JRMO Non-CTIMP Protocol Template

1. TITLE PAGE

Exploratory Double Blind Placebo Controlled Study Investigating the Regulation of Pro-resolving Mediators and White Blood Cell Responses by Fish Oil Supplements in Healthy Volunteers

Full Title	Exploratory Double Blind Placebo Controlled Study Investigating the Regulation of Pro-resolving Mediators and White Blood Cell Responses by Fish Oil Supplements in Healthy Volunteers
Short Title/Acronym	SPM regulation by Fish Oil Supplements in Healthy Volunteers
Sponsor	*Name the sponsor organisation and contact person:* ▪ *Queen Mary, University of London* *Contact person of the above sponsor organisations is:* *Head of Research Resources* *Joint Research Management Office* *5 Walden Street* *London* *E1 2EF* *Phone: 020 7882 7260* *Email: sponsorsrep@bartshealth.nhs.uk*
REC Reference	*Insert once known*
IRAS Reference Number	193174
Chief Investigator	Dr. David Collier, Barts & The London School of Medicine and Dentistry, Queen Mary University of London, Clinical Pharmacology, William Harvey Clinical Research Centre, William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ Email: d.j.collier@qmul.ac.uk Phone: 020 7882 5668
Insert as applicable list of	*A) Sites* Barts and The London School of Medicine Queen Mary University of London WHRI Heart Centre Charterhouse Square London EC1M 6BQ *B) Laboratories and/or technical departments*

William Harvey Research Institute
Center for Biochemical Pharmacology
Charterhouse Square
London. EC1M 6BQ

William Harvey Research Institute
Center for Clinical Pharmacology
Charterhouse Square
London. EC1M 6BQ

William Harvey Research Institute
Center for Biochemical Pharmacology
Lipid Mediator Metabololipidomics Unit
Charterhouse Square,
London. EC1M 6BQ

C) Central facilities

Contents Page

2. GLOSSARY of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
JRMO	Joint Research Management Office
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

4. SUMMARY/SYNOPSIS

Short Title	SPM regulation by Fish Oil Supplements in Healthy Volunteers
Methodology	A randomised, double-blind, placebo-controlled study to determine whether fish oil supplementation regulates peripheral levels of specialized pro-resolving mediators and white blood cell responses in healthy volunteers
Research Sites	Barts and The London School of Medicine Queen Mary University of London William Harvey Research Institute Charterhouse Square London EC1M 6BQ
Objectives/Aims	The objective of this study is to learn more about the biology of fish oil supplementation in human healthy volunteers by assessing the actions of these supplements in healthy volunteers.
Number of Participants/Patients	40
Main Inclusion Criteria	Able to provide informed consent, willingness to abstain from fish for 2 days before each study visit, physical examination, laboratory tests.
Statistical Methodology and Analysis (if applicable)	The results will be tabulated and assessed for biological activity of the fish oil supplement and any relationship between dose and primary variables. Because the study is exploratory in nature, there will be no formal statistical analysis of the data.
Proposed Start Date	July 2016
Proposed End Date	April 2016
Study Duration	9 months

3. SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol **(Version 001, dated 06/04/2016)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: Dr. David Collier

Chief Investigator Site: William Harvey Heart Centre, WHRI, QMUL

Signature and Date: 16 SEPT 2016

Principal Investigator Agreement *(if different from Chief investigator)*

The clinical study as detailed within this research protocol **(Version 001, dated 06/04/2016)**,, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Principal Investigator Name: Dr Jesmond Dalli

Principal Investigator Site: Centre for Biochemical Pharmacology WHRI, QMUL

Signature and Date: 16/09/2016

5. INTRODUCTION

5.1 Background

Acute inflammation is the body's response to injury and/or infection that is orchestrated by mediators produced at the site where the inflammatory insult occurred and instructs white blood cell and stromal cell behavior (1-3). When this response is self-contained it is protective and leads to disposal of the inciting stimulus, clearance of white blood cells from the site, repair of damaged tissue(s) and regain of function. If the response becomes dysregulated it leads to uncontrolled white blood cell activation, tissue damage and malaise. Unbridled inflammation is widely appreciated as the unifying component in many chronic conditions including metabolic syndrome, vascular disease and rheumatoid arthritis (1-3).

