UNIVERSITY OF Southampton

PROTOCOL

Final version 3.0: 5th March 2019

Title of study

Influence of an omega-3 fatty acid triglyceride formulation on EPA and DHA appearance in human plasma after single dosing

Short title

Incorporation of omega-3 fatty acids

Study identifiers

REC number: 19/LO/0939
R&D number: NUT0070
Sponsors number: CTN800218102

Sponsor of the study

BASF AS, Lilleakerveien 2c, 0283 Oslo, Norway

Principal investigator

Professor P.C. Calder, Human Development & Health, Faculty of Medicine, University of Southampton, IDS Building. MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
Email: pcc@soton.ac.uk
Phone: +44 (0)2381205250

PI Signature

5 March 2019
Date

Places where research will be conducted

IDS Building, Faculty of Medicine, University of Southampton, Southampton, UK

NIHR Clinical Research Facility, University Hospital Southampton NHS Foundation Trust. Southampton General Hospital, Southampton, UK

Researchers involved

Professor Philip C. Calder, Human Development & Health, Faculty of Medicine, University of Southampton, IDS Building. MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
Email: pcc@soton.ac.uk
Phone: +44 (0)2381 205250

Miss Helena Fisk, Human Development & Health, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
Email: H.fisk@soton.ac.uk
Phone: +44 (0)2381 205252

Research nurses at the NIHR Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, Southampton General Hospital, Southampton SO16 6YD, UK
Phone: +44 (0)2381 204989

Protocol Information

This protocol describes the above study and provides information about procedures for entering study participants and the procedures involved once they are entered. Amendments may be necessary and will be signed by the investigator and BASF AS.

Compliance

This study will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It is subject to University Hospital Southampton NHS Foundation Trust R&D approval and will be conducted in compliance with the protocol, the Data Protection Act and all other regulatory requirements, as appropriate.

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse Event
DHA	Docosahexaenoic acid
EE	Ethyl Ester
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
GCP	Good Clinical Practice
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICH GCP	International Conference on Harmonisation of Good Clinical Practice
IFS	International Food Standard
MHRA	Medicines and Healthcare products Regulatory Authority
NHS	National Health Service
PI	Principal investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SSAR	Suspected serious adverse reaction
SUSAR	Suspected unexpected serious adverse reaction
TG	Triglyceride
UHS	University Hospital Southampton NHS Foundation Trust

Keywords

Omega-3 fatty acids; Fish oil; Omega-3 index; Fatty acid status; Emulsification, Bioavailability; Absorption; Kinetics

Project synopsis

Full title	Influence of an omega-3 fatty acid triglyceride formulation on EPA and DHA appearance in human plasma after single dosing
Sponsor	BASF AS
REC number	19/LO/0939
R&D number	NUT0070
Sponsor reference number	CTN800218102
Principal Investigator	Professor Philip Calder
Study phase if not mentioned in title	Biodistribution study of dietary supplement
Funder	BASF AS
Project Type:	Random order blinded cross-over trial in healthy human volunteers
Primary Objective:	To follow the change in omega-3 fatty acids (EPA and DHA) hourly between 1-12 hours in plasma after single dosing with fish oil concentrates as a standard ethyl-ester form and a triglyceride formulation.
Rationale:	Omega-3 fatty acids from fish oils are associated with improved human health. Poor absorption of the fatty acids from standard supplements may limit effectiveness. Pre-emulsification of the oil delivering the n-3 fatty acids results in higher blood levels of EPA and DHA after both single dosing and repeated daily dosing. Most omega-3 oil is in the triglyceride form. By preparing a triglyceride formulation that can promote oil emulsification might promote EPA and DHA delivery to the bloodstream. This will be tested by head-to-head comparison of standard ethyl-ester oil and a triglyceride oil formulation.
Study Design:	Blinded, random order, single centre, comparative study with a cross-over design.
Inclusion Criteria:	• Healthy males and females • Age 50 to 70 years • Body mass index 20 to 35 kg/m^2 • Not eating more than one oily fish meal per week • Willing to adhere to the study protocol • Being able to provide written informed consent • Omega-3 index $\leq$6.5 at screening visit
Exclusion Criteria:	• Being diabetic (type 1 or type 2) • Being vegetarian or vegan and unwilling to

	consume capsules with a beef gelatine coating • Use of prescribed medicine to control inflammation • Smokers • Alcohol consumption > 14 units/week • Chronic gastrointestinal problems (e.g. IBD, IBS, celiac disease, cancer) • Allergic to fish • Use of fish oil or other oil supplement • Participation in another clinical trial (currently or in the 12 weeks prior to study entry) • Pregnancy or lactation • Blood donations during 3 months prior to or during the study period
Total No. of Sites:	One
Study Duration	Subjects will receive a single dose of one of two oil formulations at two single dosing visits separated by a 2 week washout period. Each dosing visit will last 12 hours.
Study dose	4 capsules at each of the 2 single dosing visits
Data collection	Age, height, weight, general health status
Biological samples	Blood samples (to prepare plasma)
Number of participants	20 healthy subjects
Primary endpoint	Concentration of EPA and DHA in plasma
Secondary endpoint	Tolerability
Statistical Methods:	Repeated-measures two-factor analysis of variance (Factors: time and treatment group) with subsequent comparisons over time within groups (one-factor analysis) or between groups at each time (one-factor analysis). Subsequent pair-wise comparisons as appropriate.

