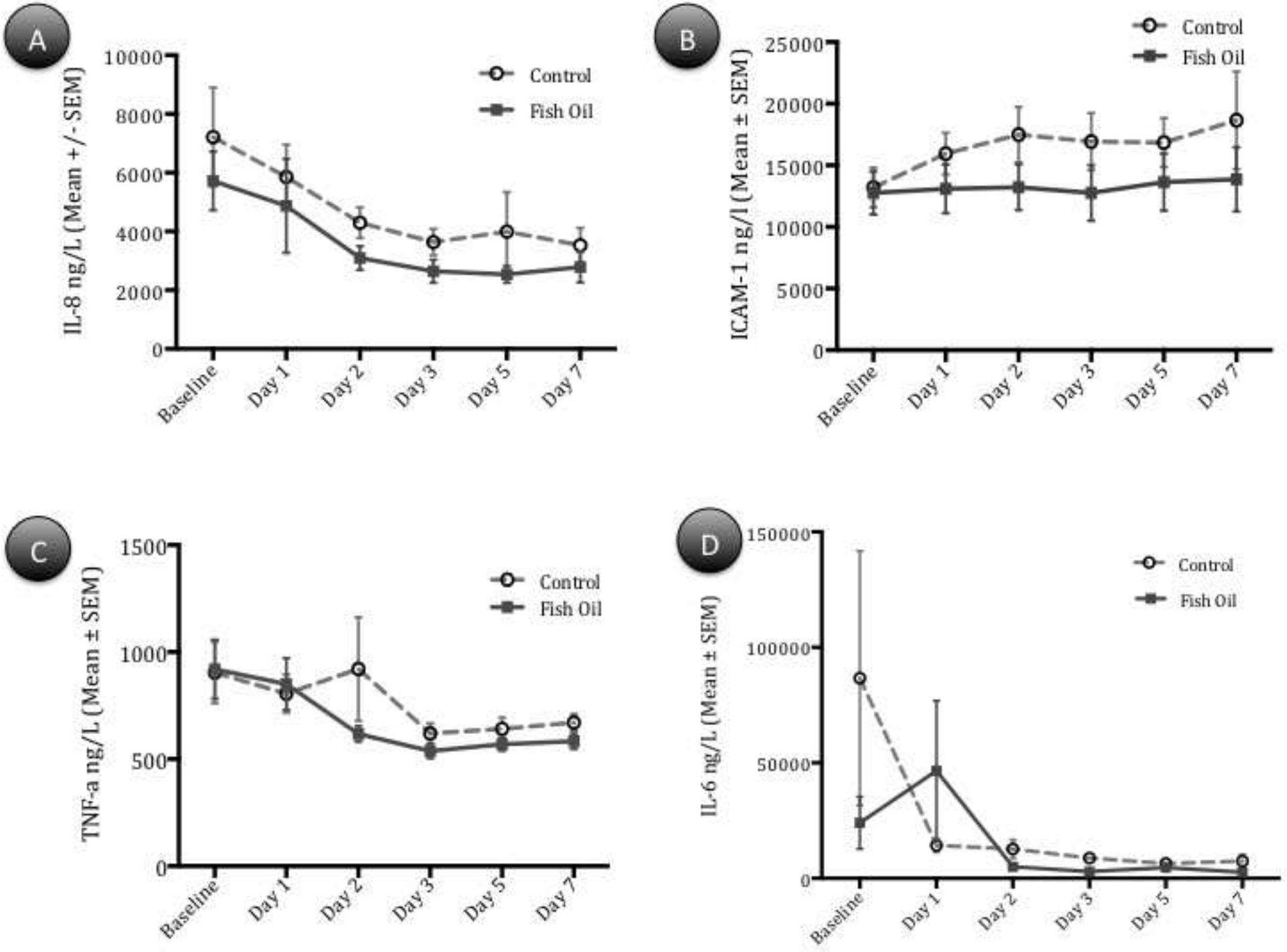

Figure 5

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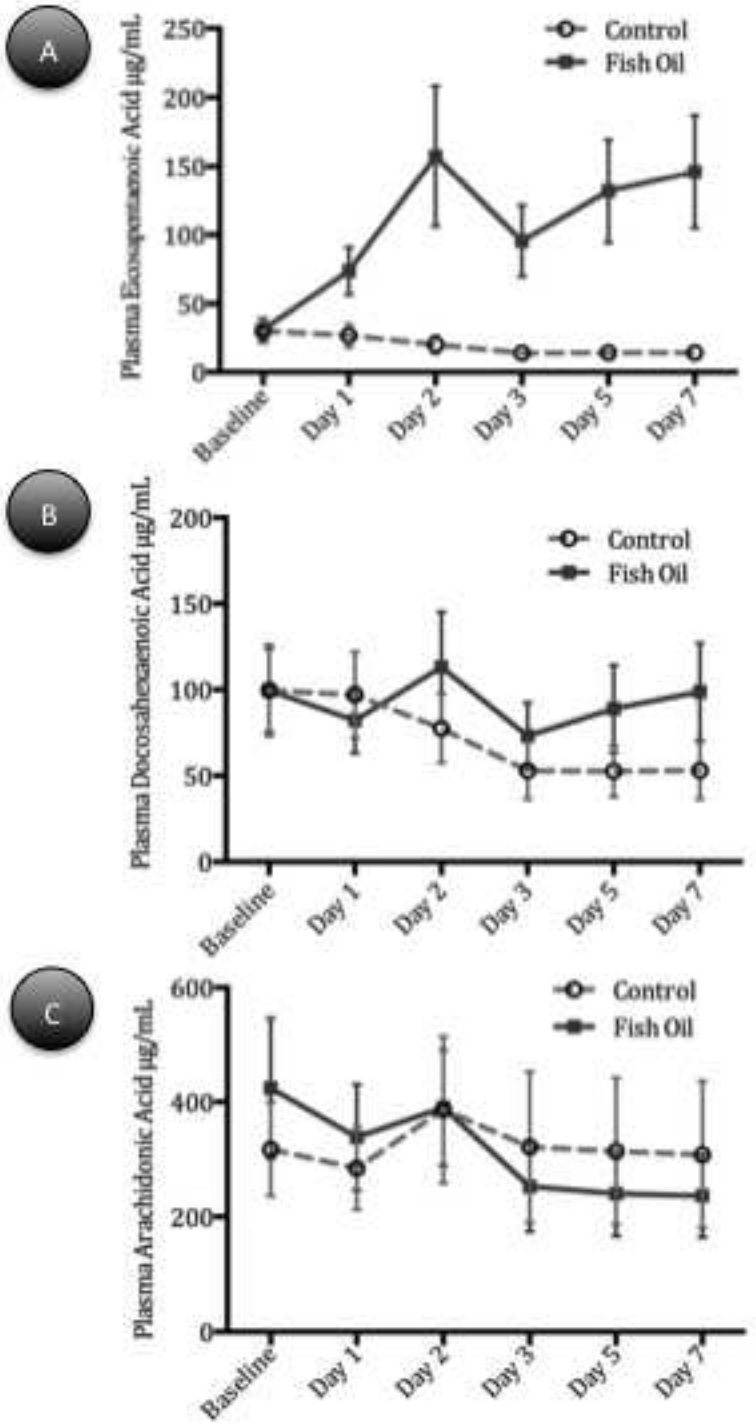

Tables (1&2)

	Fish oil group (n=22)	**Control group (n=22)**	***p* value**
Age (y) (median (range))	66 (28-88)	69 (30-87)	0.987[a]
Sex F/M	10/12	9/13	-
Aetiology:			
Gall stones	10 (45%)	11(50%)	
Alcohol	5 (23%)	3 (14%)	-
Other	2 (9%)	2 (9%)	
Unknown	5 (23%)	6 (27%)	
APACHE II score on admission (mean ± SEM)	9.9 ± 0.7	10.3 ± 0.7	0.727[a]
Glasgow score on admission (mean ± SEM)	2.8 ± 0.3	2.8 ± 0.2	0.997[a]
Ranson score on admission (mean ± SEM)	3.2 ± 0.3	3.1 ± 0.4	0.901[a]
Patients with organ failure on admission	8 (36%)	6 (27%)	0.525[b]
Organ failure score on admission (mean ± SEM)	1.2 ± 0.2	1.7 ± 0.2	0.079[a]
Early warning score on admission (mean ± SEM)	2.1 ± 0.4	2.7 ± 0.3	0.293[a]

Table 1. Patient demographics at study entry. [a] Two tailed t test, [b] Fisher's exact test.

	Fish Oil group (n = 22)						Control group (n = 22)					
Parameter (normal range)	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
WBC (x 10^9/L) (4 - 11)	17.9 ± 1.7	13.1 ± 1.3	10.7 ± 1	8.4 ± 0.7	9.6 ± 0.8	10.2 ± 0.9*	19.6 ± 2	13.2 ± 1.4	12.1 ± 1.5	12.5 ± 1.4	13.8 ± 1.4	16.7 ± 2.3
Hematocrit (L/L) (0.40 – 0.54)	0.40 ± 0.01	0.33± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.42 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.33 ± 0.01
eGFR (mL/min) (>90)	75.3 ± 9.3	99.5 ± 10.5	101.9 ± 10.9	105.1 ± 10.9	103.4 ± 12.4	98.9 ± 10.4	72.6 + 8.6	90.2 ± 10.8	97.8 ± 11.9	107.5 ± 11.6	101.9 ± 11.3	103.0 ± 11.2
APTT (sec) (25 - 35)	37.7 ± 2	40.9 ± 2.2	38.8 ± 2.8	38.2 ± 3.5	35.7 ± 1.1	33.6 ± 1.3	35.1 ± 1.6	37 ± 1.1	36.3 ± 1.3	35.2 ± 1.3	33.3 ± 1.1	33.6 ± 1.2
Fibrinogen (g/L) (2 - 4)	6.6 ± 0.8	6.6 ± 0.8	7.1 ± 0.4	7.0 ± 0.3	6.3 ± 0.7	6.7 ± 1	7.4 ± 0.8	7.4 ± 0.8	7.8 ± 0.9	8.0 ± 0.9	8.3 ± 0.7	7.9 ± 0.6
Adjusted calcium (mmol/L) (2.2 -2.6)	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.04	2.2 ± 0.04	2.3 ± 0.03	2.3 ± 0.07	2.3 ± 0.02	2.2 ± 0.04	2.2 ± 0.05	2.3 ± 0.03	2.2 ± 0.05	2.3 ± 0.04
Glucose (mmol/L) (3.3 – 6.0)	9.8 ± 1.1	7.2 ± 1.1	7.6 ± 1.1	9.6 ± 1.2	7.7 ± 1.1	7.7 ± 1.2	10.3 ± 2.3	7.8 ± 1.1	5.7 ± 0.4	7.0 ± 0.8	8.7 ± 1.6	8.7 ± 1.6
pO_2 (kPa) (>10)	16.0 ± 6.5	11.2 ± 1.1	16 ± 1.9	15.8 ± 2.3	16.0 ± 2.0	15.8 ± 2.3	10.5 ± 0.5	10.3 ± 0.7	11.6 ± 1	11.6 ± 1.5	12.8 ± 1.5	11.7 ± 1.5
HCO_3 (mmol/L) (22–26)	23.3 ± 1.6	26.5 ± 2.7	27.3 ± 2.4	28 ± 1.6	28.7 ± 2.3	28.4 ± 2.5	19.9 ± 0.8	20.3 ± 1	22.6 ± 1.1	23.3 ± 0.7	24.3 ± 1.2	24.2 ± 1.4
Base excess (mmol/L) (± 2)	-2.2 ± 2.2	1.3 ± 3.5	1.1 ± 2.9	3.2 ± 1.8	3.1 ± 1.9	2.5 ± 2.5	-4.2 ± 1	-4.3 ± 1.1	-2 ± 1.3	-1.3 ± 0.9	0.01 ± 1.3	0.01 ± 1.5

Table 2. Laboratory outcomes in patients with SAP receiving control or fish oil containing lipid emulsions daily for 7 days. Data are mean ± SEM. *indicates significantly different from control (P=0.035).

