UNIVERSITY OF Southampton

PROTOCOL

Version 4 : 27th May 2016

Title of study

Incorporation of omega-3 fatty acids in healthy humans following oral dosing of dietary supplements

Short title

Incorporation of omega-3 fatty acids in healthy humans

Study identifiers

REC number: 15/SC/0775
R&D number: To be identified
Sponsors number: CTN00715201

Funder of the study

Pronova BioPharma, Lilleakerveien 2c, 0283 Oslo, Norway

Sponsor of the study

Pronova BioPharma, Lilleakerveien 2c, 0283 Oslo, Norway

Principal investigator

Professor P.C. Calder, Human Development & Health Academic Unit, 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 — 27 May 2016 Date

Sponsor representative signature — 27 May 2016 Date

SUMMARY OF, AND RATIONALE FOR, CHANGES FROM VERSION 3

Modification of postprandial meal timing and the addition of fruit and an extra hot drink during the 12 hour investigation period.

Protocol Section	**Brief description of changes from protocol Version 3 (17 February 2016)**
Section 3.4	From: Subjects will be allowed a low fat meal of toast and jam with tea or coffee after the 3 hour and 8 hour blood samples are collected. To: Subjects will be allowed a low fat meal of toast and jam with tea or coffee after the 3 hour blood samples are collected. Subjects will be allowed a low fat meal of toast and jam with an apple or orange and tea or coffee after the 6 hour blood samples are collected. Subjects will be allowed an additional tea or coffee at the 8 hour time point.

Places where research will be conducted

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

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

Department of Bioanalysis and Immunology, Charles River, Tranent, Edinburgh, UK

Researchers involved

Professor Philip C. Calder, Human Development & Health Academic Unit, 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 Annette West, Human Development & Health Academic Unit, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
Email: A.West@soton.ac.uk
Phone : +44 (0)2381 205252

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

Hannah Mather, Department of Bioanalysis and Immunology, Charles River, Tranent, Edinburgh EH33 2NE, UK
Email: hannah.mather@crl.com
Phone: +44 (0)1875886125

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. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the project as they are approved.

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.

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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
MHRA	Medicines and Healthcare products Regulatory Authority
NHS	National Health Service
PI	Principal investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SMEDDS	Self Micro Emulsifying Drug Delivery System
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	Incorporation of omega-3 fatty acids into blood lipid pools in healthy humans following oral dosing of dietary supplements
Sponsor	Pronova BioPharma
REC number	15/SC/0775
R&D number	
Sponsor reference number	CTN00715201
Principal Investigator	Professor Philip Calder
Study phase if not mentioned in title	Phase II: Biodistribution study of dietary supplements
Funder	Pronova BioPharma
Project Type:	Randomised blinded trial in healthy human volunteers
Primary Objective:	To follow the change in omega-3 fatty acids (EPA and DHA) over 12 weeks in plasma and red and white blood cells during supplementation with fish oil concentrates as pure oils or corresponding pre-emulsified formulations
Rationale:	Omega-3 fatty acids from fish oils are associated with improved human health. Absorption of the fatty acids from standard supplements may limit effectiveness. Pre-emulsification may aid digestion and absorption. This will be tested by head-to-head comparison of standard and pre-emulsified oils.
Project Design:	Blinded, randomized, single centre, comparative study with a parallel design.
Inclusion Criteria:	• Healthy males and females • Age 18 to 65 years • Body mass index 20 to 35 kg/m^2 • Not consuming fish oil or similar supplements • Not eating more than one oily fish meal per week • Willing to adhere to the study protocol • Being able to provide written informed consent
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 • Chronic gastrointestinal problems (e.g. IBD, IBS, celiac disease, cancer) • Allergic to fish

	• Allergic to soybean • 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 repeated daily doses for 12 weeks.
Data collection	Age, height, weight, general health status
Biological samples	Blood samples (plasma, red blood cells, white blood cells)
Number of participants	20 healthy subjects per group, 4 groups = 80
Primary endpoint	Concentration of EPA and DHA in red blood cells
Secondary endpoint	Concentration of EPA and DHA in plasma and white blood cells
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

Omega-3 (n-3) fatty acids are widely considered to be important for human health. This is particularly so for 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]. They 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], including by the UK Government [13]. 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 for n-3 fatty acid intake. 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. One such strategy is pre-emulsification of the oil delivering the n-3 fatty acids. This could serve to enhance the digestive process and may make delivery of the bioactive n-3 fatty acids more effective.