The acute inflammatory response has classically been divided into two phases, initiation and resolution. The initiation phase is catalysed by local acting mediators including the essential fatty acid derived autacoids as well as cytokines and chemokines. Among these autacoids the arachidonic acid derived prostaglandins (PG; i.e. PGD_2 and PGE_2) and cysteinyl leukotrienes (LT) mediate vascular leakage, LTB_4 is a potent leukocyte chemo-attractant and thromboxane A_2 is a potent pro-thrombotic agent (4). On the other hand, the resolution phase is now appreciated to be a coordinated cellular and biochemical response that involves leukocyte trafficking to and from the site of injury/infection and production of autacoids that reprogram the immune response(s) (2).

Given the important role that pro-inflammatory mediators play in the initiation of inflammation it was thought that chronic inflammation is a result of unabated proinflammatory mediator production both at the site of inflammation and systemically (4, 5). Thus, a great deal of effort has been devoted over the last decades to identifying therapeutics that inhibit production of proinflammatory mediators (4, 5). This has led to the development of nonsteroidal anti-inflammatory drugs (NSAIDS), glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs) that are widely used in the management and treatment of rheumatoid arthritis. The first two classes are primarily used to relieve symptoms, whereas DMARDs reduce or even prevent disease progression caused by unregulated inflammation when used early on (6). While these approaches are successful at controlling inflammation in the short term, the majority of anti-inflammatory therapeutics in clinical use to date have demonstrated adverse effects when administered over extended periods of time (1-3). Indeed chronic inhibition of these pro-inflammatory pathways results in gastro-intestinal barrier breakdown (7) and immune suppression amongst others (1-3). There is also significant variability in patient responsiveness to these therapeutics and their ability to limit inflammation significantly declines with disease severity (8, 9). In addition, these anti-inflammatories may also delay tissue repair and/or regeneration, thus underscoring the importance of developing alternative strategies that are able to reprogram the inflammatory response and promote tissue repair and regeneration (10). Given that under ideal conditions the body engages protective mechanisms that limit inflammation without unwanted side effects (1-3), gaining insights into these processes is of great interest.

5.2 Identification and actions of pro-resolving mediators.

Recently efforts to better understand processes that regulate the termination of the inflammatory response and the re-establishment of homeostasis, provided

mechanistic evidence that the resolution of inflammation is not simply a passive dissipation of signals but a coordinated cellular and biochemical process (11-16). Indeed, cells require signals that instruct their behaviour at the site of inflammation. Systematic analysis of inflammatory fluids collected from the site of injury/infection during self-resolving inflammation and assessment of cell trafficking at site of inflammation lead to the identification of a novel genus of local mediators that potently and stereospecifically regulate key steps in the resolution of inflammation. Thus these novel mediators produced by the body does not simply inhibit inflammation but activates resolution responses, thereby displaying both anti-inflammatory and pro-resolving actions (2, 11-16). Given that these the potent biological actions these novel molecules are coined as specialized pro-resolving mediators (SPM). The potent biological actions exerted on different components of the inflammatory response by these mediators further underscore the notion that inhibition of inflammation, or anti-inflammation, may not be the only approach to treat uncontrolled and chronic inflammation. Indeed these mediators provide evidence that under ideal conditions our body can regulate the inflammatory response by actively terminating it rather then inhibiting it, thereby sparing the immune response by repurposing the biological actions of leukocytes to re-establish homeostasis.