Figure captions

Figure 1. Flow of participants through the study.

Figure 2. Serum CRP concentrations in patients with predicted SAP receiving control or fish oil containing lipid emulsions daily for 7 days. The effect of fish oil on the CRP concentrations was (F=6.37, P=0.013) between both groups, two-way ANOVA RM. Data are mean ± SEM.

Figure 3. MODS, SOFA, EWS and SIRS in patients with predicted SAP receiving control or fish oil containing lipid emulsions daily for 7 days. Multiple organ dysfunction score (F=4.83, P=0.029), sequential organ failure assessment score (F=8.32, P=0.004) early warning score (F=6.89, P=0.014) and systemic inflammatory response syndrome (F=5.51, P=0.025) were reduced in the fish oil group when compared to the control group, using two-way ANOVA RM. Data are mean ± SEM.

Figure 4. Serum IL-8, ICAM-1, TNF-α and IL-6 concentrations in patients with predicted SAP receiving control or fish oil containing lipid emulsions daily for 7 days. The effect of fish oil on IL-8 (F=4.52, P=0.051), ICAM-1 (F=11.78, P=0.013), TNF-α (F=0.60, P=0.229) and IL-6 (F=0.01, P=0.491), two-way ANOVA RM. Data are mean ± SEM.

Figure 5. Plasma PC EPA, DHA and arachidonic acid in patients with predicted SAP receiving control or fish oil containing lipid emulsions daily for 7 days. Fish oil emulsion increased EPA and DHA concentrations (F=4.04, P=0.001) and (F=0.56, P=0.463) respectively and reduced AA concentration (F=1.55, P= 0.176) two-way ANOVA RM. Data are mean ± SEM.

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***Revision Note**

Date: 08/02/2018

Dear Editor, Journal of Clinical Nutrition

Re: MS. Ref. No.: YCLNU-D-17-00739. "Intravenous omega-3 fatty acids are associated with better clinical outcome and less inflammation in patients with predicted severe acute pancreatitis:A randomised double blind controlled trial"

We would like to thank you for your email dated 10/11/2017, and the opportunity to resubmit a revised copy of this manuscript. We would also like to take this opportunity to express our thanks to the reviewers for the positive feedback and helpful comments. We believe this have resulted in an improved revised manuscript. The manuscript has been revised to address the reviewer comments, which are appended alongside our responses to this letter.

Reviewer comments

1- Please show the exact constituents of both intravenous lipid emulsions and total nutritional intake of enteral nutrition and/or synbiotics except intravenous lipid emulsion. Those data are important for future usage of this result.
Response: Types of enteral and parenteral nutrition have been explained in lines 275-276 and 279-280 respectively. In addition, nutritional compositions have been uploaded and attached to "Additional file" document.

2- Do you indicate the diagnostic criteria for acute pancreatitis and severe acute pancreatitis?
Response: Study protocol with criteria used to define severe acute pancreatitis is uploaded and attached to "Additional file" document.

3- Line 320 to 331 and line 342 to 343 in the discussion should be moved to the introduction.
Response: This has been edited according to above suggestions.

4- Please discuss the difference among your study and studies referred as 10, 28, 29 in detail in the discussion including the mechanism of your omega-3 fatty acids intravenous lipid emulsion.
Response: A paragraph has been added to the discussion section address the above line 425-442 and 446- 448.

Is it true of line 110 to 110 with reference 10, 28, 29?
Response: We apologise for not clarifying the commentary made about the above studies. This has now been addressed and clarified in line 109-113.

We very much hope the revised manuscript is accepted for publication in your respected Journal.

Sincerely yours,

Mr Dhya Al-Leswas

on behalf of the authors
dhya@doctors.org.uk

Supplemental Reference File

Click here to download Supplemental Reference File: ADDITIONAL FILE.docx

Intravenous omega-3 fatty acids are associated with better clinical outcome and less inflammation in patients with predicted severe acute pancreatitis: A randomised double blind controlled trial

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Authors' contributions

DA-L, JAS, OA-T, MSM and ARD designed the study and conducted study approval processes. DA-L, AME, CP, MSM and ARD recruited patients and oversaw the intervention. DA-L, AME, AA, JAS and OA-T collected blood samples and collated the clinical data under the supervision of ARD. DA-L, AME and W-YC processed the blood samples and conducted laboratory assays. HLF performed fatty acid composition analyses under the supervision of PCC. DA-L, AME and GG conducted the statistical

analysis under supervision of ARD. DA-L drafted the manuscript; GG, PCC and ARD had significant input into the manuscript. All authors agreed upon and approved the final manuscript.

Conflict of Interest

DA-L, AA, JAS, OA-T, MSM and ARD received support from BBraun, Melsungen for investigational products (Lipidem® and Lipofundin®) used in this trial. PCC has received speaking honoraria from BBraun, Fresenius-Kabi, and Baxter Healthcare. The other authors have no competing interests

ABSTRACT

Background and Aims

Omega-3 fatty acids (FA) can ameliorate the hyper-inflammatory response that occurs in conditions such as severe acute pancreatitis (SAP) and this may improve clinical outcome. We tested the hypothesis that parenteral omega-3 FA from a lipid emulsion that includes fish oil could be beneficial in patients with predicted SAP by reducing C-reactive protein (CRP) concentration (primary outcome), and modulating the inflammatory response and improving clinical outcome (secondary outcomes).

Methods

In a phase II randomized double-blind single-centre controlled trial, patients with predicted SAP were randomised to receive a daily infusion of fish oil containing lipid emulsion (Lipidem® 20%, BBraun) for 7 days (n=23) or a daily infusion of a lipid emulsion without fish oil (Lipofundin® MCT 20%, BBraun) (n=22).

Results

On admission, both groups had comparable pancreatitis predicted severity and APACHE II scores. Administration of fish oil resulted in lower total blood leukocyte number (P=0.04), CRP (P=0.013), interleukin-8 (P=0.05) and intercellular adhesion molecule 1 (P=0.01) concentrations, multiple organ dysfunction score, sequential organ failure assessment score (P=0.004), early warning score (P=0.01), and systemic inflammatory response syndrome (P=0.03) compared to the control group. The fish oil group had fewer new organ failures (P=0.07), lower critical care admission rate (P=0.06)., shorter critical care stay (P=0.03) and shorter total hospital stay (P=0.04).

Conclusions

It is concluded that intravenous administration of a fish oil containing lipid emulsion, a source of omega-3 FA, improves clinical outcomes in patients with predicted SAP, benefits that may be linked to reduced inflammation.

ClinicalTrials.gov number: NCT01745861

EU Clinical Trials Register: EudraCT (2010-018660-16)

Keywords: C-reactive protein; organ failure; systemic inflammatory response syndrome; omega-3; fish oil; severe acute pancreatitis

Abbreviations used: CRP, C-reactive protein; DHA, docosahexenoic acid; EPA, eicosapentenoic acid; EWS: early warning score; SIRS, systemic inflammatory response syndrome; FA, fatty acid; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MCT, medium chain triglyceride; MODS, multiple organ dysfunction score; PC, phosphatidylcholine; SOFA, sequential organ failure assessment.

INTRODUCTION

Severe acute pancreatitis (SAP) is an inflammatory disorder of the pancreas with a potentially complicated clinical course and variable outcome ranging from complete resolution to multiple organ failure and death. Despite improvements in general and critical care management, morbidity and mortality from SAP remain high (1,2). An early severe systemic inflammatory response syndrome (SIRS) and multiple organ failure are considered to be responsible for most deaths in SAP (1,2). Consequently, current management of AP is focussed on meticulous supportive care and prevention of pancreatic necrosis, infection, and organ failure (3).

Nutrition support has traditionally focussed on supplying energy and micronutrients to the patient. Lipids are an important component of nutrition support because the fatty acid (FA) constituents of the lipids are good energy sources and reduce the need for carbohydrate. In intravenous nutrition support, lipids are present as stable emulsions. It is now appreciated that FA have biological activities related to their effects on membrane structure and function, production of signalling molecules, and regulation of gene expression (4). Consequently, the mix of FA within an intravenous lipid emulsion will influence the host's metabolic, immune and inflammatory responses (5,6). In this regard, the omega-3 FA found within fish oil, eicosapentenoic acid (EPA) and docosahexenoic acid (DHA), have a range of biological activities (5,7). The central mechanism of action of EPA and DHA relates to their incorporation into plasma lipids and into the membranes of cells and tissues from where they exert their biological actions (8). With regard to inflammation, EPA and DHA have multiple actions as discussed in detail elsewhere (9). As a result of these actions the omega-3 FA EPA and DHA may be useful in controlling SIRS and improving outcome in patients with SAP. Indeed, Wang et al. reported some benefits from parenteral nutrition with omega-3 FA

~~intravenous fish oil~~ in patients with SAP (10). However, parenteral nutrition in acute pancreatitis is not recommended routinely by the gastroenterology societies due to reports and studies that linked it to poor outcome (3). Therefore, there is a need for a well-designed study that test the effect of omega-3 FA in SAP in settings that resemble the daily clinical practice and in-line with the current pancreatitis management guidelines. In this study we investigated the effect of daily intravenous infusion of a lipid emulsion containing fish oil for seven days in patients with predicted SAP. The emulsion used has the commercial name Lipidem® or Lipoplus®. Lipidem® has been used previously in post-operative surgical (11-14) and critically ill septic (15,16) patients. In those studies, Lipidem® was found to decrease the concentrations of pro-inflammatory cytokines (13,16) and pro-inflammatory lipid mediators (11,13,16), to improve gas exchange (16) and to reduce the length of hospital stay (14). A different lipid emulsion based on fish oil (Omegaven®, Fresenius Kabi, Germany) has been used in patients with predicted SAP (10, 17, 18) , critically ill patients (19), septic patients (20,21) and post-operative surgical patients (22-24). In those studies, Omegaven® decreased pro-inflammatory cytokine concentrations (17, 20,22), increased anti-inflammatory cytokine concentrations (18), decreased pro-inflammatory lipid mediator concentrations (21,24), improved immune function (20-23) and improved clinical outcomes (17,20-23,25)~~However, the application of intravenous fish oil in patients with predicted SAP is not well explored. Therefore, in this study we investigated the effect of daily intravenous infusion of a lipid emulsion containing fish oil for seven days in patients with predicted SAP.~~

Both omega-3 FA emulsions (Lipidem® and Omegaven®) have demonstrated better outcome in various clinical settings but authors of this study opted to use Lipidem® to ease the randomisation and double blinding processes.

~~; we evaluated plasma FAs, serum inflammatory markers and clinical outcomes.~~ The hypothesis of the current study was that intravenous fish oil would lower serum CRP concentration. We have also evaluated plasma FAs, serum inflammatory markers and clinical outcomes.

PATIENTS AND METHODS

Study design and outcomes

This was a phase II, single center, double-blinded, randomized controlled trial conducted in accordance with the recommendations of the EEC Committee for Proprietary Medicinal Products (26~~11~~); the trial was registered as NCT01745861, received ethical approval from the Leicestershire, Northamptonshire and Rutland Research Ethics Committee and was approved by the Medicines and Healthcare Products Regulatory Agency (MHRA). The primary objective was to determine if intravenous fish oil given daily starting within 72 hours of the onset of symptoms of predicted SAP could reduce the concentration of the inflammatory marker C-reactive protein (CRP) by day 7. The secondary objectives were to assess the effects of the omega-3 rich fish oil emulsion on sequential organ failure assessment (SOFA) score (27~~12~~), multiple organ dysfunction score (MODS) (28~~13~~), early warning scores (EWS) (29~~14~~), SIRS (30~~15~~), development of new organ dysfunction, escalation of the patients' care, length of stay, circulating pro-inflammatory cytokines and adhesion molecules, and plasma phospholipid (phosphatidylcholine (PC)) EPA, DHA and arachidonic acid levels. Sepsis was defined as the presence of infection, documented or strongly suspected, with one or more SIRS features (31~~16~~). Patients were assessed daily for 7 days for any adverse events or complications. Severity scores and blood samples were obtained on days 0, 1, 2, 3, 5 and 7 of the infusion.