1. Introduction

1.1. Background information

1.1.1 The main research question

The two long chain omega-3 (n-3) fatty acids of most importance to human health are eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). These fatty acids have been shown to lower risk of morbidity and mortality from cardiovascular disease [1-3]. They exert their protective effects by beneficially altering some of the recognised cardiovascular risk factors [1,2,4]. These fatty acids also exert benefit in inflammatory conditions [5] and perhaps in some cancers [6]. There is emerging evidence that they are important in development of learning and behaviour in childhood [7], in preventing psychiatric and psychological disorders in adults [8] and in slowing cognitive decline in the elderly [9]. As a result of these beneficial effects on human health, particularly the cardioprotective effects, there have been recommendations that individuals should increase their intake of long chain n-3 fatty acids [1,10-14]. The only naturally rich source of EPA and DHA is seafood, especially oily fish. Thus, one strategy to increase intake of these fatty acids is to increase fish consumption, and there are recommendations to do so [13,14]. However, many consumers are resistant to taking this option despite the likely benefit. Also the n-3 fatty acid content is highly variable amongst fish species and even within species depending upon time of year, location at which caught etc. Thus, consumption of oily fish once or twice a week, as recommended, results in irregular intake of an unknown (to the consumer) amount of EPA plus DHA. In addition, some fish species are contaminated with heavy metals and other pollutants [13] and so their intake should be limited [13,14]. An alternative strategy to increase EPA plus DHA intake is to supplement with “fish oil” capsules. These present a useful strategy because capsules can provide a regular (daily) intake of a known amount of n-3 fatty acids. Furthermore, because of fish oil processing technologies, contaminants are largely removed, and so capsules represent a safe alternative to fish. There are many “fish oils” available and these may present the n-3 fatty acids in different chemical forms, in different concentrations and in different ratios (of EPA to DHA). The biological effect of n-3 fatty acids, and so their clinical impact, depends upon effective incorporation of the fatty acids into cells and tissues; in general, the higher the amount incorporated the greater the effect. Therefore, strategies to enhance incorporation are of interest to consumers, to industry and to regulators. We previously demonstrated that a strategy promoting emulsification of the oil delivering the n-3 fatty acids results in higher blood levels of EPA and DHA after both single dosing and repeated daily dosing [15]. Promoting emulsification would serve to enhance the digestive process and make delivery of the bioactive n-3 fatty acids more effective, so explaining these findings. This previous study used pro-emulsifying excipients within the oil capsule. Most omega-3 oil is in the triglyceride form. A triglyceride formulation can promote emulsification that might promote EPA and DHA delivery to the bloodstream. Therefore, we propose to conduct research in human volunteers that will address this. We plan to

investigate the appearance of EPA and DHA in the bloodstream over the immediate period (up to 12 hours) after consumption of identical amounts of those fatty acids presented in either standard ethyl ester form or as a triglyceride.