Pronova BioPharma Norge AS (hereafter Pronova) is the developer of the Pronovum Omega 3 food supplements. These are soft gelatine capsules containing fish oil based, highly concentrated n-3 fatty acids in the form of either ethyl esters (EE) or triglycerides (TG) as the

active (nutritional) ingredient. The n-3 fatty acid oil and capsule fill ingredients form a “Self Micro Emulsifying Drug Delivery System” (SMEDDS), designed to improve the bioavailability of n-3 fatty acids compared with standard fish oil supplements. This has been demonstrated in unpublished short term (over several hours) studies in humans. The longer term impact of pre-emulsified n-3 fatty acids on incorporation of EPA and DHA into plasma and cells compared with standard material is not known. Therefore, we propose to conduct research in healthy human volunteers that will address this question. We plan to investigate the appearance of EPA and DHA in the bloodstream over a period of 12 weeks of daily consumption of those fatty acids presented in either standard or SMEDDS form.

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

Fish oil based highly concentrated n-3 fatty acids in the form of either EE or TG are well established ingredients of food supplement products in the European Union (EU). The additional ingredients in Pronovum capsules are considered as either food ingredients or food additives. The use of the food additives is permitted in food supplements according to Regulation (EC) No 1333/2008 on Food Additives. Pronovum capsules are intended for use in the adult population.

Pronovum 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 10 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 [15] 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 [15]. 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 [15].

Results from animal studies and clinical trials indicate that there will be an enhanced bioavailability in humans of EPA and DHA from the supplement products formulated as SMEDDS. 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, and studies have been performed with one of the present formulation with higher concentrates of omega-3 fatty acids (CTN02012101).

The overall conclusion is that the use of Pronovum capsules 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 weeks is planned. Omega-3 fatty acids are incorporated into blood lipids, white blood cells and red blood cells over the course of weeks to months. A new steady state is reached over that time period but the exact time when that occurs depends upon the exact pool being considered (blood lipids < white blood cells < red blood cells) as described in detail by Browning et al. [16]. Thus, a treatment period of 12 weeks is planned, this being sufficient to obtain steady state EPA and DHA incorporation into blood lipids ((i.e plasma), white blood cells and red cells. A shorter duration would be insufficient for red blood cells to reach maximum incorporation [16].

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 18 to 65 years, body mass index 20 to 35 kg/m^2).