SPM production occurs via stereospecific conversion of essential fatty acids, including eicosapentaenoic acid (EPA), n-3 docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) by leukocyte enzymes, primarily lipoxygenases and cyclooxygenases. The omega-3 derived SPM are divided into four main families D- and E-series resolvins (Rv), protectins (PD) and maresins (MaR) and display both overlapping as well as characteristic biological actions. Each actively limits further neutrophil recruitment to the site of inflammation (1-3), they reduce chemo-attractant production and adhesion molecule expression, counter-regulate the production of pro-inflammatory mediators by regulating their formation and clearance, and promote neutrophil apoptosis and their clearance by macrophages as well as pain. In addition the maresins actively promote the repair and regeneration of damaged tissues, the protectins display potent anti-viral actions the D-series resolvins regulate B-cell antibody production and the E-series resolvins regulate T-cell and dendritic cell responses (1-3).

Studies investigating the production of these molecules during unresolved or chronic inflammatory conditions in experimental settings demonstrate that dysregulation in SPM production may be an underlying cause for disease propagation. For example in delayed or non resolving *Escherichia coli* infections SPM production becomes dysregulated leading to exacerbated local and systemic inflammation, failure of the host response to clear the invading pathogen and eventually death. Results with human tissues lend further support to this hypothesis, where SPM production is found to be dysregulated in a number of clinical settings including Alzheimer's disease and sepsis.

5.3 Fish oil supplementation to boost endogenous SPM

Evidence that chronic inflammation may result from inability of the body to produce adequate levels of SPM to counter regulate the ongoing inflammation suggests that increasing endogenous omega-3 levels may help promote SPM formation and thereby facilitate the termination of inflammation. A Pubmed search for the term 'fish oils supplementation + inflammation' yielded over 500 hits highlighting the fact that Fish oil supplementation is of general interest to both the academic and clinical fields and has be extensively studied. Animal studies using both fish oil as the main source of omega-3 essential fatty acids as well as other omega-3 enriched supplements

provide further evidence for the relationship between increased SPM production following supplementation and a reduction in inflammation (17-19). Studies with humans also indicate that fish oil supplementation may exert protective actions in chronic conditions such as rheumatoid arthritis (20) and certain cardiovascular diseases (21).

Omega-3 essential fatty acid supplementation has also been associated with increase SPM production in humans. In healthy volunteers taking 4 g of fish oil per day for three weeks plasma levels of three SPM, RvD1, RvD2 and AT-RvD1 were found to increase (22). In a separate study patients with chronic kidney disease were given 4 g of essential fatty acids and the investigators found significant increases in markers SPM pathways and RvD1 (23). In patients diagnosed with Alzheimer's or minor cognitive impairment, omega-3 fatty acid supplementation improved or stabilized cognitive function, it also lead to an increased ability of peripheral blood monocyte derived macrophages to phagocytose Amyloid □, and increase RVd1 levels (24). We recently developed a new platform that allows for the simultaneous measurement of all the pro-resolving mediators in addition to their pathway markers (>90 molecules) in human tissues. Using this platform we found that supplementation of healthy volunteers of healthy volunteers with 1 g essential fatty acids and aspirin increased plasma SPM levels, including RvD1, RvD2, RvD5 and RvE2 ((25), as early as 4 hours after intake providing the first evidence in humans that omega-3 supplementation can rapidly increases plasma SPM levels.

5.4 Rationale for the study

The relationship between omega-3 essential fatty acid supplementation, and specifically fish oil supplementation, and SPM production in humans is very poorly understood. Given that the body produces SPM from omega-3 essential fatty acids to regulate inflammation and also to repair damaged tissues, it is critical to gain further insights on how the body utilizes dietary supplementation of omega-3 fatty acids from fish oils for SPM formation. With the availability of the platform we recently developed (25) we are now in a unique position to better understand the biology of fish oil supplementation by monitoring the levels of SPM in plasma. This understanding may in turn shed light into the beneficial actions of omega-3 supplementation. It may also provide new leads for the control of excessive inflammation, as found in chronic inflammatory disorders, via dietary supplementation to exploit the body's own defense systems.