1.1.2 Summary of the known and potential risks and benefits, if any, to human subjects

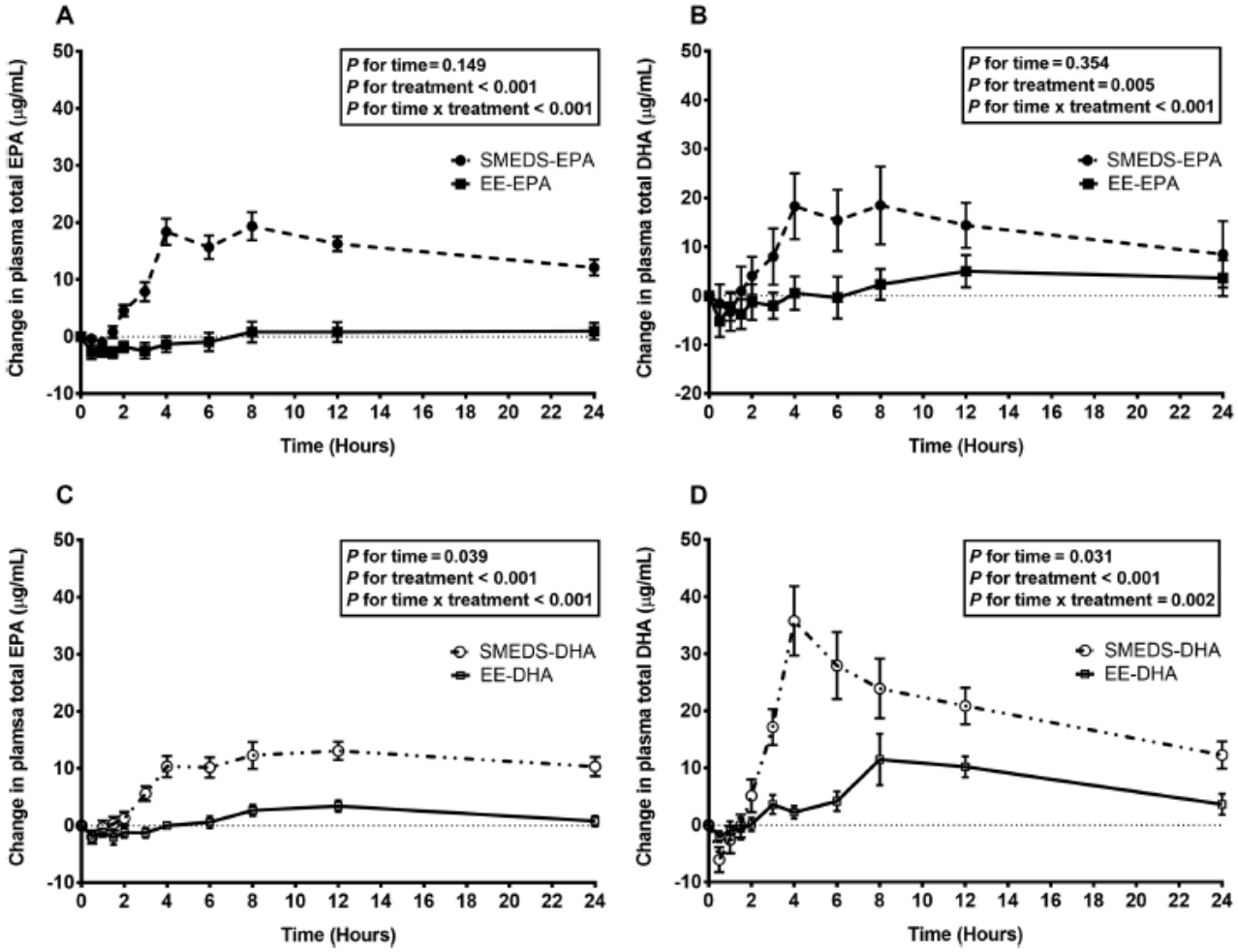
Capsules containing formulations of EE and TG have been tested in humans with no serious adverse events observed. Information considered pertinent to the safety of DHA and EPA includes regulatory or authoritative body opinions published in the last 15 years. None of the regulatory or authoritative bodies established an upper limit for DHA and EPA; however, several provided upper intake levels that were considered to be safe. The most recent opinion from the European Food Safety Authority (EFSA) [16] concluded that supplemental intakes of DHA and EPA combined at up to 5 g/day "do not raise safety concerns for the adult population". Bleeding complications have been suggested as a potential adverse effect of consuming high doses of DHA and EPA after reports of an increased tendency to bleed in Greenland Inuits with high dietary intakes of fatty fish (mean intake 6.5 g/day n-3 fatty acids). However, EFSA noted that other uncontrolled confounding factors may have been responsible for the observed effects in Greenland Inuits [16]. Based on more recent and controlled intervention studies, EFSA concluded that supplemental intakes of EPA and DHA combined up to about 5 g/day for up to 2 years and about 7 g/day for up to 6 months do not increase the risk of spontaneous bleeding episodes or bleeding complications, even in subjects at high risk of bleeding [16].

Results from animal studies and clinical trials indicate that there will be an enhanced bioavailability in humans of EPA and DHA from pre-emulsified supplement products. It is not expected that an enhanced bioavailability will change the safety profile of EPA and DHA supplement products. Even with an increased absorption the safety margin is substantial.

The overall conclusion is that the use of capsules containing a triglyceride formulation in the present study does not raise any safety concerns to the subjects involved.

1.1.3 Description of and justification for the regime and treatment period

A treatment period of 12 hours repeated after a 2 week wash out period is planned. Omega-3 fatty acids are incorporated into plasma lipids following single dosing and we previously demonstrated that supplementation with EE formulations results in higher plasma levels of EPA and DHA after both single dosing and repeated daily dosing [15]. The key findings are shown in the figure below. This demonstrates the time course of appearance of EPA and DHA in plasma from a single dose of different preparations of ethyl esters (EE) compared with self-emulsifying EE formulations (SMEDS) [15]. It is evident that blood sampling out to 12 hours is sufficient to identify a difference in availability between two formulations.



Therefore, a treatment period of 12 hours is planned, this being sufficient to evaluate the postprandial incorporation of EPA and DHA into plasma.

1.1.4 Statement of compliance

This study will be conducted in compliance with the protocol, GCP, the applicable regulatory requirement(s), and the relevant approvals.

1.1.5 Description of the population to be studied

The study will be conducted in healthy human subjects (male and female, aged 50 to 70 years, body mass index 20 to 35 kg/m^2).