Inclusion and exclusion criteria

All conscious patients aged 18 to 90 years admitted with predicted SAP proven by compatible clinical features (abdominal pain with or without vomiting) associated with amylase activity at least three times greater than the upper limit of the normal value and one or more of the severity criteria as outlined in the Atlanta severity criteria for AP (32~~17~~) or Glasgow (Imrie) score ≥ 3 were considered eligible for the study. Patients were excluded for any of the following reasons: age < 18 or > 90 years; unconscious or unable to consent; allergic to fish, egg or soy protein; uncontrolled hyperlipidemia; severe primary blood coagulation disorders; acute pancreatitis accompanied with hyperlipidemia; ketoacidosis; acute thromboembolic disease; severe liver failure; acute phase of myocardial infarction or stroke; pregnancy or lactation; severe renal failure without access to hemofiltration or dialysis.

The intervention

Forty-five patients admitted to University Hospitals of Leicester NHS Trust with predicted SAP were randomized into two groups, fish oil and control. Patients were allocated to either group by a computer-based randomization system (Wellspring Clinical Services, Doncaster, UK; see Supplementary Material). The fish oil group (n = 23, one patient withdrew consent prior to any intervention) received a lipid emulsion enriched with omega-3 FAs (Lipidem® 200 mg/ml: 50% medium chain triglycerides (MCT), 40% soybean oil and 10% fish oil; B Braun, Melsungen, Germany). The control group (n = 22) received an isocaloric lipid emulsion without fish oil (Lipofundin® 200 mg/ml; 50% MCT and 50% soybean oil; B Braun, Melsungen, Germany). Lipidem® and Lipofundin® were infused at a rate of 10 ml per kg body weight over 14 hours each day

for a maximum of 7 days or until the patient was clinically fit for discharge if sooner; this corresponds to 2 g lipid per kg body weight over 14 hours each day. Standard management for these patients continued. Patients were withdrawn if serum triglycerides persisted above 3 mmol/L despite temporary cessation of lipid infusion.

Laboratory analyses

Routine hematology, biochemistry (including serum CRP), coagulation, random lipid profiles, urine analysis and arterial blood gases were performed at University Hospitals of Leicester NHS Trust laboratories. Serum and plasma samples were stored at –80°C. Pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8) and the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) were measured in serum using an ultra-sensitive multi-array assay (Meso Scale Discovery, Gaithersburg, MD, USA). Fatty acid composition of plasma PC was determined by gas chromatography as described elsewhere (33~~18~~).

Randomisation and blinding processes

The randomisation and blinding processes was assigned to an independent pharmaceutical company "Wellspring Clinical Services, Doncaster, England, UK", which has created sequential kit numbers. Lipidem® and Lipofundin® bottles were randomly allocated to these kit numbers. Consecutive patients entering the trial were allocated to the sequential kit number provided by Wellspring. This process was created prior to the start of the study.

Wellspring Clinical Services also created the over-labels, essential for the blinding process, and this was approved by MHRA, study sponsor and research team.

A clear protocol, developed by Wellspring Clinical Services, with description of the conditions and procedures for emergency unblinding was available within the pharmacy department. Trial pharmacists or the on-call pharmacists (all blinded) can only do the unblinding process after following the unblinding protocol. Both patients and research team were also blinded throughout the study. The randomisation and blinding procedures were not compromised in this study. The blinding procedure and all aspects of the trial were inspected and agreed upon by MHRA auditors.

Power calculation and statistical methods

Based upon the existing literature (19,20), we considered that a 20% lower mean concentration of serum CRP in the fish oil group than in the control group at day 7 (trial exit point) would be clinically meaningful. Assuming an SD of 15 mg/L in CRP concentration it was calculated that 22 patients would give 90% power to identify a significant effect given α=0.05.

Continuous variables are presented as mean ± SEM and categorical variables as numbers and percentages. D'Agostino & Pearson omnibus normality test was used to determine the distribution of continuous data. Normally distributed data were analyzed using the 2-tailed Student's *t* test and non-normally distributed data were analyzed using the Mann-Whitney *U* test. Categorical data were analysed using the χ^2 test and Fisher's exact probability test. Mann-Whitney *U* or 2-tailed *t* tests were used for comparisons between time points and for comparisons between groups at a particular time point. Differences in parameters between the fish oil and control groups during the 7 days of intervention were tested for significance by 2-factor (time × treatment) repeated measures (RM) ANOVA followed by post-hoc analysis using Bonferroni's correction for multiple comparisons. In all cases, a value of $P < 0.05$ was taken to

indicate statistical significance. Statistical analyses were performed using Prism 6 (version 6.0e, 1994 – 2014 GraphPad Software, Inc.). Data were analysed with the intention-to-treat and analysis was performed only after study completion and before unblinding. All patients that received intervention were included in the analysis and missing data were treated by the last observation carried forward (LOCF) approach.

RESULTS

Patient demographics

One hundred and ninety-eight patients with AP were admitted to the study centre (Leicester, UK). One hundred and thirty nine patients did not meet SAP inclusion criteria, 3 patients had one or more exclusion criteria and 11 patients refused to participate in the trial (Figure 1). The remaining 45 patients were randomized to the fish oil group (n=23) or the control group (n=22). One patient in the fish oil group withdrew prior to any intervention and was therefore excluded from the results (Figure 1). Five patients did not finish the 7 day trial period: two patients in the fish oil group withdrew on day 3 and 1 patient in the control group withdrew on day 5; one patient in the fish oil group had features of haemorrhagic severe pancreatitis and died on day 2; one patient in the control group was deemed to be fit for discharge on day 5. Missing data for these patients were substituted by their last observation.

Patient demographics and baseline clinical characteristics in the fish oil and control groups are shown in Table 1. The mean baseline triglyceride level was 1.9 ± 0.7 and 1.5 ± 0.3 mmol/L in the fish oil and control groups, respectively.

The average caloric contents of Lipidem® and Lipofundin® are around 1900 kcal/l and this does not meet the hyper catabolic state of this disease with estimated energy needs of 30-35 kcal/kg/day. Researchers observed that early oral nutrition was encouraged to

all patients without restrictions. However, if oral nutrition was deemed to be inadequate, NG or NJ feed was subsequently started in these patients. Five patients (two in treatment group and 3 in the control group) deemed to have inadequate oral intake and feeding via NG tube was started at different time points in the trial (earliest was day 3 and the latest was day 6). Nutrison® 1kcal/ml (see appendices) was the standard enteral nutrition regimen used by the dietician and clinical team. Two patients (one in fish oil group and one in control group) had prolonged ileus and NG/NJ feed deemed to be inadequate, ~~total~~ parenteral nutrition (TPN) was subsequently started on day 5 and 6 respectively. Triomel® Baxter standard PN was the main parenteral nutrition given to these patients. The decision about the enteral or parenteral nutrition laid~~lay~~ between patients' clinicians and on-call dietician. The on-call dieticians reviewed and adjusted NG/NJ feed or TPN to accommodate the administration of Lipidem®/Lipofundin®.

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Primary outcome measure: CRP concentration

On admission, there was no significant difference in CRP concentrations between the two groups (148.5 ± 30.5 mg/L in the fish oil group and 142.9 ± 31.6 mg/L in the control group (P=0.90)). Two factor ANOVA revealed that CRP concentration changed over time (P=0.004) and was different between treatment groups (P=0.013) (Figure 2). At day 7, CRP concentration was 34% lower in the fish oil than in the control group; however the concentrations (104.1 ± 23 mg/L in the fish oil group and 157.6 ± 26.8 mg/L in control group) were not significantly different (P=0.15).

Secondary outcome measures: serology

Many serological parameters changed significantly over time (Table 2), only HCO_3 concentration was affected by fish oil treatment (P=0.03), although there was a strong

283 trend for an effect of treatment group on total blood leukocyte count (P=0.08). At each
284 time point, the fish oil group had lower blood leukocyte number than the control group,
285 but the difference only reached statistical significance on day 7 (P=0.04) (Table 2).
286
287 **Secondary outcome measures: organ failure scores (MODS and SOFA), EWS and**
288 **SIRS**
289 On admission, the number of patients with one or more organ failure was 8 (36%) in
290 the fish oil group and 6 (27%) in the control group. There was a strong trend for fewer
291 patients in the fish oil group to develop new organ failure (6 (27%) vs 13 (59%);
292 (P=0.07). SOFA (P=0.03), EWS (P<0.001) and SIRS (P<0.001) all decreased over time
293 (Figure 3). Fish oil affected MODS (P=0.03), SOFA (P=0.004), EWS (P=0.01) and SIRS
294 (P=0.03), which were all lower in the fish oil group than the control group (Figure 3).
295 EWS was lower in the fish oil group at days 1 (P=0.01), 2 (P=0.05) and 3 (P=0.04) and
296 tended to be lower at day 5 (P=0.08). SIRS was lower in the fish oil group at days 1
297 (P=0.01) and 2 (P=0.05) and tended to be lower at days 5 and 7 (both P=0.05).
298 **Secondary outcome measures: septic complications**
299 Eleven (50%) patients in the control group developed sepsis compared with 8 (36%) in
300 the fish oil group, but the groups were not significantly different (P=0.36). The median
301 duration of antibiotic administration (intravenous or oral) was shorter in the fish oil
302 group than the control group: 5 [95% CI, 3.3 to 5.3] days vs 10 [95% CI, 7.2 to 16.7]
303 days, respectively (P<0.01).
304 **Secondary outcome measures: escalation of care and length of stay**
305 Fewer patients in the fish oil group (n=5 (23%)) than in the control group (n=11
306 (50%)) required escalation of their care from a normal ward to a higher-level of care
307 (intensive/critical care or high dependency units), a difference that approached

statistical significance (P=0.06). The median length of stay (LOS) in a higher-level of care (intensive/critical care or high dependency units) was 3 [95% CI, -0.9 to 6.9] days in the fish oil group compared with 9 [95% CI, 6.7 to 23.4] days in the control group (P=0.03). The median inpatient (hospital) stay was also shorter in the fish oil group than the control group: 12 [95% CI, 9.6 to 15.3] days vs 18 [95% CI, 15.5 to 27.2] days, respectively (P=0.04).

Secondary outcome measures: serum cytokines and ICAM-1

There were no differences between groups at study entry for the serum concentrations of any of the cytokines or intercellular adhesion molecule (ICAM)-1. There was a significant effect of time on the concentrations of TNF-α (P=0.006), IL-8 (P<0.001) and ICAM-1 (P=0.04) with a trend towards an effect on IL-6 concentration (P=0.08) (Figure 4). The concentrations of TNF-α and IL-8 declined over time (Figure 4). There was an effect of treatment on the concentration of IL-8 (P=0.05) and ICAM-1 (P=0.01), which was lower in the fish oil group (Figure 4).

Secondary outcome measure: plasma phosphatidylcholine (PC) fatty acid composition

Plasma PC contributes about 75% of plasma phospholipid and acts as a transporter for FAs including EPA, DHA and arachidonic acid to target cells and tissues such as leukocytes (36~~21~~). There was a significant effect of time (P=0.03) and treatment group (P=0.001) and a significant time x treatment group interaction for plasma PC EPA (P=0.002) (Figure 5). Plasma PC EPA was significantly higher in the fish oil group at days 1, 2, 3, 5, 7 (Figure 5). In contrast, neither DHA nor arachidonic acids were affected by time or treatment group (Figure 5).