5.5 Rationale for choice of doses

Given that in a study using a different fish oil source and formulation we found that 1 g of essential fatty acids gave a mild but significant increase in plasma SPM levels (25) we chose the lowest dose in the study to be of 1.5g. We then chose to investigate two other doses that were within the **European Food Safety Authority's Tolerable Upper Intake Level for supplements containing both EPA and DHA (See Appendix 2 and 3)**. Given that this limit is of 5 g and previous study with both healthy volunteers and patients demonstrated that doses up to 4 g are well tolerated (22-24), we chose the remaining 2 doses to be 3.0 g and 4.5 g. In addition, this supplement was awarded a **Generally Recognized as Safe Status (see appendix 1)** in the for a dose of up to 5 g. Similar doses of the emulsion from of the fish oil supplement are also being used in an ongoing clinical study in the USA (ClinicalTrials.gov Identifier: NCT02719665) measuring different outcomes to those being investigated in the present study.

5.6 Fish oil supplement manufacture

The fish oil and placebo will be provided by Metagenics and will be manufactured as detailed below:
Fish oils are manufactured following current Good Manufacturing Practice (cGMP), using semi-refined fish oil (anchovy/sardine oil) or pre-concentrated omega-3 fatty acid ethyl ester as a starting material. When semi-refined fish oil is used, the first step in the process is chemical esterification followed by a distillation step, in order to meet the same specifications as is the case for the pre-concentrated omega-3 fatty acid ethyl ester starting material. Concentration steps follow which are dependent on the degree of concentration of the starting material (natural heterogeneous material). The intermediates generated in the process are processed using carbon dioxide (CO_2) in a supercritical fluid extraction processes (SFE). The process benefits from the fact that CO_2 in supercritical conditions can operate at moderate temperatures without stressing the products and avoids further degradation of the products due to the inert properties in front of oxidation. A bleaching unit and or a deodorization step can be included if applicable. A bleaching step is usually performed to control and reduce oxidative parameters in oils, and to reduce the colour. A deodorization step is performed to reduce volatile components of the product and consequently its odour. The last step is homogenization with an added quantity of natural antioxidants such as a mixture of tocopherols, in order to improve the oxidative stability of the final product. The product is then packaged in a clean room into steel drums with an appropriate internal coating.
Reagents/processing aids are suitable for use in the production of the fish oil supplement. They are commonly used in food ingredient manufacturing processes as described in the table below.

Reagents/Processing Aids		
Reagent/Processing Aid	*CAS Number(s)*	*21 CFR/GRN Citation(s)*
Ethanol, food grade	64-17-5	184.1293
Sodium ethylate	141-52-6	NA*
Carbon dioxide	124-38-9	184.1240
Diatomaceous earth	NA	GRN No. 87; SCOGS No. 61
Nitrogen	7727-37-9	184.1540
Mixed natural tocopherols	59-02-9; 16698-35-4; 54-28-4; 119-13-1	182.3890; 184.1890

*Basic catalyst employed for esterification purposes; employed in food and pharmaceutical manufacturing processes.

The Specifications for the fish oil supplement (monohydroxylated ethyl ester of omega-3) are presented below in the table below.

Specifications for Fish Oil Supplement	
Parameter (Assay Method)	**Specification**
General Characteristics	
Appearance (Visual)	Slightly yellow
Odour	Slight fish odor
Solubility	Almost insoluble in water. Miscible with acetone, ethanol, heptane, and methanol
Acid value (mg KOH/g) (Eur.Ph 2.5.1)	Max. 3
Peroxide index (meq O_2/kg) (Eur.Ph 2.5.5)	Max. 10