1.1.6 References to literature relevant to the trial, and that provide background for the trial

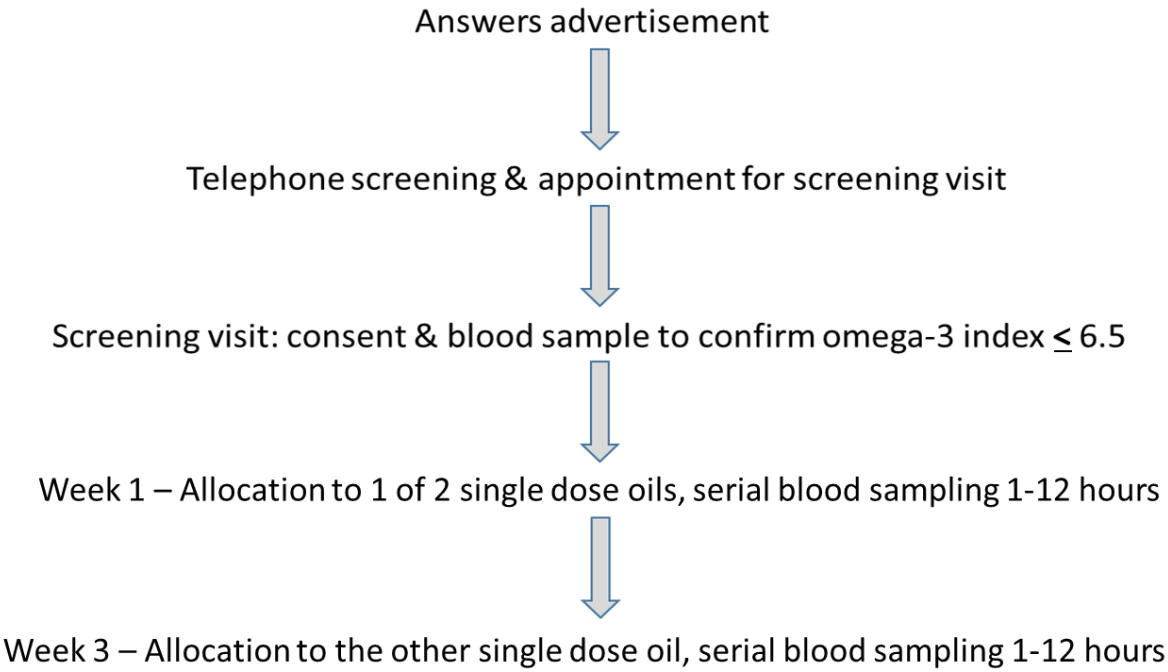
1. Kris-Etherton, Harris, Appel, American Heart Association Nutrition Committee (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747-57.

2. Calder (2004) N-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clinical Science 107: 1-11.
3. Bucher, Hengstler, Schindler, Meier (2002) N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. American Journal of Medicine 112: 298-304.
4. Studer, Briel, Leimenstoll, Glass, Bucher (2005) Effect of different antilipidemic agents and diets on mortality: a systematic review. Archives of Internal Medicine 65: 725-730
5. Calder (2006) N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. American Journal of Clinical Nutrition 83: 1505S-1519S.
6. Roynette, Calder, Dupertuis, Pichard (2004) N-3 polyunsaturated fatty acids and colon cancer prevention. Clinical Nutrition 23: 139-151.
7. Helland, Smith, Saarem, Saugstad, Drevon (2003) Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 111: e39-44.
8. Nemets, Stahl, Belmaker (2002) Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. American Journal of Psychiatry 159: 477-479.
9. Conquer, Tierney, Zecevic, Bettge, Fisher (2000) Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 35: 1305-1312.
10. Simopolous, Leaf, Salem (1999) Essentiality and recommended dietary intakes for omega-6 and omega-3 fatty acids. Annals of Nutrition and Metabolism 43: 127-130.
11. British Nutrition Foundation (1999) N-3 fatty acids and health. British Nutrition Foundation, London.
12. van de werf, Ardissino, Betriu, Cokkinos, Falk, Fox, Julian, Lengyel, Neumann, Ruzyllo, Thygesen, Underwood, Vahanian, Verheugt, Wijns, Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology (2003) Management of acute myocardial infarction in patients presenting with ST-segment elevation. European Heart Journal 24: 26-66.
13. Scientific Advisory Committee on Nutrition/Committee on Toxicity (2004) Advice on fish consumption; benefits and risks. TSO, London.
14. Committee on Nutrient Relationships in Seafood (2006) Seafood choices: Balancing benefits and risks. http://www.nap.edu/catalog/11762.htm.
15. West, Kindberg, Hustvedt and Calder (2018) A novel self-micro-emulsifying delivery system enhances enrichment of eicosapentaenoic acid and docosahexaenoic acid after single and repeated dosings in healthy adults in a randomized trial. Journal of Nutrition 148:1704-1715.
16. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2012) Scientific opinion related to the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (question no EFSA-Q-2011-00834, adopted on 26 June 2012 by European Food Safety Authority). EFSA Journal 10: 2815. [48 pp.] doi:10.2903/j.efsa.2012.2815.