Safety and tolerability of the lipid emulsions

Both emulsions were well tolerated with no unexpected severe adverse events occurring. One critically ill patient in the control group developed transient hypertriglyceridaemia, which resolved after temporary cessation of the lipid infusion. Mean post-infusion serum triglycerides and random cholesterol levels did not differ significantly between groups at any time point (data not shown). There were 2 deaths, one on day 2 in the fish oil group and the one just after exiting the trial in the control group. Both patients had severe multiple organ dysfunction syndrome.

DISCUSSION

This study is the first prospective randomized double-blind controlled trial conducted with omega-3 fatty acid rich fish oil containing lipid emulsion in patients with predicted SAP. ~~The emulsion used has the commercial name Lipidem® or Lipoplus®. Lipidem® has been used previously in post-operative surgical (22-25) and critically ill septic (26, 27) patients. In those studies, Lipidem® was found to decrease the concentrations of pro-inflammatory cytokines (24, 27) and pro-inflammatory lipid mediators (22, 24, 27), to improve gas exchange (27) and to reduce the length of hospital stay (25). A different lipid emulsion based on fish oil (Omegaven®, Fresenius Kabi, Germany) has been used in patients with predicted SAP (10, 28, 29), critically ill patients (30), septic patients (31, 32) and post-operative surgical patients (33-35). In those studies, Omegaven® decreased pro-inflammatory cytokine concentrations (28, 31, 33), increased anti-inflammatory cytokine concentrations (29), decreased pro inflammatory lipid mediator concentrations (32, 35), improved immune function (31-34) and improved clinical outcomes (28, 31-34, 36).~~ In the current study, administration of Lipidem® resulted in less inflammation, less severe disease, fewer new organ failures, lower critical care

admission rate, shorter critical care stay and shorter total hospital stay compared to the control group.

Three inflammatory markers that were lower, or tended to be lower, with fish oil were blood leukocyte count and serum concentrations of CRP and IL-8. CRP is a non-specific marker of inflammation that is synthesized by liver cells (37) and its concentration rises in a variety of inflammatory conditions. IL-6 and IL-1 trigger its synthesis and it has widely been used as a predictor of the progression of an episode of moderate AP to SAP (37). The specificity, sensitivity, and positive and negative predictive values of CRP in predicting the severity of AP at 48 hours from the onset are 86%, 61%, 37%, 94%, respectively, and the positive likelihood ratio is 2.2 (38). The hypothesis of the current study was that intravenous fish oil would lower serum CRP concentration. CRP concentration was selected as the primary outcome because fish oil derived omega-3 FA are known to be anti-inflammatory (9) and because a reduction in CRP should be associated with less severe disease and improved clinical outcome in patients with predicted SAP. In accordance with the existing literature, CRP concentration was highest at day one and then declined. Peak concentrations did not differ between control and fish oil groups. After day one there was a steady decrease in CRP concentrations in both groups but with a more marked reduction in the fish oil group (one way ANOVA effect of treatment P=0.013). This observation supports the primary hypothesis of the study.

The observed reduction in inflammation in the fish oil group was linked with lower organ dysfunction scores, as measured by SOFA and MODS, and lower scores for SIRS and EWS. This finding supports data from a study of the same lipid emulsion in critically ill septic patients (6, 12~~27~~).

The current study demonstrates a likely clinical benefit of an intravenous fish oil emulsion on the SIRS score at an early stage of predicted SAP (see Figure 3). On admission, both groups had similar SIRS scores and although this reduced steadily in both groups, the reduction was more pronounced in the fish oil group. This is consistent with previous report by Wang et al. who used parenteral Omegaven® infusion and demonstrated an improvement of SIRS in SAP (10).

In the current study, the seven days infusion with a lipid emulsion containing EPA markedly increased plasma PC EPA by an average of 4.6-fold from baseline; interestingly there was no significant increase in plasma PC DHA. This is consistent with findings from another study where an average 3.8-fold increase in EPA in plasma phospholipids was observed in critically ill septic patients receiving Lipidem® for five days (12~~27~~). In that study there was also a tendency for better clinical outcome and shorter length of stay in critically ill septic patients (12~~27~~). Likewise, Barbosa et al. and Simoens et al. both observed no significant changes in DHA and AA levels and this confirms previous suggestions that better clinical outcome is associated with increased EPA status (12~~27~~, 39).

Xiong et al and Wang et al examined the effects of omega-3 FA in patients with SAP (10, 17, 18). The treatment groups in all three studies received parenteral nutrition with omega-3 FA where as the control groups either received conventional supportive treatment or parenteral nutrition without omega-3 FA. In all studies, there were better inflammatory response and clinical outcome in the pancreatitis group. However, the main concern about these studies is that parenteral nutrition is a pro-inflammatory and has shown to increase morbidity and mortality in patients with SAP (40). Furthermore, the current acute pancreatitis guidelines strongly discourage the routine use of parenteral nutrition in AP patients. They recommend enteral nutrition as a first line in

nutrition and parenteral nutrition is only reserved to patients that cannot tolerate enteral nutrition. In the above studies parenteral nutrition was routinely used in most patients and this is not in-line with daily clinical practice and the current guidelines (3). We are therefore, finding it difficult to ascertain the outcomes in the above studies are purely to omega-3 FA.

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This study has several strengths. First, it was randomized, double blind and controlled. Secondly, the withdrawal rate was low. Thirdly, a range of laboratory and clinical outcomes was measured. Fourthly, omega-3 FA status was measured alongside the laboratory and clinical outcomes. Finally, parenteral nutrition was not given routinely to all patients and this is a resemblance of the daily practice and in-line with current recommendations by the gastroenterology society.

The study also has limitations. First, the sample size was quite low and larger trials will be needed to confirm the many positive findings made before they can be transferred to clinical practice. Secondly, the primary outcome was a laboratory measure (serum CRP concentration) rather than a clinical outcome, although a number of the latter were assessed as secondary outcomes. Thirdly, this was a single centre study and therefore patient management was fairly homogeneous and may not fully reflect practice across many centres. However, the observed management of AP was in conjunction with the British Society of Gastroenterology AP management guidelines (3). Fourthly, the utilisation of the LOCF approach to replace missing data is a simple process to understand but has some disadvantages such as introduction of bias. However, this method was deemed to be suitable in handling the small proportion of missing data. Subgroup analysis was not performed due the small sample size. Fifthly, we have compared two lipid emulsions and our study does not consider whether lipids *per se* will have an impact on inflammation or clinical outcome. Finally, the original Atlanta

criteria were revised just after the recruitment process of the current study concluded (41~~40~~). Nevertheless, it is the authors' view that the revised Atlanta criteria would have no impact on the main objective and outcomes of the current study.

The current study favours the administration of omega-3 FA for clinical benefit in patients with predicted SAP. Systematic reviews and meta-analyses of other immune-modulatory agents (probiotics and anti-oxidants) revealed no beneficial effect on clinical outcome in patients with predicted SAP (42,43~~41,42~~). It is possible that the success of the current study is related to several factors including the natural components of the product used, global immune-modulatory mechanisms of action of amoega-3 FA, the early intervention and the optimisation of clinical care. Further larger studies are certainly warranted but challenges with recruitment, randomisation, costs, early intervention and potential bias need to be addressed.

CONCLUSION

It is concluded that intravenous administration of a fish oil containing lipid emulsion, a source of the bioactive omega-3 fatty acids EPA and DHA, results in fewer new organ failures, better recovery, and shorter critical care and hospital stay in patients with SAP, clinical benefits that may be linked to reduced inflammation. Larger scale, multi-centre trials investigating short and long term effects of intravenous fish oil on pancreatic late complications, progression to the disabling chronic pancreatitis, and mortality are recommended.

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References

1. Maheshwari R, Subramanian RM. Severe acute pancreatitis and necrotizing pancreatitis. Crit Care Clin. 2016;32(2):279-90 .

2. Beger HG, Rau BM. Severe acute pancreatitis: Clinical course and management. World journal of gastroenterology : WJG. 2007;13(38):5043-51.

3. Working Party of the British Society of G, Association of Surgeons of Great Britain and I, Pancreatic Society of Great Britain and I, Association of Upper GISoGBaI. UK guidelines for the management of acute pancreatitis. Gut. 2005;54 Suppl 3:iii1-9.

4. Calder PC. Functional roles of fatty acids and their effects on human health. J Parenter Enteral Nutr. 2015;39:18S-32S.

5. Calder PC. Rationale for using new lipid emulsions in parenteral nutrition and a review of the trials performed in adults. Proc Nutr Soc. 2009;68(3):252-60.

6. Calder PC. Lipids for intravenous nutrition in hospitalised adult patients: a multiple choice of options. Proc Nutr Soc. 2013;72(3):263-76.

7. Calder PC. Very long chain omega-3 (n-3) fatty acids and human health. European Journal of Lipid Science and Technology. 2014;116(10):1280-300.

529 8. Calder PC. Mechanisms of action of (n-3) fatty acids. J Nutr. 2012;142(3):592S-
530 9S.

531 9. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects,
532 mechanisms and clinical relevance. Biochim Biophys Acta. 2015;1851(4):469-84.

533 10. Wang X, Li W, Li N, Li J. Omega-3 fatty acids-supplemented parenteral nutrition
534 decreases hyperinflammatory response and attenuates systemic disease sequelae in
535 severe acute pancreatitis: a randomised and controlled study. Journal of parenteral and
536 enteral nutrition. 2008;32(3):236-41.

537 11. Koller M, Senkal M, Kemen M, Konig W, Zumtobel V, Muhr G. Impact of omega-3
538 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery.
539 Clinical Nutrition. 2003;22(1):59-64.

540 12. Senkal M, Haaker R, Linseisen J, Wolfram G, Homann HH, Stehle P. Preoperative
541 oral supplementation with long-chain Omega-3 fatty acids beneficially alters
542 phospholipid fatty acid patterns in liver, gut mucosa, and tumor tissue.Journal of
543 parenteral and enteral nutrition. 2005;29(4):236-40.

544 13. Wachtler P, König W, Senkal M, Kemen M, Köller M. Influence of a total
545 parenteral nutrition enriched with omega-3 fatty acids on leukotriene synthesis of
546 peripheral leukocytes and systemic cytokine levels in patients with major surgery. J
547 Trauma. 1997;42(2):191-8.

548 14. Wichmann MW, Thul P, Czarnetzki HD, Morlion BJ, Kemen M, Jauch KW.
549 Evaluation of clinical safety and beneficial effects of a fish oil containing lipid emulsion
550 (Lipoplus, MLF541): data from a prospective, randomised, multicenter trial. Crit Care
551 Med. 2007;35(3):700-6.

15. Tappy L, Berger MM, Schwarz JM, Schneiter P, Kim S, Revelly JP, et al. Metabolic effects of parenteral nutrition enriched with n-3 polyunsaturated fatty acids in critically ill patients. Clin Nutr. 2006;25(4):588-95.

16. Barbosa VM, Miles EA, Calhau C, Lafuente E, Calder PC. Effects of a fish oil containing lipid emulsion on plasma phospholipid fatty acids, inflammatory markers, and clinical outcomes in septic patients: a randomised, controlled clinical trial. Crit Care. 2010;14(1):R5.

17. Xiong J, Zhu S, Zhou Y, Wu H, Wang C. Regulation of omega-3 fish oil emulsion on the SIRS during the initial stage of severe acute pancreatitis. Journal of Huazhong University of Science and TechnologyMedical sciences = Hua zhong ke ji da xue xue baoYi xue Ying De wen ban = Huazhong keji daxue xuebaoYixue Yingdewen ban. 2009;29(1):35-8.