Fatty Acid Profile		
EPA (mg/g, as FFA) (Eur.Ph 2.4.29)	100 – 260	
DHA (mg/g, as FFA) (Eur.Ph 2.4.29)	300 – 450	
Total omega-3 (mg/g, as FFA)* (Eur.Ph 2.4.29)	600 – 850	
Heavy Metals		
Lead (mg/kg) (AOCS Ca18c-91)	Max. 0.1	
Arsenic (mg/kg) (AOAC 986.15)	Max. 0.1	
Cadmium (mg/kg) (AOCS Ca18d-01)	Max. 0.1	
Mercury (mg/kg) (AOAC 971.21)	Max. 0.1	
Potential Contaminants		
Copper (mg/kg) (AOCS Ca18-79)		Not specified, but analysis required
Iron (mg/kg) (AOCS Ca18-79)		Not specified, but analysis required
Cesium 134 (Bq/kg) (Gamma SPECTR)		Max. 3
Cesium 137 (Bq/kg) (Gamma SPECTR)		Max. 3
Iodine 131 (Bq/kg) (Gamma SPECTR)		Max. 3
Dioxins and Furans (pg WHO-TEQ/g) (EPA 1613)		Max. 1.75
Dioxin-like PCBs (pg WHO-TEQ/g) (EPA 1668)		Max. 3
Sum of dioxins and furans + dioxin-like PCBs (pg WHO-TEQ/g) (EPA 1613 and EPA 1668)		Max. 3
PCBs (209 congeners) (mg/g) (EPA 8082)		Max. 0.09
PAH (16 compounds) (GC/MS)		Not specified, but analysis required

* Sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:5ω3, and 22:6ω3.

The fish oil product meets the above analytical specifications. Stability testing of fish oil supplement has been conducted at 25°C for up to 18 months. After a 18-month storage period, fish oil supplement was found to be stable in terms of peroxide value, acid value, total omega-3, EPA and DHA.

6. TRIAL OBJECTIVES

6.1 Aim of research

The aim of this research is to investigate whether fish oil supplementation increases the peripheral blood levels of SPM and whether fish oil supplementation also regulates peripheral white blood cell responses (including neutrophils, monocytes and platelets) to inflammatory stimuli.

6.2 Original hypothesis

Given that fish oils are rich in omega-3 essential fatty acids that are precursors in the biosynthesis of SPM **the hypothesis underlying the present study is: *Fish oil supplementation increase peripheral blood levels of SPM precursors that may***

be converted to bioactive mediators which in turn will regulate white blood cell responses.

6.3 Primary Endpoint

The Primary endpoint of the study will be an increase in peripheral blood SPM levels that will be measured using established liquid chromatography tandem mass spectrometry based approach (see ref 17 above).

6.4 Secondary Endpoints

The secondary endpoints of this study are

1) an increase in *ex vivo* phagocytosis of *Escherichia coli* by peripheral white blood cells
2) a decrease in white blood cell activation when cells are incubated with an inflammatory stimulus (e.g. Platelet activation factor). This will be achieved by assessing the expression of adhesion molecules on peripheral blood cells using flow cytometry and fluorescently conjugated antibodies.

7. METHODOLOGY

7.1 Subject Selection

This is a single centre trial and Participants will be enrolled via adverts posted at each of the three QMUL campuses and on social media. No one with direct connections to the management or supervision of this study or any their collaborators will be eligible for recruitment. For QMUL student recruitment, permission from the Deanery will also be sought**.**

7.2 Inclusion Criteria

For participants to be included in the study they will need to meet the following criteria:

1) Able to provide informed consent
2) Men and women between the age of 18 and 45
3) Declare not to be taking aspirin, other NSAIDS, other form of medication or omega-3 fatty acid supplements for more than 2 weeks prior to screening and the duration of the participation.
4) Willingness to abstain from eating fish for 2 days before each study visit
5) Willingness to abstain from alcohol consumption for at least 24h prior to each study visit
6) Willingness to abstain from caffeine as directed before and during study

7.3 Exclusion Criteria

1) History of, chronic disorders, cardiovascular disease (e.g., heart disease, stroke), cancer, or diabetes or significant genetically inherited conditions.
2) Pregnancy or breast-feeding.
3) Hypothyroidism in the opinion of the investigator.
4) Liver disease in the opinion of the investigator.