1.2 Study schedule

Month ……	1	2	3	4	5
Recruit	X	X			
Screening visits	X	X			
Single dose visit 1-Week 1		X	X		
Single dose visit 2- Week 3			X	X	
Laboratory analysis	X	X	X	X	
Statistical analysis					X
Report writing					X

Flow of one participant through the study:



2. Study objectives and design

2.1 Objectives

The objectives of the study are to determine the appearance of EPA and DHA in plasma and the tolerability of healthy subjects over 12 hours following a single dose of omega-3 fatty acids presented in either standard ethyl-ester form or as a triglyceride formulation.

2.2 Study design

2.2.1 Type/design of study

This will be a blinded, random order, single centre, comparative study with a cross-over design.

2.2.2 Phase of study

This is a bioavailability study.

2.2.3 Treatments

Treatments will be long chain n-3 fatty acids (combination of EPA and DHA) in soft gelatine-capsules. Each capsule will contain:

- Omega-3 ethyl esters providing 500 mg EPA + 200 mg DHA
- Omega-3 triglyceride formulation providing 500 mg EPA + 200 mg DHA

Subjects will consume 4 capsules at each of the 2 single dosing visits (total of 2.8 g EPA+DHA each time).

The capsule shell contains gelatine (bovine origin), sorbitol (vegetable origin), glycerol (vegetable origin), water and traces of processing aids (medium chain TGs (vegetable origin), lecithin (sunflower oil origin)). All capsule shell ingredients and processing aids are food grade material. EuroCaps, the capsule manufacturer, only use bovine gelatine for which a certificate of suitability according to the monograph of the European pharmacopoeia is available. The certificate of suitability provides assurance that all aspects of the production of the gelatine, from country of origin, to critical stages of the manufacturing process, have been assessed and found compliant with the requirements to minimize the risk of transmissible spongiforms.

The PRB-08002S EU cap 1000 mg capsules will be produced at EuroCaps Limited in Wales, UK. The company is certified according to the BRC (British Retail Consortium) Global Standard for Food Safety. EuroCaps is also registered with the US FDA and inspected for the manufacture of dietary supplements in accordance with 21CFR111. The capsules are packaged by Wasdell,

UK. The company is certified according to BRC (British Retail Consortium) Global Standard for Food Safety.

The PPSG 1000 500:200 EE EU capsules are manufactured by Catalent Eberbach. Catalent is certified according to IFS and BRC. The company holds a Manufacturing Authorisation for pharma products and is also inspected by the FDA. The bulk capsules are packaged into bottles by GMPack, Denmark. The company is certified by the Danish Food Authority (Fødevarestyrelsen). The company holds a Manufacturing Authorisation for packaging of pharma products.

The capsules will be stored in ambient room temperature below 25 ºC and must not freeze. Randomization and labelling will be performed at University Hospital Southampton NHS Foundation Trust Research Pharmacy.

3. Details of the study

3.1 General approach to be taken

The PI's laboratory has studied the detail of omega-3 fatty acid incorporation in humans, particularly into blood plasma lipids in at least ten studies in human volunteers and also in patients with advanced atherosclerosis, with Crohn's Disease, and with cardiometabolic diseases. In these studies fish oil supplements of various types have been used, with blood being sampled at various time intervals over the course of hours to many months. The laboratory has reported both the time- and dose-dependent nature of the incorporation into plasma lipids, white blood cells, red blood cells and platelets. Appearance of EPA and DHA is typically detectable within hours of consumption [15]. The PI will use the general approach used in one previous study with human volunteers [15] to investigate the appearance of EPA and DHA in plasma lipids (total lipid), when EPA and DHA are supplemented as a standard ethyl-ester oil preparation or as a triglyceride formulation. The hypothesis is that incorporation will be greater with the triglyceride formulation.

3.2 Subjects and treatments

Healthy males and females aged 50 to 70 years with a body mass index between 20 and 35 kg/m^2 (n = 20) will be recruited. Subjects will be recruited via posters, email shots in the University of Southampton, Southampton General Hospital, and other organizations with which the researchers have contact, and advertisements in local newspapers. Subjects who express an interest will be screened by telephone interview. If they fit the inclusion and exclusion criteria (see below) they will be sent the information sheet. They will be contacted by telephone about 7 days later to confirm their interest or not, and if they remain interested an appointment will be made for them to visit the NIHR Clinical Research Facility, Southampton General Hospital for a screening visit. The screening visit will be used to obtain written informed consent and to collect a blood sample to confirm that the subject has a relatively low omega-3 status (defined as red blood cell EPA + DHA $\leq$ 6.5).
Recruited subjects will be randomized to one of two treatment groups at their first single dosing visit at week 1, with a crossover occurring at their second single dosing visit occurring at about week 3. Therefore, all subjects will consume both treatments over the duration of the study. In total 20 subjects will be treated (n=20 per treatment).

Treatments:

- Omega-3 ethyl esters providing 500 mg EPA + 200 mg DHA
- Omega-3 triglyceride formulation providing 500 mg EPA + 200 mg DHA

The omega-3 fatty acid preparations to be used will each be provided in capsules. In all groups four capsules will be taken at each single dosing visit supplying a total of about 2.8 g EPA plus DHA. Subjects, researchers and clinical staff will be blinded to capsule allocation.