18. Wang X, Li W, Zhang F, Pan L, Li N, Li J. Fish oil-supplemented parenteral nutrition in severe acute pancreatitis patients and effects on immune function and infectious risk: a randomised controlled trial. Inflammation. 2009;32(5):304-9

19. Friesecke S, Lotze C, Köhler J, Heinrich A, Felix SB, Abel P. Fish oil supplementation in the parenteral nutrition of critically ill medical patients: a randomised controlled trial. Intensive Care Med. 2008;34(8):1411-20.

20. Mayer K, Gokorsch S, Fegbeutel C, Hattar K, Rosseau S, Walmrath D, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. Am J Respir Crit Care Med. 2003;167(10):1321-8.

21. Mayer K, Fegbeutel C, Hattar K, Sibelius U, Kramer HJ, Heuer KU, et al. Omega-3 vs. omega-6 lipid emulsions exert differential influence on neutrophils in septic shock

patients: impact on plasma fatty acids and lipid mediator generation. Intensive care medicine. 2003;29(9):1472-81.

22. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr. 2002;87 Suppl 1:S89-94.

23. Schauder P, Rohn U, Schafer G, Korff G, Schenk HD. Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period. The British journal of nutrition. 2002;87 Suppl 1:S103-10.

24. Morlion BJ, Torwesten E, Lessire H, Sturm G, Peskar BM, Fürst P, et al. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. Metabolism. 1996;45(10):1208-13.

25. Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. Clin Nutr. 2004;23(3):325-30.

26~~11~~. EEC note for guidance: good clinical practice for trials on medicinal products in the European Community. CPMP Working Party on Efficacy of Medicinal Products. Pharmacology & toxicology. 1990;67(4):361-72.

27~~12~~. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Medicine. 1996;22(7):707-10.

28~~13~~. Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Critical Care Medicine. 1995;23(10):1638-52.

29~~14~~. Garcea G, Jackson B, Pattenden CJ, Sutton CD, Neal CP, Dennison AR, et al. Early warning scores predict outcome in acute pancreatitis. Journal of Gastrointestinal Surgery. 2006;10(7):1008-15.

30~~15~~. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644-55.

31~~16~~. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Critical Care Medicine. 2003;31(4):1250-6.

32~~17~~. Bradley EL, 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Archives of Surgery. 1993;128(5):586-90.

33~~18~~. Fisk HL, West AL, Childs CE, Burdge GC, Calder PC. The use of gas chromatography to analyze compositional changes of fatty acids in rat liver tissue during pregnancy. Journal of Visulaised Experiments. 2014(85).

34~~19~~. Muller CA, Uhl W, Printzen G, Gloor B, Bischofberger H, Tcholakov O, et al. Role of procalcitonin and granulocyte colony stimulating factor in the early prediction of infected necrosis in severe acute pancreatitis. Gut. 2000;46(2):233-8.

35~~20~~. Digalakis MK, Katsoulis IE, Biliri K, Themeli-Digalaki K. Serum profiles of C-reactive protein, interleukin-8, and tumor necrosis factor-alpha in patients with acute pancreatitis. HPB Surg. 2009;2009:878490.

36~~21~~. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids. 2000;35(12):1305-12.

~~22. Koller M, Senkal M, Kemen M, Konig W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. Clinical Nutrition. 2003;22(1):59-64.~~

~~23. Senkal M, Haaker R, Linseisen J, Wolfram G, Homann HH, Stehle P. Preoperative oral supplementation with long-chain Omega-3 fatty acids beneficially alters phospholipid fatty acid patterns in liver, gut mucosa, and tumor tissue.Journal of parenteral and enteral nutrition. 2005;29(4):236-40.~~

~~24. Wachtler P, König W, Senkal M, Kemen M, Köller M. Influence of a total parenteral nutrition enriched with omega 3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. J Trauma. 1997;42(2):191-8.~~

~~25. Wichmann MW, Thul P, Czarnetzki HD, Morlion BJ, Kemen M, Jauch KW. Evaluation of clinical safety and beneficial effects of a fish oil containing lipid emulsion (Lipoplus, MLF541): data from a prospective, randomised, multicenter trial. Crit Care Med. 2007;35(3):700-6.~~

26. Tappy L, Berger MM, Schwarz JM, Schneiter P, Kim S, Revelly JP, et al. Metabolic effects of parenteral nutrition enriched with n-3 polyunsaturated fatty acids in critically ill patients. Clin Nutr. 2006;25(4):588-95.

27. Barbosa VM, Miles EA, Calhau C, Lafuente E, Calder PC. Effects of a fish oil containing lipid emulsion on plasma phospholipid fatty acids, inflammatory markers, and clinical outcomes in septic patients: a randomised, controlled clinical trial. Crit Care. 2010;14(1):R5.

28. Xiong J, Zhu S, Zhou Y, Wu H, Wang C. Regulation of omega-3 fish oil emulsion on the SIRS during the initial stage of severe acute pancreatitis. Journal of Huazhong University of Science and TechnologyMedical sciences = Hua-zhong ke ji da xue xue baoYi xue Ying De wen ban = Huazhong keji daxue xuebaoYixue Yingdewen ban. 2009;29(1):35-8.

29. Wang X, Li W, Zhang F, Pan L, Li N, Li J. Fish oil-supplemented parenteral nutrition in severe acute pancreatitis patients and effects on immune function and infectious risk: a randomised controlled trial. Inflammation. 2009;32(5):304-9

30. Friesecke S, Lotze C, Köhler J, Heinrich A, Felix SB, Abel P. Fish oil supplementation in the parenteral nutrition of critically ill medical patients: a randomised controlled trial. Intensive Care Med. 2008;34(8):1411-20.

31. Mayer K, Gokorsch S, Fegbeutel C, Hattar K, Rosseau S, Walmrath D, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. Am J Respir Crit Care Med. 2003;167(10):1321-8.

32. Mayer K, Fegbeutel C, Hattar K, Sibelius U, Kramer HJ, Heuer KU, et al. Omega-3 vs. omega-6 lipid emulsions exert differential influence on neutrophils in septic shock

~~patients: impact on plasma fatty acids and lipid mediator generation. Intensive care medicine. 2003;29(9):1472-81.~~

~~33. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr. 2002;87 Suppl 1:S89-94.~~

~~34. Schauder P, Rohn U, Schafer G, Korff G, Schenk HD. Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period. The British journal of nutrition. 2002;87 Suppl 1:S103-10.~~

~~35. Morlion BJ, Torwesten E, Lessire H, Sturm G, Peskar BM, Fürst P, et al. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. Metabolism. 1996;45(10):1208-13.~~

~~36. Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. Clin Nutr. 2004;23(3):325-30.~~

37. Pepys MB. C-reactive protein fifty years on. Lancet. 1981;1(8221):653-7.

38. Neoptolemos JP, Kemppainen EA, Mayer JM, Fitzpatrick JM, Raraty MG, Slavin J, et al. Early prediction of severity in acute pancreatitis by urinary trypsinogen activation peptide: a multicentre study. Lancet. 2000;355(9219):1955-60.

39. Simoens CM, Deckelbaum RJ, Massaut JJ, Carpentier YA: Inclusion of 10% fish oil in mixed medium-chain triacylglycerol-long-chain triacylglycerol emulsions increases

plasma triacylglycerol clearance and induces rapid eicosapentaenoic acid (20:5n-3) incorporation into blood cell phospholipids. AmJClinNutr2008,88:282-288.

40. Y. Cao, Y. Xu, T. Lu, F. Gao, and Z. Mo, "Meta-analysis of enteral nutrition versus total parenteral nutrition in patients with severe acute pancreatitis," Annals of Nutrition and Metabolism, vol. 53, no. 3-4, pp. 268–275, 2009

410. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102-11.

421. Gou S, Yang Z, Liu T, Wu H, Wang C. Use of probiotics in the treatment of severe acute pancreatitis: a systematic review and meta-analysis of randomized controlled trials. Crit Care. 2014 Mar 31;18(2).

432. Maziar Gooshe, Amir Hossein Abdolghaffari, Shekoufeh Nikfar, Parvin Mahdaviani, and Mohammad Abdollahi. Antioxidant therapy in acute, chronic and post-endoscopic retrograde cholangiopancreatography pancreatitis: An updated systematic review and meta-analysis. World J Gastroenterol. 2015 Aug 14; 21(30): 9189–9208.

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Intravenous omega-3 fatty acids are associated with better clinical outcome and less inflammation in patients with predicted severe acute pancreatitis: A randomised double blind controlled trial

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Authors' contributions

DA-L, JAS, OA-T, MSM and ARD designed the study and conducted study approval processes. DA-L, AME, CP, MSM and ARD recruited patients and oversaw the intervention. DA-L, AME, AA, JAS and OA-T collected blood samples and collated the clinical data under the supervision of ARD. DA-L, AME and W-YC processed the blood samples and conducted laboratory assays. HLF performed fatty acid composition analyses under the supervision of PCC. DA-L, AME and GG conducted the statistical

analysis under supervision of ARD. DA-L drafted the manuscript; GG, PCC and ARD had significant input into the manuscript. All authors agreed upon and approved the final manuscript.

Conflict of Interest

DA-L, AA, JAS, OA-T, MSM and ARD received support from BBraun, Melsungen for investigational products (Lipidem® and Lipofundin®) used in this trial. PCC has received speaking honoraria from BBraun, Fresenius-Kabi, and Baxter Healthcare. The other authors have no competing interests

ABSTRACT

Background and Aims

Omega-3 fatty acids (FA) can ameliorate the hyper-inflammatory response that occurs in conditions such as severe acute pancreatitis (SAP) and this may improve clinical outcome. We tested the hypothesis that parenteral omega-3 FA from a lipid emulsion that includes fish oil could be beneficial in patients with predicted SAP by reducing C-reactive protein (CRP) concentration (primary outcome), and modulating the inflammatory response and improving clinical outcome (secondary outcomes).

Methods

In a phase II randomized double-blind single-centre controlled trial, patients with predicted SAP were randomised to receive a daily infusion of fish oil containing lipid emulsion (Lipidem® 20%, BBraun) for 7 days (n=23) or a daily infusion of a lipid emulsion without fish oil (Lipofundin® MCT 20%, BBraun) (n=22).

Results

On admission, both groups had comparable pancreatitis predicted severity and APACHE II scores. Administration of fish oil resulted in lower total blood leukocyte number (P=0.04), CRP (P=0.013), interleukin-8 (P=0.05) and intercellular adhesion molecule 1 (P=0.01) concentrations, multiple organ dysfunction score, sequential organ failure assessment score (P=0.004), early warning score (P=0.01), and systemic inflammatory response syndrome (P=0.03) compared to the control group. The fish oil group had fewer new organ failures (P=0.07), lower critical care admission rate (P=0.06)., shorter critical care stay (P=0.03) and shorter total hospital stay (P=0.04).

Conclusions

It is concluded that intravenous administration of a fish oil containing lipid emulsion, a source of omega-3 FA, improves clinical outcomes in patients with predicted SAP, benefits that may be linked to reduced inflammation.

ClinicalTrials.gov number: NCT01745861

EU Clinical Trials Register: EudraCT (2010-018660-16)

Keywords: C-reactive protein; organ failure; systemic inflammatory response syndrome; omega-3; fish oil; severe acute pancreatitis

Abbreviations used: CRP, C-reactive protein; DHA, docosahexenoic acid; EPA, eicosapentenoic acid; EWS: early warning score; SIRS, systemic inflammatory response syndrome; FA, fatty acid; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MCT, medium chain triglyceride; MODS, multiple organ dysfunction score; PC, phosphatidylcholine; SOFA, sequential organ failure assessment.

INTRODUCTION

Severe acute pancreatitis (SAP) is an inflammatory disorder of the pancreas with a potentially complicated clinical course and variable outcome ranging from complete resolution to multiple organ failure and death. Despite improvements in general and critical care management, morbidity and mortality from SAP remain high (1,2). An early severe systemic inflammatory response syndrome (SIRS) and multiple organ failure are considered to be responsible for most deaths in SAP (1,2). Consequently, current management of AP is focussed on meticulous supportive care and prevention of pancreatic necrosis, infection, and organ failure (3).