5) Any abnormality or pre-existing disease which, in the opinion of the investigator, might either expose the subject to risk, or influence the validity of the results.
6) Women of childbearing potential not taking adequate methods of contraception
7) Inability to read and write in English
8) Participation in a clinical study of a new chemical entity, biological product or a prescription medicine, or loss of more than 400 mL blood, within the previous 3 months
9) Smoking
10) Presence or history of drug or alcohol abuse or intake of more than the amount of alcohol in the current guidelines on alcohol consumption

7.4 Study Design / Plan – Study Visits

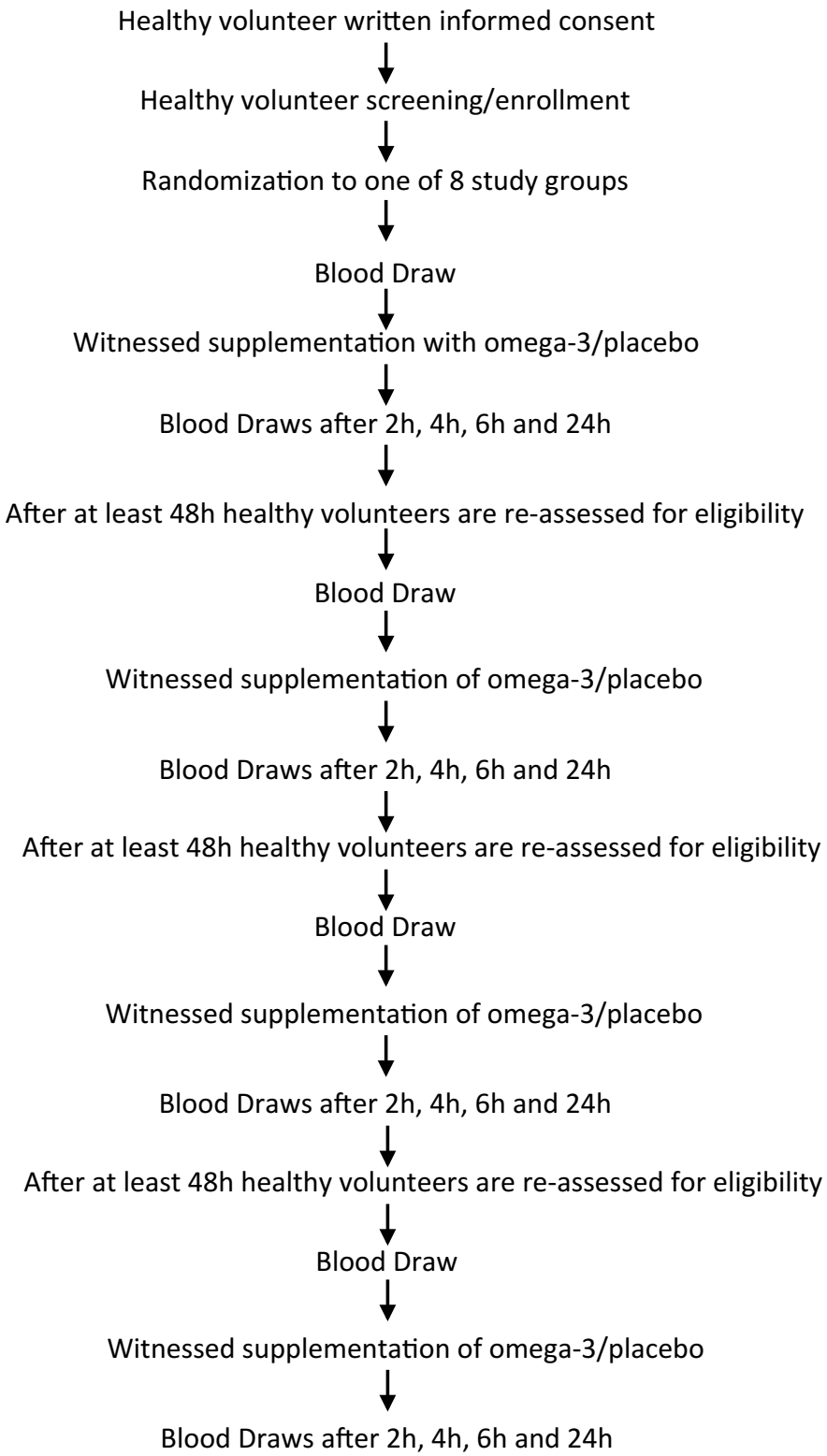
This is an open label double-blind, placebo controlled, crossover study that aims to determine the dose of omega-3 fatty acids that leads to an increase in peripheral blood SPM levels and the kinetics of this regulation.