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

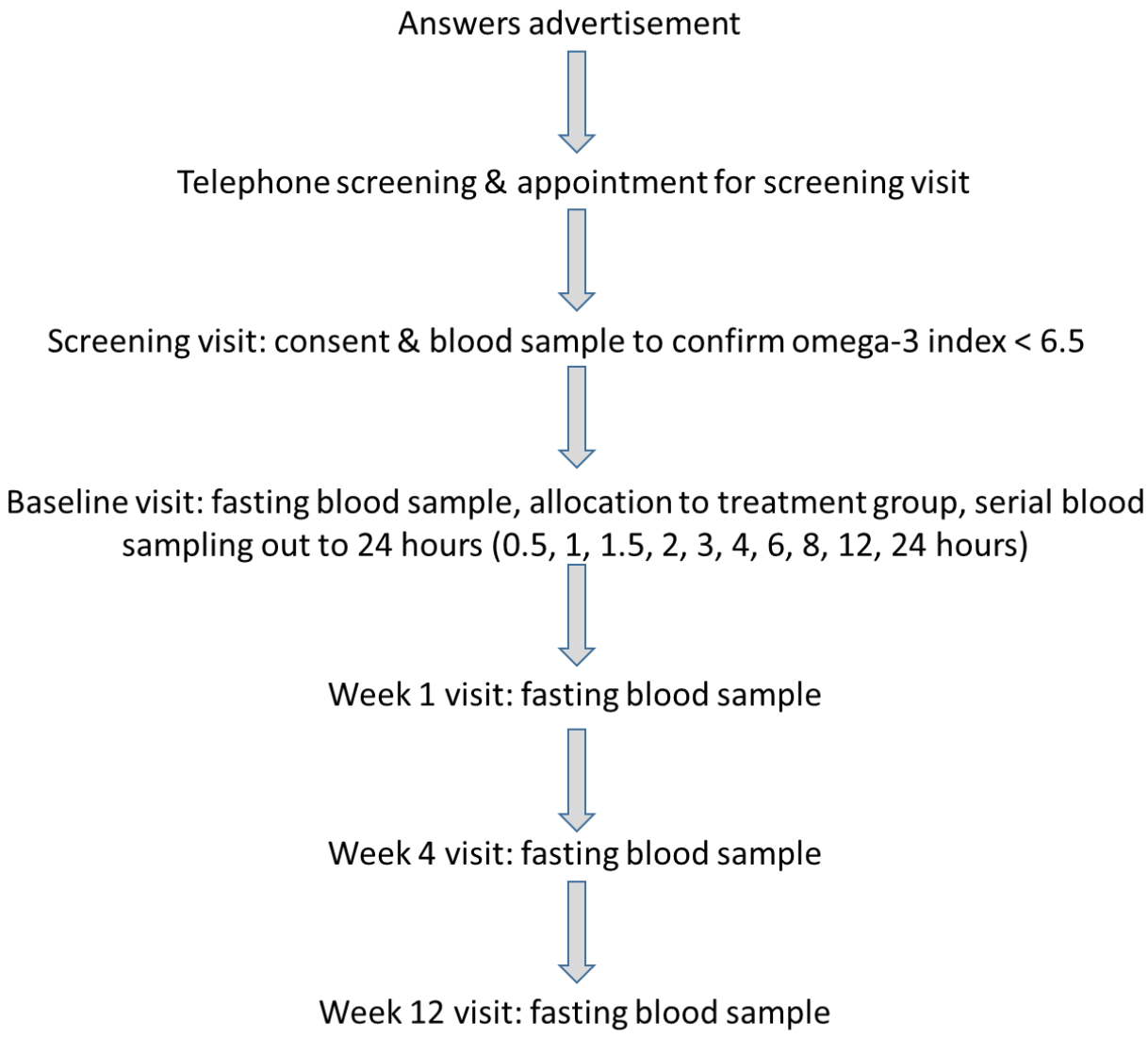
1. Kris-Etherton, Harris, Appel, American Heart Association Nutrition Committee (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747-2757.
2. Calder (2004) N-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clinical 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. 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.
16. Browning, Walker, Mander, West, Madden, Gambell, Young, Wang, Jebb, Calder (2012) P.C. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. American Journal of Clinical Nutrition 96: 748-758.

1.2 Study schedule

Month ……	1	2	3	4	5	6	7	8	9	10	11	12
Recruit	X											
Screening visits	X	X										
Baseline visits		X	X	X								
Week 1 visits		X	X	X								
Week 4 visits			X	X	X							
Week 12 visits					X	X	X					
Laboratory analysis	X	X					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 objective of the study is to determine the appearance of EPA and DHA in plasma, white blood cells and red blood cells over 12 weeks in healthy subjects consuming omega-3 fatty acids presented in either standard or pre-emulsified (SMEDDS) form.

2.2 Study design

2.2.1 Type/design of study

This will be a blinded, randomized, single centre, comparative study with a parallel design.

2.2.2 Phase of study

This is a phase II 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:
Cohort A:

- Omega-3 ethyl esters providing 230 mg EPA + 190 mg DHA
- SMEDDS Omega-3 ethyl esters providing 230 mg EPA + 190 mg DHA

Cohort B:

- Omega-3 ethyl esters providing 90 mg EPA + 300 mg DHA
- SMEDDS Omega-3 ethyl esters providing 90 mg EPA + 300 mg DHA

Subjects will consume 3 capsules per day.

The capsule shell contains gelatine (bovine 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 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; last audit was 11-12 June 2013. EuroCaps is also registered with the US FDA, and inspected for the manufacture of dietary supplements in accordance with 21CFR111.