Nutrition support has traditionally focussed on supplying energy and micronutrients to the patient. Lipids are an important component of nutrition support because the fatty acid (FA) constituents of the lipids are good energy sources and reduce the need for carbohydrate. In intravenous nutrition support, lipids are present as stable emulsions. It is now appreciated that FA have biological activities related to their effects on membrane structure and function, production of signalling molecules, and regulation of gene expression (4). Consequently, the mix of FA within an intravenous lipid emulsion will influence the host's metabolic, immune and inflammatory responses (5,6). In this regard, the omega-3 FA found within fish oil, eicosapentenoic acid (EPA) and docosahexenoic acid (DHA), have a range of biological activities (5,7). The central mechanism of action of EPA and DHA relates to their incorporation into plasma lipids and into the membranes of cells and tissues from where they exert their biological actions (8). With regard to inflammation, EPA and DHA have multiple actions as discussed in detail elsewhere (9). As a result of these actions the omega-3 FA EPA and DHA may be useful in controlling SIRS and improving outcome in patients with SAP. Indeed, Wang et al. reported some benefits from parenteral nutrition with omega-3 FA

~~intravenous fish oil~~ in patients with SAP (10). However, parenteral nutrition in acute pancreatitis is not recommended routinely by the gastroenterology societies due to reports and studies that linked it to poor outcome (3). Therefore, there is a need for a well-designed study that test the effect of omega-3 FA in SAP in settings that resemble the daily clinical practice and in-line with the current pancreatitis management guidelines. In this study we investigated the effect of daily intravenous infusion of a lipid emulsion containing fish oil for seven days in patients with predicted SAP. The emulsion used has the commercial name Lipidem® or Lipoplus®. Lipidem® has been used previously in post-operative surgical (11-14) and critically ill septic (15,16) patients. In those studies, Lipidem® was found to decrease the concentrations of pro-inflammatory cytokines (13,16) and pro-inflammatory lipid mediators (11,13,16), to improve gas exchange (16) and to reduce the length of hospital stay (14). A different lipid emulsion based on fish oil (Omegaven®, Fresenius Kabi, Germany) has been used in patients with predicted SAP (10, 17, 18) , critically ill patients (19), septic patients (20,21) and post-operative surgical patients (22-24). In those studies, Omegaven® decreased pro-inflammatory cytokine concentrations (17, 20,22), increased anti-inflammatory cytokine concentrations (18), decreased pro-inflammatory lipid mediator concentrations (21,24), improved immune function (20-23) and improved clinical outcomes (17,20-23,25).

Both omega-3 FA emulsions (Lipidem® and Omegaven®) have demonstrated better outcome in various clinical settings but authors of this study opted to use Lipidem® to ease the randomisation and double blinding processes.

The hypothesis of the current study was that intravenous fish oil would lower serum CRP concentration. We have also evaluated plasma FAs, serum inflammatory markers and clinical outcomes.

PATIENTS AND METHODS

Study design and outcomes

This was a phase II, single center, double-blinded, randomized controlled trial conducted in accordance with the recommendations of the EEC Committee for Proprietary Medicinal Products (26~~11~~); the trial was registered as NCT01745861, received ethical approval from the Leicestershire, Northamptonshire and Rutland Research Ethics Committee and was approved by the Medicines and Healthcare Products Regulatory Agency (MHRA). The primary objective was to determine if intravenous fish oil given daily starting within 72 hours of the onset of symptoms of predicted SAP could reduce the concentration of the inflammatory marker C-reactive protein (CRP) by day 7. The secondary objectives were to assess the effects of the omega-3 rich fish oil emulsion on sequential organ failure assessment (SOFA) score (27~~12~~), multiple organ dysfunction score (MODS) (28~~13~~), early warning scores (EWS) (29~~14~~), SIRS (30~~15~~), development of new organ dysfunction, escalation of the patients' care, length of stay, circulating pro-inflammatory cytokines and adhesion molecules, and plasma phospholipid (phosphatidylcholine (PC)) EPA, DHA and arachidonic acid levels. Sepsis was defined as the presence of infection, documented or strongly suspected, with one or more SIRS features (31~~16~~). Patients were assessed daily for 7 days for any adverse events or complications. Severity scores and blood samples were obtained on days 0, 1, 2, 3, 5 and 7 of the infusion.

Inclusion and exclusion criteria

All conscious patients aged 18 to 90 years admitted with predicted SAP proven by compatible clinical features (abdominal pain with or without vomiting) associated with

amylase activity at least three times greater than the upper limit of the normal value and one or more of the severity criteria as outlined in the Atlanta severity criteria for AP (32~~17~~) or Glasgow (Imrie) score ≥ 3 were considered eligible for the study. Patients were excluded for any of the following reasons: age < 18 or > 90 years; unconscious or unable to consent; allergic to fish, egg or soy protein; uncontrolled hyperlipidemia; severe primary blood coagulation disorders; acute pancreatitis accompanied with hyperlipidemia; ketoacidosis; acute thromboembolic disease; severe liver failure; acute phase of myocardial infarction or stroke; pregnancy or lactation; severe renal failure without access to hemofiltration or dialysis.

The intervention

Forty-five patients admitted to University Hospitals of Leicester NHS Trust with predicted SAP were randomized into two groups, fish oil and control. Patients were allocated to either group by a computer-based randomization system (Wellspring Clinical Services, Doncaster, UK; see Supplementary Material). The fish oil group (n = 23, one patient withdrew consent prior to any intervention) received a lipid emulsion enriched with omega-3 FAs (Lipidem® 200 mg/ml: 50% medium chain triglycerides (MCT), 40% soybean oil and 10% fish oil; B Braun, Melsungen, Germany). The control group (n = 22) received an isocaloric lipid emulsion without fish oil (Lipofundin® 200 mg/ml; 50% MCT and 50% soybean oil; B Braun, Melsungen, Germany). Lipidem® and Lipofundin® were infused at a rate of 10 ml per kg body weight over 14 hours each day for a maximum of 7 days or until the patient was clinically fit for discharge if sooner; this corresponds to 2 g lipid per kg body weight over 14 hours each day. Standard management for these patients continued. Patients were withdrawn if serum triglycerides persisted above 3 mmol/L despite temporary cessation of lipid infusion.

184

185 **Laboratory analyses**

186 Routine hematology, biochemistry (including serum CRP), coagulation, random lipid
187 profiles, urine analysis and arterial blood gases were performed at University Hospitals
188 of Leicester NHS Trust laboratories. Serum and plasma samples were stored at –80°C.
189 Pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8)
190 and the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) were measured
191 in serum using an ultra-sensitive multi-array assay (Meso Scale Discovery,
192 Gaithersburg, MD, USA). Fatty acid composition of plasma PC was determined by gas
193 chromatography as described elsewhere (33~~18~~).

194

195 **Randomisation and blinding processes**

196 The randomisation and blinding processes was assigned to an independent
197 pharmaceutical company "Wellspring Clinical Services, Doncaster, England, UK", which
198 has created sequential kit numbers. Lipidem® and Lipofundin® bottles were randomly
199 allocated to these kit numbers. Consecutive patients entering the trial were allocated to
200 the sequential kit number provided by Wellspring. This process was created prior to the
201 start of the study.
202 Wellspring Clinical Services also created the over-labels, essential for the blinding
203 process, and this was approved by MHRA, study sponsor and research team.
204 A clear protocol, developed by Wellspring Clinical Services, with description of the
205 conditions and procedures for emergency unblinding was available within the
206 pharmacy department. Trial pharmacists or the on-call pharmacists (all blinded) can
207 only do the unblinding process after following the unblinding protocol. Both patients
208 and research team were also blinded throughout the study. The randomisation and

blinding procedures were not compromised in this study. The blinding procedure and all aspects of the trial were inspected and agreed upon by MHRA auditors.

Power calculation and statistical methods

Based upon the existing literature (19,20), we considered that a 20% lower mean concentration of serum CRP in the fish oil group than in the control group at day 7 (trial exit point) would be clinically meaningful. Assuming an SD of 15 mg/L in CRP concentration it was calculated that 22 patients would give 90% power to identify a significant effect given α=0.05.

Continuous variables are presented as mean ± SEM and categorical variables as numbers and percentages. D'Agostino & Pearson omnibus normality test was used to determine the distribution of continuous data. Normally distributed data were analyzed using the 2-tailed Student's *t* test and non-normally distributed data were analyzed using the Mann-Whitney *U* test. Categorical data were analysed using the χ^2 test and Fisher's exact probability test. Mann-Whitney *U* or 2-tailed *t* tests were used for comparisons between time points and for comparisons between groups at a particular time point. Differences in parameters between the fish oil and control groups during the 7 days of intervention were tested for significance by 2-factor (time × treatment) repeated measures (RM) ANOVA followed by post-hoc analysis using Bonferroni's correction for multiple comparisons. In all cases, a value of $P < 0.05$ was taken to indicate statistical significance. Statistical analyses were performed using Prism 6 (version 6.0e, 1994 – 2014 GraphPad Software, Inc.). Data were analysed with the intention-to-treat and analysis was performed only after study completion and before unblinding. All patients that received intervention were included in the analysis and missing data were treated by the last observation carried forward (LOCF) approach.

RESULTS

Patient demographics

One hundred and ninety-eight patients with AP were admitted to the study centre (Leicester, UK). One hundred and thirty nine patients did not meet SAP inclusion criteria, 3 patients had one or more exclusion criteria and 11 patients refused to participate in the trial (Figure 1). The remaining 45 patients were randomized to the fish oil group (n=23) or the control group (n=22). One patient in the fish oil group withdrew prior to any intervention and was therefore excluded from the results (Figure 1). Five patients did not finish the 7 day trial period: two patients in the fish oil group withdrew on day 3 and 1 patient in the control group withdrew on day 5; one patient in the fish oil group had features of haemorrhagic severe pancreatitis and died on day 2; one patient in the control group was deemed to be fit for discharge on day 5. Missing data for these patients were substituted by their last observation.

Patient demographics and baseline clinical characteristics in the fish oil and control groups are shown in Table 1. The mean baseline triglyceride level was 1.9 ± 0.7 and 1.5 ± 0.3 mmol/L in the fish oil and control groups, respectively.

The average caloric contents of Lipidem® and Lipofundin® are around 1900 kcal/l and this does not meet the hyper catabolic state of this disease with estimated energy needs of 30-35 kcal/kg/day. Researchers observed that early oral nutrition was encouraged to all patients without restrictions. However, if oral nutrition was deemed to be inadequate, NG or NJ feed was subsequently started in these patients. Five patients (two in treatment group and 3 in the control group) deemed to have inadequate oral intake and feeding via NG tube was started at different time points in the trial (earliest was day 3 and the latest was day 6). Nutrison® 1kcal/ml (see appendices) was the standard

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enteral nutrition regimen used by the dietician and clinical team. -Two patients (one in fish oil group and one in control group) had prolonged ileus and NG/NJ feed deemed to be inadequate, ~~total~~ parenteral nutrition (TPN) was subsequently started on day 5 and 6 respectively. Triomel® Baxter standard PN was the main parenteral nutrition given to these patients. The decision about the enteral or parenteral nutrition laid~~lay~~ between patients' clinicians and on-call dietician. The on-call dieticians reviewed and adjusted NG/NJ feed or TPN to accommodate the administration of Lipidem®/Lipofundin®.