3.3 Inclusion and exclusion criteria

Inclusion criteria

1. Healthy males and females
2. Aged 50 to 70 years
3. Body mass index 20 to 35 kg/m^2
4. Not eating more than one oily fish meal per week
5. Willing to adhere to the study protocol
6. Being able to provide written informed consent
7. Omega-3 Index ≤ 6.5 at screening visit

Exclusion criteria

1. Being diabetic (type 1 or type 2)
2. Being vegetarian or vegan and unwilling to consume capsules with a beef gelatine coating
3. Use of prescribed medicine to control inflammation
4. Chronic gastrointestinal problems (e.g. IBD, IBS, celiac disease, cancer)
5. Allergic to fish
6. Use of fish oil or other oil supplement
7. Participation in another clinical trial (currently or in the 12 weeks prior to study entry)
8. Use of fish oil or other oil supplements
9. Pregnancy or lactation
10. Blood donation in the 3 months prior to, or during, the study
11. Smoking
12. Alcohol consumption > 14 units/week
13. Blood donations during 3 months prior to or during the study period

3.4 Subject participation schedule

Subjects who express an interest will be screened by telephone interview. If they fit the inclusion and exclusion criteria, they will be sent the information sheet. They will be contacted about 7 days later to confirm their interest or not and if they remain interested an appointment will be made for them to visit the NIHR Clinical Research Facility, Southampton General Hospital for a screening visit.

The first (screening) visit will be used to obtain written informed consent and to collect a 5 ml blood sample to confirm that the subject does not have an elevated omega-3 fatty acid status (inclusion defined by red blood cell EPA + DHA ≤ 6.5); at this visit height and weight will also be measured.

Recruited subjects will be randomized to receive one of two treatments at visit 2 (n = 10 per group at each visit). Subjects will be stratified for gender and then randomly allocated to treatment groups to ensure even grouping. Subjects will be randomized by University Hospital Southampton NHS Foundation Trust Research Pharmacy.

Subjects will subsequently visit the NIHR Clinical Research Facility, Southampton General Hospital on two occasions each between 7 and 9 am. On each occasion, they will be in the fasted state (no food or drink except water since 9 pm the evening before the visit). The two visits will be at least 2 weeks apart. On each occasion subjects will consume the same meal the evening before the clinic visit.

On the study entry visit, female subjects less than 2 years from (self reported) menopause will undergo a standard test to confirm that they are not pregnant. Blood (~20 ml) will be taken into EDTA tubes and subjects will take their first batch of four capsules with a glass of water (150 to 250 ml) under supervision. Further blood samples will be taken at 1, 2, 3, 4, 5, 6, 8 and 12 hours. On each occasion ~5 ml blood will be collected into EDTA tubes. Thus, on this visit a total of ~70 ml blood will be collected. Subjects will be allowed a low fat meal of toast and jam with decaffeinated tea or coffee after the 3 hour blood sample is collected. Subjects will be allowed a low fat meal of toast and jam with an apple or orange and decaffeinated tea or coffee after the 6 hour blood sample is collected and again at around the 10 hour time point. Subjects will be allowed to leave the Clinical Research Facility after the final 12-hour blood sample is collected.

3.5 Expected duration of subject participation

The duration of involvement (i.e. screening visit to visit 3) will be approx. 4 weeks. Subjects will cease to be involved in the project when the final clinic visit and blood sampling is completed.

The project will be completed when all fatty acid composition analyses are completed, data are entered into a database and initial statistical analysis is completed.

3.6 Compliance to treatment

Subjects will receive a single dose of omega-3 fatty acids on two occasions each time within the Clinical Research Facility under supervision from a research nurse. Thus compliance will be 100%.

3.7 Subject withdrawal

Recruited subjects will be able to withdraw from the study at any time without giving a reason. Withdrawal will be noted on a sheet designed for this purpose; withdrawing subjects

will be given the option of having data and/or samples already collected retained for study purposes or destroyed. Withdrawn subjects will not be replaced unless they have not yet attended visit 2.

3.8 Sample analysis

3.8.1 Overview of samples to be collected

Blood will be used to prepare plasma (visits 2 and 3), and red blood cells (screening visit only). Red blood cells will be prepared from screening blood samples.
Plasma will be prepared from all blood samples except the screening samples.

3.8.2 Processing of blood collected at time points up to 12 hours at the study entry clinic visit

Blood will be collected into K2 ethylenediaminetetraacetic acid (EDTA) vacuettes and after mixing, these will be placed in a cool box containing crushed ice/water. The samples will be centrifuged within 60 minutes of collection, at 1900 g for 15 minutes at approximately 4ºC.

Plasma will be aliquoted into 2 x 0.5 ml aliquots into each of two (Set 1 and Set 2) 2 ml labelled polypropylene tubes and stored within 60 minutes at -20ºC.