The capsules will be stored in ambient room temperature and will have a shelf life of at least 6 months. They will be packaged into screw-topped plastic bottles with 100 capsules per bottle. Each subject will receive 3 bottles. Packaging 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

Long term (several weeks to several months) studies are required to investigate and to compare the incorporation of n-3 fatty acids into transport pools (blood lipids) and functional pools (e.g. blood cells) which is a true measure of n-3 fatty acid status. When included in the human diet EPA and DHA are incorporated into plasma lipid fractions, platelets, white cells, red cells and many other cell and tissue types. The PI's laboratory has studied the detail of such fatty acid incorporation, particularly for plasma phospholipids and for white blood cells 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 phospholipids, white blood cells, red blood cells and platelets (e.g. see [16]). Incorporation of EPA and DHA is typically detectable within weeks. However, the rate of incorporation and the time to reach a maximum varies according to turnover of the pool investigated [16]. Thus, incorporation into white cells is faster than into red cells. The PI will use the general approach used in these previous studies with human volunteers to investigate the appearance of EPA and DHA in plasma lipids (total lipid), in white blood cells, and in red blood cells when EPA and DHA are supplemented as standard oil preparations or as those same preparations pre-emulsified (SMEDDS). The hypothesis is that incorporation will be greater with the SMEDDs preparation.

3.2 Subjects and treatments

Healthy males and females aged 18 to 65 years with a body mass index between 20 and 35 kg/m^2 (n = 80) will be recruited. Subjects will be recruited via posters, email shots in the University of Southampton, Southampton General Hospital, and other organisations 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 Wellcome Trust 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 < 6.5).
Recruited subjects will be allocated into two cohorts, A and B. Recruitment for cohort A will be finalized before initiation of cohort B. Within each cohort the subjects will be randomized to one of two treatment groups. In total 80 subjects will be treated (n=20 per treatment).

Cohort A:

- Omega-3 ethyl esters providing 230 mg EPA + 190 mg DHA
- SMEDDS Omega-3 ethyl esters providing 230 mg EPA + 190 mg DHA

Cohort B:

- Omega-3 ethyl esters providing 90 mg EPA + 300 mg DHA
- SMEDDS Omega-3 ethyl esters providing 90 mg EPA + 300 mg DHA

The omega-3 fatty acid preparations to be used will each be provided in capsules. In all groups three capsules will be taken daily supplying a total of about 1.2 to 1.3 g EPA plus DHA. Subjects, researchers and clinical staff will be blinded to group allocation.

3.3 Inclusion and exclusion criteria

Inclusion criteria

1. Aged 18 to 65 years
2. Body mass index 20 to 35 kg/m^2
3. Not consuming fish oil or similar supplements
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

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. Allergic to soybean
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

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 Wellcome Trust 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 for cohort A will be randomized to one of two treatment groups and subsequent subjects recruited for cohort B will be randomized to one of two treatment groups as described in section 2.2.3 and 3.2 (n = 20 per group). Subjects will be stratified for gender and age then randomly allocated to treatment groups to ensure even grouping. Subjects will be randomised by University Hospital Southampton NHS Foundation Trust Research Pharmacy.

Subjects will subsequently visit the Wellcome Trust Clinical Research Facility, Southampton General Hospital on four occasions each between 8 and 10 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). Visits will be at study entry and after approx. one week (7 $\pm$ 1 day), four weeks (28 $\pm$ 3 days) and twelve weeks (84 $\pm$ 7 days) of supplementation.

On the study entry visit, female subjects will undergo a standard test to confirm that they are not pregnant. On the study entry visit, subjects will receive a supply of supplements for the entire 12 weeks along with instructions on how to take them (all three capsules to be swallowed daily with a glass of water first thing in the morning and at least half an hour before breakfast). Blood (~20 ml) will be taken into EDTA tubes and subjects will take their first batch of three capsules with a glass of water (150 to 250 ml) under supervision. Further blood samples will be taken at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 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 tea or coffee after the 3 hour blood samples are collected. Subjects will be allowed a low fat meal of toast and jam with an apple or orange and tea or coffee after the6 hour blood samples are collected. Subjects will be allowed an additional tea or coffee at the 8 hour time point. Subjects will be allowed to leave the Clinical Research Facility once the 4 hour blood sample is collected, but will be required to return with sufficient time for collection of the 6, 8, 12 and 24 hour samples. Subjects will be allowed to drink water throughout the 24 hour period. Subjects will be permitted to consume an evening meal after the 12 hour sample has been collected. Subjects will be advised to avoid fish or any foods that contain omega-3 fatty acids. Subjects will be required to be fasted for a minimum of 11 hours prior to returning for 24 hour blood sample. After the 24 hour blood sample is collected subjects will then be offered toast with jam and a hot drink.

On the visits at weeks one, four and twelve, blood (20 ml) will be taken into EDTA tubes. Subjects will then be offered toast with jam and a hot drink.