Primary outcome measure: CRP concentration

On admission, there was no significant difference in CRP concentrations between the two groups (148.5 ± 30.5 mg/L in the fish oil group and 142.9 ± 31.6 mg/L in the control group (P=0.90)). Two factor ANOVA revealed that CRP concentration changed over time (P=0.004) and was different between treatment groups (P=0.013) (Figure 2). At day 7, CRP concentration was 34% lower in the fish oil than in the control group; however the concentrations (104.1 ± 23 mg/L in the fish oil group and 157.6 ± 26.8 mg/L in control group) were not significantly different (P=0.15).

Secondary outcome measures: serology

Many serological parameters changed significantly over time (Table 2), only HCO_3 concentration was affected by fish oil treatment (P=0.03), although there was a strong trend for an effect of treatment group on total blood leukocyte count (P=0.08). At each time point, the fish oil group had lower blood leukocyte number than the control group, but the difference only reached statistical significance on day 7 (P=0.04) (Table 2).

Secondary outcome measures: organ failure scores (MODS and SOFA), EWS and SIRS

On admission, the number of patients with one or more organ failure was 8 (36%) in the fish oil group and 6 (27%) in the control group. There was a strong trend for fewer patients in the fish oil group to develop new organ failure (6 (27%) vs 13 (59%); (P=0.07). SOFA (P=0.03), EWS (P<0.001) and SIRS (P<0.001) all decreased over time (Figure 3). Fish oil affected MODS (P=0.03), SOFA (P=0.004), EWS (P=0.01) and SIRS (P=0.03), which were all lower in the fish oil group than the control group (Figure 3). EWS was lower in the fish oil group at days 1 (P=0.01), 2 (P=0.05) and 3 (P=0.04) and tended to be lower at day 5 (P=0.08). SIRS was lower in the fish oil group at days 1 (P=0.01) and 2 (P=0.05) and tended to be lower at days 5 and 7 (both P=0.05).

Secondary outcome measures: septic complications

Eleven (50%) patients in the control group developed sepsis compared with 8 (36%) in the fish oil group, but the groups were not significantly different (P=0.36). The median duration of antibiotic administration (intravenous or oral) was shorter in the fish oil group than the control group: 5 [95% CI, 3.3 to 5.3] days vs 10 [95% CI, 7.2 to 16.7] days, respectively (P<0.01).

Secondary outcome measures: escalation of care and length of stay

Fewer patients in the fish oil group (n=5 (23%)) than in the control group (n=11 (50%)) required escalation of their care from a normal ward to a higher-level of care (intensive/critical care or high dependency units), a difference that approached statistical significance (P=0.06). The median length of stay (LOS) in a higher-level of care (intensive/critical care or high dependency units) was 3 [95% CI, -0.9 to 6.9] days in the fish oil group compared with 9 [95% CI, 6.7 to 23.4] days in the control group (P=0.03). The median inpatient (hospital) stay was also shorter in the fish oil group

than the control group: 12 [95% CI, 9.6 to 15.3] days vs 18 [95% CI, 15.5 to 27.2] days, respectively (P=0.04).

Secondary outcome measures: serum cytokines and ICAM-1

There were no differences between groups at study entry for the serum concentrations of any of the cytokines or intercellular adhesion molecule (ICAM)-1. There was a significant effect of time on the concentrations of TNF-α (P=0.006), IL-8 (P<0.001) and ICAM-1 (P=0.04) with a trend towards an effect on IL-6 concentration (P=0.08) (Figure 4). The concentrations of TNF-α and IL-8 declined over time (Figure 4). There was an effect of treatment on the concentration of IL-8 (P=0.05) and ICAM-1 (P=0.01), which was lower in the fish oil group (Figure 4).

Secondary outcome measure: plasma phosphatidylcholine (PC) fatty acid composition

Plasma PC contributes about 75% of plasma phospholipid and acts as a transporter for FAs including EPA, DHA and arachidonic acid to target cells and tissues such as leukocytes (36~~21~~). There was a significant effect of time (P=0.03) and treatment group (P=0.001) and a significant time x treatment group interaction for plasma PC EPA (P=0.002) (Figure 5). Plasma PC EPA was significantly higher in the fish oil group at days 1, 2, 3, 5, 7 (Figure 5). In contrast, neither DHA nor arachidonic acids were affected by time or treatment group (Figure 5).

Safety and tolerability of the lipid emulsions

Both emulsions were well tolerated with no unexpected severe adverse events occurring. One critically ill patient in the control group developed transient hypertriglyceridaemia, which resolved after temporary cessation of the lipid infusion.

Mean post-infusion serum triglycerides and random cholesterol levels did not differ significantly between groups at any time point (data not shown). There were 2 deaths, one on day 2 in the fish oil group and the one just after exiting the trial in the control group. Both patients had severe multiple organ dysfunction syndrome.

DISCUSSION

This study is the first prospective randomized double-blind controlled trial conducted with omega-3 fatty acid rich fish oil containing lipid emulsion in patients with predicted SAP. ~~The emulsion used has the commercial name Lipidem® or Lipoplus®. Lipidem® has been used previously in post-operative surgical (22-25) and critically ill septic (26, 27) patients. In those studies, Lipidem® was found to decrease the concentrations of pro-inflammatory cytokines (24, 27) and pro-inflammatory lipid mediators (22, 24, 27), to improve gas exchange (27) and to reduce the length of hospital stay (25). A different lipid emulsion based on fish oil (Omegaven®, Fresenius Kabi, Germany) has been used in patients with predicted SAP (10, 28, 29), critically ill patients (30), septic patients (31, 32) and post-operative surgical patients (33-35). In those studies, Omegaven® decreased pro-inflammatory cytokine concentrations (28, 31, 33), increased anti-inflammatory cytokine concentrations (29), decreased pro-inflammatory lipid mediator concentrations (32, 35), improved immune function (31-34) and improved clinical outcomes (28, 31-34, 36).~~ In the current study, administration of Lipidem® resulted in less inflammation, less severe disease, fewer new organ failures, lower critical care admission rate, shorter critical care stay and shorter total hospital stay compared to the control group.

Three inflammatory markers that were lower, or tended to be lower, with fish oil were blood leukocyte count and serum concentrations of CRP and IL-8. CRP is a non-specific

marker of inflammation that is synthesized by liver cells (37) and its concentration rises in a variety of inflammatory conditions. IL-6 and IL-1 trigger its synthesis and it has widely been used as a predictor of the progression of an episode of moderate AP to SAP (37). The specificity, sensitivity, and positive and negative predictive values of CRP in predicting the severity of AP at 48 hours from the onset are 86%, 61%, 37%, 94%, respectively, and the positive likelihood ratio is 2.2 (38). The hypothesis of the current study was that intravenous fish oil would lower serum CRP concentration. CRP concentration was selected as the primary outcome because fish oil derived omega-3 FA are known to be anti-inflammatory (9) and because a reduction in CRP should be associated with less severe disease and improved clinical outcome in patients with predicted SAP. In accordance with the existing literature, CRP concentration was highest at day one and then declined. Peak concentrations did not differ between control and fish oil groups. After day one there was a steady decrease in CRP concentrations in both groups but with a more marked reduction in the fish oil group (one way ANOVA effect of treatment P=0.013). This observation supports the primary hypothesis of the study.

The observed reduction in inflammation in the fish oil group was linked with lower organ dysfunction scores, as measured by SOFA and MODS, and lower scores for SIRS and EWS. This finding supports data from a study of the same lipid emulsion in critically ill septic patients (6, 12~~27~~).

The current study demonstrates a likely clinical benefit of an intravenous fish oil emulsion on the SIRS score at an early stage of predicted SAP (see Figure 3). On admission, both groups had similar SIRS scores and although this reduced steadily in both groups, the reduction was more pronounced in the fish oil group. This is

383 consistent with previous report by Wang et al. who used parenteral Omegaven®
384 infusion and demonstrated an improvement of SIRS in SAP (10).
385 In the current study, the seven days infusion with a lipid emulsion containing EPA
386 markedly increased plasma PC EPA by an average of 4.6-fold from baseline;
387 interestingly there was no significant increase in plasma PC DHA. This is consistent with
388 findings from another study where an average 3.8-fold increase in EPA in plasma
389 phospholipids was observed in critically ill septic patients receiving Lipidem® for five
390 days (12~~27~~). In that study there was also a tendency for better clinical outcome and
391 shorter length of stay in critically ill septic patients (12~~27~~). Likewise, Barbosa et al. and
392 Simoens et al. both observed no significant changes in DHA and AA levels and this
393 confirms previous suggestions that better clinical outcome is associated with increased
394 EPA status (12~~27~~, 39).
395 Xiong et al and Wang et al examined the effects of omega-3 FA in patients with SAP (10,
396 17, 18). The treatment groups in all three studies received parenteral nutrition with
397 omega-3 FA where as the control groups either received conventional supportive
398 treatment or parenteral nutrition without omega-3 FA. In all studies, there were better
399 inflammatory response and clinical outcome in the pancreatitis group. However, the
400 main concern about these studies is that parenteral nutrition is a pro-inflammatory and
401 has shown to increase morbidity and mortality in patients with SAP (40). Furthermore,
402 the current acute pancreatitis guidelines strongly discourage the routine use of
403 parenteral nutrition in AP patients. They recommend enteral nutrition as a first line in
404 nutrition and parenteral nutrition is only reserved to patients that cannot tolerate
405 enteral nutrition. In the above studies parenteral nutrition was routinely used in most
406 patients and this is not in-line with daily clinical practice and the current guidelines (3).
407 We are therefore, finding it difficult to ascertain the outcomes in the above studies are

purely to omega-3 FA.

This study has several strengths. First, it was randomized, double blind and controlled. Secondly, the withdrawal rate was low. Thirdly, a range of laboratory and clinical outcomes was measured. Fourthly, omega-3 FA status was measured alongside the laboratory and clinical outcomes. Finally, parenteral nutrition was not given routinely to all patients and this is a resemblance of the daily practice and in-line with current recommendations by the gastroenterology society.

The study also has limitations. First, the sample size was quite low and larger trials will be needed to confirm the many positive findings made before they can be transferred to clinical practice. Secondly, the primary outcome was a laboratory measure (serum CRP concentration) rather than a clinical outcome, although a number of the latter were assessed as secondary outcomes. Thirdly, this was a single centre study and therefore patient management was fairly homogeneous and may not fully reflect practice across many centres. However, the observed management of AP was in conjunction with the British Society of Gastroenterology AP management guidelines (3). Fourthly, the utilisation of the LOCF approach to replace missing data is a simple process to understand but has some disadvantages such as introduction of bias. However, this method was deemed to be suitable in handling the small proportion of missing data. Subgroup analysis was not performed due the small sample size. Fifthly, we have compared two lipid emulsions and our study does not consider whether lipids *per se* will have an impact on inflammation or clinical outcome. Finally, the original Atlanta criteria were revised just after the recruitment process of the current study concluded (41~~40~~). Nevertheless, it is the authors' view that the revised Atlanta criteria would have no impact on the main objective and outcomes of the current study.

The current study favours the administration of omega-3 FA for clinical benefit in

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patients with predicted SAP. Systematic reviews and meta-analyses of other immune-modulatory agents (probiotics and anti-oxidants) revealed no beneficial effect on clinical outcome in patients with predicted SAP (42,43~~41,42~~). It is possible that the success of the current study is related to several factors including the natural components of the product used, global immune-modulatory mechanisms of action of amoega-3 FA, the early intervention and the optimisation of clinical care. Further larger studies are certainly warranted but challenges with recruitment, randomisation, costs, early intervention and potential bias need to be addressed.

CONCLUSION

It is concluded that intravenous administration of a fish oil containing lipid emulsion, a source of the bioactive omega-3 fatty acids EPA and DHA, results in fewer new organ failures, better recovery, and shorter critical care and hospital stay in patients with SAP, clinical benefits that may be linked to reduced inflammation. Larger scale, multi-centre trials investigating short and long term effects of intravenous fish oil on pancreatic late complications, progression to the disabling chronic pancreatitis, and mortality are recommended.