3.8.3 Fatty acid composition analysis

Lipid will be extracted from plasma and red blood cells using chloroform/methanol. The fatty acid composition of each sample will be determined by gas chromatography according to in-house standard operating procedures.

Analysis of screening red blood cells and of plasma collected at the single dosing visits at week 1 and 3 will take place in the PI's laboratory at Faculty of Medicine, University of Southampton, Southampton. Exploratory analysis of other outcomes such as blood lipids and inflammatory markers (both in EDTA plasma) may be conducted in the PI's laboratory at Faculty of Medicine, University of Southampton, Southampton.

Data reported will include: total EPA, total DHA, and total EPA+DHA in red blood cells at screening and total EPA, total DHA and total EPA+DHA in plasma samples collected over 12 hours at the two single dosing studies.

All laboratory analysis will be performed blind to treatment allocation.

3.9 Data handling and record keeping

Miss Helena Fisk will be responsible for data collection, recording and quality, under the supervision of the PI.

All data will be entered onto a spreadsheet (Microsoft Excel) by the researchers involved. The spreadsheet will be kept on a password-protected computer and will accessed only by the PI and the researcher involved.

All data will only be linked to study codes and thus not identifiable with the source subject. However, the caveat to this will be a data set recording the subject name and study code without any other subject details.

All data recorded on paper will be kept in a locked filing cabinet in the researchers' office and/or in a dedicated, restricted access, clinical data storage area on Level D of the IDS Building, University of Southampton.

Data of an identifiable nature (i.e. subject names, contact details, addresses) will be destroyed 12 months after the study is reported. All other data will be kept securely for 15 years and then destroyed.

Data will be obtained, handled and stored in adherence to the principle set out in the General Data Protection Regulation of 2018.

The investigators will permit monitoring, audits, REC and MHRA review (as applicable) and provide direct access to source data and documents.

3.10 Statistical analysis

3.10.1 Sample size calculation

The study is powered according to the anticipated change in EPA + DHA content of plasma over the 12 hour period following single dosing (see earlier figure from [15]). Assuming a similar difference between the two preparations to be used as was seen in the previous study, a sample size of 20 will give 90% power of detecting this difference as statistically significant, by a pairwise comparison and setting $P < 0.05$, as is usual. In order to allow for a drop-out rate of 20% 25 subjects will be recruited.

3.10.2 Data analysis

The statistical analysis will involve comparison of EPA, DHA and the sum of EPA+DHA in plasma according to time and treatment (two-factor ANOVA) followed by one-way ANOVA

and pairwise comparisons. All statistical comparisons will be performed at the end of the study using the latest version of the programme SPSS. Researchers will be blind to treatment allocation until after the analysis is complete.

3.11 Reporting and dissemination

The funding award is subject to a signed contract between University of Southampton and BASF AS.

The study will be registered at www.clinicaltrials.gov or a similar trial registration site.

Results will be provided to the study funder and subject to approval subsequently presented at scientific conferences and published in relevant scientific journals. The contract between the funder and the University of Southampton specifies the conditions that govern such dissemination.

4. Adverse events

4.1 What is an adverse event?

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical study subject administered an investigational product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

An adverse reaction is defined as all untoward and unintended responses to an investigational product related to any dose administered, i.e. where a causal relationship between the investigational product and an adverse event is at least a reasonable possibility.

An unexpected adverse reaction is an adverse reaction, the nature or severity of which is not consistent with the information about the investigational product or intervention in question set out in the Summary of Product Characteristics (SmPC) or Investigator's Brochure (IB).

If a subject is found to be pregnant, the subject will be withdrawn from the study immediately. The pregnancy will be followed to term or until the pregnancy has been terminated, and the outcome will be recorded. If a subject becomes pregnant during the study while receiving investigational product, this will be reported to the sponsor on a SAE form (even if the pregnancy itself is not considered as serious).

An adverse event, adverse reaction, unexpected adverse reaction, is defined as serious if it:

a) results in death;
b) is life-threatening;
   Life threatening in the definition of a serious adverse event (SAE)/serious adverse reaction (SAR) refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
c) requires hospitalisation or prolongation of existing hospitalisation;
   In general, hospitalisation signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment which would not have been appropriate at the investigator site. When in doubt as to whether hospitalisation occurred or was necessary, the adverse event should be considered as serious. Hospitalisation for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AE and should

be recorded on a Clinical Assessment form and added to the study file. If something untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'non-serious' attributed according to the usual criteria.

d) results in persistent or significant disability or incapacity;
e) consists of a congenital anomaly or birth defect.

Medical judgement should be exercised in deciding whether an SAE/SAR is serious in other situations. Important SAE/SARs that are not immediately life-threatening or do not result in death or prolonged hospitalisation but may jeopardise the subject or may require intervention to prevent one or the other outcomes listed in the definition above, should also be considered serious.

A suspected serious adverse reaction (SSAR), is any serious adverse reaction that is suspected (possibly or probably) to be related to the investigational product.