3.5 Expected duration of subject participation

The duration of the intervention phase will be approx. 12 weeks (84 $\pm$ 7 days). As the intervention phase will begin about 2 weeks after the screening visit, subjects recruited into the study will be actively involved for 13 to 15 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

Compliance will be assessed by counting of returned capsules.

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 the baseline visit.

3.8 Sample analysis

3.8.1 Overview of samples to be collected

Blood will be used to prepare plasma, white blood cells, and red blood cells.

Red blood cells will be prepared from screening blood samples.

Plasma will be prepared from all blood samples except the screening samples.

White blood cells and red blood cells will be prepared from samples collected at zero-time on visits at study entry and weeks one, four and twelve.

3.8.2 Processing of blood collected at time points up to 24 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 4 x 0.5 ml aliquots as follows:
For free fraction EPA/DHA determination: 2 x 0.5 ml aliquots into each of two (Set 1 and Set 2) 2 ml labelled polypropylene tubes each containing 12.5 µL of inhibitor cocktail and stored within 60 minutes at -80ºC. Inhibitor cocktail is: 0.5 g sodium fluoride, 1.0 g L-ascorbic acid and 0.25 g 5-methylisoxasole-3-carboxylic acid in 10 mL of deionised water.
For total EPA/DHA determination: 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 Processing of (fasting) blood collected at the study entry clinic visit and at clinic visits at weeks one, four and twelve

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 blood (20 ml) will be layered onto 10 ml Histopaque solution and the samples will be centrifuged within 60 minutes of collection, at 1500 rpm for 15 minutes at room temperature with the centrifuge brake off. Plasma will be collected from the upper layer and aliquoted into 5 x 1.5 ml aliquots and frozen at minus 80^{o}C. Mononuclear cells (“White blood cells”) will be collected from the

interface and washed once with phosphate-buffered saline before freezing at minus 80°C. Red blood cells will be collected from the bottom of the tube and washed twice with phosphate-buffered saline before freezing red cell membranes at minus 80°C.

3.8.4 Fatty acid composition analysis

Lipid will be extracted from plasma, white blood cells, 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 selected plasma samples from the repeated blood samples collected at the study entry visit will be done at Department of Bioanalysis and Immunology, Charles River, Tranent, Edinburgh.

Analysis of screening red blood cells; of fasting plasma collected at study entry and at weeks one, four and twelve; and of white blood cells and red blood cells collected, at study entry and at weeks one, four and twelve 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 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 and in plasma samples collected over 24 hours at study entry free EPA and free DHA.

All laboratory analysis will be performed blind to subject group allocation.

3.9 Data handling and record keeping

Miss Annette West 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 researchers 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 end of the study. 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 Data Protection Act 1998.

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 red blood cells (the so called omega-3 index). Based upon previous studies of this sort, a standard supplement providing 1 to 1.5 g EPA plus DHA is expected to increase the omega-3 index by 3 (e.g. from 6.5 to 9.5). It is estimated that pre-emulsification (i.e. SMEDDS) will increase the omega-3 index by a further 30% i.e. by 4. Using a SD of 1.5 for both changes, a sample size of 15 per group 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 25% 20 subjects per group will be recruited (80 subjects in total).

3.10.2 Data analysis

The statistical analysis will involve comparison of EPA, DHA and the sum of EPA+DHA in each fraction studied 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 Pronova BioPharma.

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 investigation subject administered a pharmaceutical 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 a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An adverse reaction is defined as a noxious and unintended response to any medicinal (investigational) product related to any dose, i.e. where a causal relationship between the medicinal product and an adverse event is at least a reasonable possibility.

An unexpected adverse reaction is an adverse reaction, the nature and severity of which is not consistent with the information about the medicinal 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, and the outcome will be recorded.

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 anything 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 medicinal 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 medicinal 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 drug/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 conclusion of the study all adverse events/reactions, recorded during the study will be subject to statistical analysis and that analysis and subsequent conclusions 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 transferred to Pronova.

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).

Any SUSAR, SAE reports or urgent safety measures should be transmitted to the following address at Pronova: pharmacovigilance@pronova.com.

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.

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 MHRA and 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:	As above; but no expedited reporting to the MHRA and REC.

	- the PI - the Sponsor - UHS R&D Department - the University of Southampton	
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 MHRA - 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 MHRA/NRES websites. 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 finalised.

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 Pronova BioPharma.

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 recognised 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.