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References

1. Maheshwari R, Subramanian RM. Severe acute pancreatitis and necrotizing pancreatitis. Crit Care Clin. 2016;32(2):279-90 .

2. Beger HG, Rau BM. Severe acute pancreatitis: Clinical course and management. World journal of gastroenterology : WJG. 2007;13(38):5043-51.

3. Working Party of the British Society of G, Association of Surgeons of Great Britain and I, Pancreatic Society of Great Britain and I, Association of Upper GISoGBaI. UK guidelines for the management of acute pancreatitis. Gut. 2005;54 Suppl 3:iii1-9.

4. Calder PC. Functional roles of fatty acids and their effects on human health. J Parenter Enteral Nutr. 2015;39:18S-32S.

5. Calder PC. Rationale for using new lipid emulsions in parenteral nutrition and a review of the trials performed in adults. Proc Nutr Soc. 2009;68(3):252-60.

6. Calder PC. Lipids for intravenous nutrition in hospitalised adult patients: a multiple choice of options. Proc Nutr Soc. 2013;72(3):263-76.

7. Calder PC. Very long chain omega-3 (n-3) fatty acids and human health. European Journal of Lipid Science and Technology. 2014;116(10):1280-300.

8. Calder PC. Mechanisms of action of (n-3) fatty acids. J Nutr. 2012;142(3):592S-9S.

9. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015;1851(4):469-84.

10. Wang X, Li W, Li N, Li J. Omega-3 fatty acids-supplemented parenteral nutrition decreases hyperinflammatory response and attenuates systemic disease sequelae in severe acute pancreatitis: a randomised and controlled study. Journal of parenteral and enteral nutrition. 2008;32(3):236-41.

11. Koller M, Senkal M, Kemen M, Konig W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. Clinical Nutrition. 2003;22(1):59-64.

12. Senkal M, Haaker R, Linseisen J, Wolfram G, Homann HH, Stehle P. Preoperative oral supplementation with long-chain Omega-3 fatty acids beneficially alters phospholipid fatty acid patterns in liver, gut mucosa, and tumor tissue.Journal of parenteral and enteral nutrition. 2005;29(4):236-40.

13. Wachtler P, König W, Senkal M, Kemen M, Köller M. Influence of a total parenteral nutrition enriched with omega-3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. J Trauma. 1997;42(2):191-8.

14. Wichmann MW, Thul P, Czarnetzki HD, Morlion BJ, Kemen M, Jauch KW. Evaluation of clinical safety and beneficial effects of a fish oil containing lipid emulsion (Lipoplus, MLF541): data from a prospective, randomised, multicenter trial. Crit Care Med. 2007;35(3):700-6.

15. Tappy L, Berger MM, Schwarz JM, Schneiter P, Kim S, Revelly JP, et al. Metabolic effects of parenteral nutrition enriched with n-3 polyunsaturated fatty acids in critically ill patients. Clin Nutr. 2006;25(4):588-95.

16. Barbosa VM, Miles EA, Calhau C, Lafuente E, Calder PC. Effects of a fish oil containing lipid emulsion on plasma phospholipid fatty acids, inflammatory markers, and clinical outcomes in septic patients: a randomised, controlled clinical trial. Crit Care. 2010;14(1):R5.

17. Xiong J, Zhu S, Zhou Y, Wu H, Wang C. Regulation of omega-3 fish oil emulsion on the SIRS during the initial stage of severe acute pancreatitis. Journal of Huazhong University of Science and TechnologyMedical sciences = Hua zhong ke ji da xue xue baoYi xue Ying De wen ban = Huazhong keji daxue xuebaoYixue Yingdewen ban. 2009;29(1):35-8.

18. Wang X, Li W, Zhang F, Pan L, Li N, Li J. Fish oil-supplemented parenteral nutrition in severe acute pancreatitis patients and effects on immune function and infectious risk: a randomised controlled trial. Inflammation. 2009;32(5):304-9

19. Friesecke S, Lotze C, Köhler J, Heinrich A, Felix SB, Abel P. Fish oil supplementation in the parenteral nutrition of critically ill medical patients: a randomised controlled trial. Intensive Care Med. 2008;34(8):1411-20.

20. Mayer K, Gokorsch S, Fegbeutel C, Hattar K, Rosseau S, Walmrath D, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. Am J Respir Crit Care Med. 2003;167(10):1321-8.

21. Mayer K, Fegbeutel C, Hattar K, Sibelius U, Kramer HJ, Heuer KU, et al. Omega-3 vs. omega-6 lipid emulsions exert differential influence on neutrophils in septic shock patients: impact on plasma fatty acids and lipid mediator generation. Intensive care medicine. 2003;29(9):1472-81.

22. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr. 2002;87 Suppl 1:S89-94.

23. Schauder P, Rohn U, Schafer G, Korff G, Schenk HD. Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period. The British journal of nutrition. 2002;87 Suppl 1:S103-10.

24. Morlion BJ, Torwesten E, Lessire H, Sturm G, Peskar BM, Fürst P, et al. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. Metabolism. 1996;45(10):1208-13.

25. Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. Clin Nutr. 2004;23(3):325-30.

26~~11~~. EEC note for guidance: good clinical practice for trials on medicinal products in the European Community. CPMP Working Party on Efficacy of Medicinal Products. Pharmacology & toxicology. 1990;67(4):361-72.

27~~12~~. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Medicine. 1996;22(7):707-10.

28~~13~~. Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Critical Care Medicine. 1995;23(10):1638-52.

29~~14~~. Garcea G, Jackson B, Pattenden CJ, Sutton CD, Neal CP, Dennison AR, et al. Early warning scores predict outcome in acute pancreatitis. Journal of Gastrointestinal Surgery. 2006;10(7):1008-15.

30~~15~~. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644-55.

31~~16~~. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Critical Care Medicine. 2003;31(4):1250-6.

32~~17~~. Bradley EL, 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Archives of Surgery. 1993;128(5):586-90.

33~~18~~. Fisk HL, West AL, Childs CE, Burdge GC, Calder PC. The use of gas chromatography to analyze compositional changes of fatty acids in rat liver tissue during pregnancy. Journal of Visulaised Experiments. 2014(85).

34~~19~~. Muller CA, Uhl W, Printzen G, Gloor B, Bischofberger H, Tcholakov O, et al. Role of procalcitonin and granulocyte colony stimulating factor in the early prediction of infected necrosis in severe acute pancreatitis. Gut. 2000;46(2):233-8.

~~35~~20. Digalakis MK, Katsoulis IE, Biliri K, Themeli-Digalaki K. Serum profiles of C-reactive protein, interleukin-8, and tumor necrosis factor-alpha in patients with acute pancreatitis. HPB Surg. 2009;2009:878490.

~~36~~21. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids. 2000;35(12):1305-12.

~~22. Koller M, Senkal M, Kemen M, Konig W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. Clinical Nutrition. 2003;22(1):59-64.~~

~~23. Senkal M, Haaker R, Linseisen J, Wolfram G, Homann HH, Stehle P. Preoperative oral supplementation with long-chain Omega-3 fatty acids beneficially alters phospholipid fatty acid patterns in liver, gut mucosa, and tumor tissue.Journal of parenteral and enteral nutrition. 2005;29(4):236-40.~~

~~24. Wachtler P, König W, Senkal M, Kemen M, Köller M. Influence of a total parenteral nutrition enriched with omega 3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. J Trauma. 1997;42(2):191-8.~~

~~25. Wichmann MW, Thul P, Czarnetzki HD, Morlion BJ, Kemen M, Jauch KW. Evaluation of clinical safety and beneficial effects of a fish oil containing lipid emulsion (Lipoplus, MLF541): data from a prospective, randomised, multicenter trial. Crit Care Med. 2007;35(3):700-6.~~

26. Tappy L, Berger MM, Schwarz JM, Schneiter P, Kim S, Revelly JP, et al. Metabolic effects of parenteral nutrition enriched with n-3 polyunsaturated fatty acids in critically ill patients. Clin Nutr. 2006;25(4):588-95.

27. Barbosa VM, Miles EA, Calhau C, Lafuente E, Calder PC. Effects of a fish oil containing lipid emulsion on plasma phospholipid fatty acids, inflammatory markers, and clinical outcomes in septic patients: a randomised, controlled clinical trial. Crit Care. 2010;14(1):R5.

28. Xiong J, Zhu S, Zhou Y, Wu H, Wang C. Regulation of omega-3 fish oil emulsion on the SIRS during the initial stage of severe acute pancreatitis. Journal of Huazhong University of Science and TechnologyMedical sciences = Hua-zhong ke ji da xue xue baoYi xue Ying De wen ban = Huazhong keji daxue xuebaoYixue Yingdewen ban. 2009;29(1):35-8.

29. Wang X, Li W, Zhang F, Pan L, Li N, Li J. Fish oil-supplemented parenteral nutrition in severe acute pancreatitis patients and effects on immune function and infectious risk: a randomised controlled trial. Inflammation. 2009;32(5):304-9

30. Friesecke S, Lotze C, Köhler J, Heinrich A, Felix SB, Abel P. Fish oil supplementation in the parenteral nutrition of critically ill medical patients: a randomised controlled trial. Intensive Care Med. 2008;34(8):1411-20.

31. Mayer K, Gokorsch S, Fegbeutel C, Hattar K, Rosseau S, Walmrath D, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. Am J Respir Crit Care Med. 2003;167(10):1321-8.

32. Mayer K, Fegbeutel C, Hattar K, Sibelius U, Kramer HJ, Heuer KU, et al. Omega-3 vs. omega-6 lipid emulsions exert differential influence on neutrophils in septic shock

patients: impact on plasma fatty acids and lipid mediator generation. Intensive care medicine. 2003;29(9):1472-81.

33. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr. 2002;87 Suppl 1:S89-94.

34. Schauder P, Rohn U, Schafer G, Korff G, Schenk HD. Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period. The British journal of nutrition. 2002;87 Suppl 1:S103-10.

35. Morlion BJ, Torwesten E, Lessire H, Sturm G, Peskar BM, Fürst P, et al. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. Metabolism. 1996;45(10):1208-13.

36. Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. Clin Nutr. 2004;23(3):325-30.

37. Pepys MB. C-reactive protein fifty years on. Lancet. 1981;1(8221):653-7.

38. Neoptolemos JP, Kemppainen EA, Mayer JM, Fitzpatrick JM, Raraty MG, Slavin J, et al. Early prediction of severity in acute pancreatitis by urinary trypsinogen activation peptide: a multicentre study. Lancet. 2000;355(9219):1955-60.

39. Simoens CM, Deckelbaum RJ, Massaut JJ, Carpentier YA: Inclusion of 10% fish oil in mixed medium-chain triacylglycerol-long-chain triacylglycerol emulsions increases

plasma triacylglycerol clearance and induces rapid eicosapentaenoic acid (20:5n-3) incorporation into blood cell phospholipids. AmJClinNutr2008,88:282-288.

40. Y. Cao, Y. Xu, T. Lu, F. Gao, and Z. Mo, "Meta-analysis of enteral nutrition versus total parenteral nutrition in patients with severe acute pancreatitis," Annals of Nutrition and Metabolism, vol. 53, no. 3-4, pp. 268–275, 2009

410. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102-11.

421. Gou S, Yang Z, Liu T, Wu H, Wang C. Use of probiotics in the treatment of severe acute pancreatitis: a systematic review and meta-analysis of randomized controlled trials. Crit Care. 2014 Mar 31;18(2).

432. Maziar Gooshe, Amir Hossein Abdolghaffari, Shekoufeh Nikfar, Parvin Mahdaviani, and Mohammad Abdollahi. Antioxidant therapy in acute, chronic and post-endoscopic retrograde cholangiopancreatography pancreatitis: An updated systematic review and meta-analysis. World J Gastroenterol. 2015 Aug 14; 21(30): 9189–9208.

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