A suspected unexpected serious adverse reaction (SUSAR) is an SSAR which is also "unexpected", meaning that its nature and severity are not consistent with the information about the investigational product in question set out in the IB.

4.2. Intensity

The assessment of intensity will be based on the investigator's clinical judgement using the following definitions:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.

- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

- Severe: An event that prevents normal everyday activities.

The term severity is often used to describe the intensity (severity) of a specific event. This is not the same as 'seriousness', which is based on participant/event outcome or action criteria.

4.3. Causality

The relationship between the investigational product/procedure and the occurrence of each AE will be assessed and categorised as below by the investigator. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural

history of the underlying diseases, concomitant therapy, other risk factors etc. will be considered. The Investigator will also consult the IB or other product information.

- Not related: Temporal relationship of the onset of the event, relative to administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.

- Unlikely: Temporal relationship of the onset of the event, relative to administration of the product, is likely to have another cause which can by itself explain the occurrence of the event.

- Possibly related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable but the event could have been due to another, equally likely cause.

- Probably related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable and the event is more likely explained by the product than any other cause.

- Definitely related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable and there is no other cause to explain the event, or a re-challenge (if feasible) is positive.

- Where an event is assessed as possibly related, probably related, definitely related the event is an adverse reaction.

4.4. Expectedness

Adverse reactions must be considered as unexpected if they add significant information on the specificity or severity of an expected adverse reaction. The expectedness of an adverse reaction shall be determined according to the reference documents (e.g. IB).

- Expected: Reaction previously identified and described in protocol and/or reference documents (e.g. IB, SmPC).

- Unexpected: Reaction not previously described in the protocol or reference documents.

All AEs occurring during the period from screening visit to the trial completion will be registered and reported if applicable.

For all adverse event/reactions the investigator will make an assessment of intensity, causality, expectedness and seriousness.

The PI will keep the Sponsor and the REC informed of any significant findings.

At the end of the study all adverse events recorded during the study will be subject to statistical analysis and analysis and subsequent conclusions will be included in the final study report. All AEs experienced by study subjects will be registered. After trial completion these study subjects will be unblinded and the list of treatment allocation should be transferred to BASF AS.

4.5. Expedited reporting of serious adverse events

All patient safety related incidents will be reported according to University Hospital Southampton NHS Foundation Trust (UHS) Incident Reporting and Management Policy. In addition to the Trust Incident reporting, SAEs are expedited to the people and departments identified below. The only exception is where the protocol or IB identifies an event as not requiring immediate expedited reporting (see below).

All SUSARs, SAE reports, urgent safety measures, periodic safety reports (if applicable) and the final study report shall be prepared by the PI (or delegated person) and transmitted to the following email address at BASF AS: Omega3.PV@basf.com, or other e-mail address if communicated by BASF AS.

The PI (or delegated person) will make an initial report, orally or in writing. The initial report will include as much information as is available at the time.

The PI (or delegated person) will report the following:

SUSAR	Immediately report to: - the PI - the sponsor - UHS R&D department - UHS patient safety team (using Trust incident Reporting form) - the University of Southampton UHS will be responsible to further expedite the Reporting of SUSAR to the REC that gave approval as soon as possible but within 7 days	The investigator (or delegated person) will make an initial report, orally or in writing. The initial report will include as much information as is available at the time. Oral reports will be followed up in writing within a further 24 hours of the initial report. After the initial report the investigator will actively follow up the subject. The Investigator (or delegated person) will provide information missing from the initial report within five working days of the initial report. Written reports will be made by completing an SAE/SUSAR reporting form provided by University Hospital Southampton R&D. In addition, the REC receives a completed NRES CTIMP safety report to REC

		Http://www.nres.npsa.nhs.uk/applications/afteR-ethical-review/safetyreports/safety-reportsfor-ctimps/#safetyctimpssubmission UHS incident report template available from UHS Staffnet or departmental log books
SAE	Within 24 hours report to: - the PI - the Sponsor - UHS R&D Department - the University of Southampton	As above; but no expedited reporting to the REC.
Urgent Safety Measures/ Temporary Halt of the Trial	Implement and report immediately as a substantial amendment to: - the PI - the Sponsor The PI must inform as soon as possible but within 3 days: - the REC that granted approval - the University of Southampton	The Sponsor and the PI must be notified of any urgent safety measures/temporary halt of a trial that have had to be taken that are not part of the protocol. The report must include the reasons for the urgent safety measure and the plan for further action. The standard CTIMP substantial amendment form must be used, as available from NRES website. http://www.nres.npsa.nhs.uk/applications/after-ethical-review/amendments/

5. Ethical and governance considerations

The study will be approved by an NHS Ethics Committee; such approval will be sought as soon as the protocol is finalized.

The study will be approved by Southampton University Hospital R&D if appropriate.

The study will be approved by the University of Southampton Research Governance Office.

The study sponsor will be BASF AS.

The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 as revised and recognized by governing laws and EU Directives; and the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

The PI will submit a final report at conclusion of the trial to the REC within the timelines defined in the Regulations.