- Each participant will give a baseline blood sample then they will be given one of the randomly allocated three doses of fish oil supplement, or a matching placebo in one of 8 study groups [this will be on a 1:1:1:1:1:1:1:1 ratio]. Blood will be collected again at 2h, 4h, 6h and 24h after supplementation/placebo.
- This design allows to compare the levels of SPM pre and post supplementation and therefore to account for inter participant variation in circulating SPM levels as well as for any differences in white blood cell responses ex vivo.
- Using a crossover design and a placebo, diurnal variation in the levels of SPM (currently not known) will be checked. In addition, using each volunteer as their own control for comparison with each dose of supplement will further improve the power of the study.
- Each participant is expected to be involved in the study for a maximum of 11 weeks from initial consent and screening.
- Participants will be asked to refrain from consuming caffeine containing drinks at least 2h prior to their visit.
- Participants will be expected to refrain from alcohol intake for at least 24h before screening for the study, and for at least 24h before each cycle of the study.
- Participants will also be asked not to take other supplements, medications for the duration of the study, and to avoid fish containing meals for 2 days before each study cycle.
- For each cycle, the participants will make two visits, the first visit will be of a maximum of 8hrs. Here baseline (0hrs) blood sample will be obtained and the supplement/placebo given.
- Blood will be collected 2hrs, 4hrs and 6hrs following supplement/placebo intake and the participant will be free to leave after the 6hrs blood collection.
- During this time participants will be asked to refrain from drinking caffeine containing drinks such as coffee or tea.
- Participants will then be asked to return the next day (24h after fish oil supplement/placebo intake).
- The 24h blood draw should take a maximum of 30 min.
- After a minimum of 48 hours, and not more than two weeks participants will be asked to return, their eligibility criteria re-assessed and then administered the next supplement/placebo dose in the cycle as determined by the group they were assigned to at the beginning of the study (see Section 13.5.1)

- A first follow-up phone-call to the volunteer will be made to check well-being at 2 weeks (+/- 1 week) after the end of the volunteers last cycle
- A Second follow-up phone-call to the volunteer will be made to check well-being at 4 weeks (+/- 1 week) after the end of the volunteers last cycle
- If any adverse effects are reported by the volunteer they will be asked return for further evaluation.

The fish oil supplements that will be used in the present studies are isolated from Peruvian anchovies. These are then packaged as emulsions and supplied by Metagenics with whom we will have contractual agreement in place before the start of the study. Metagencis will also supply the emulsion that does not contain fish oil supplements to be used as placebo. Both of these produces are not considered to be drugs or medicines and classified as foodstuffs.

7.5 Study Scheme Diagram



8. STUDY PROCEDURES

8.1 Recruitment

Participants will be enrolled via adverts posted at each of the three QMUL campuses and social media. No one with direct connections to the management or supervision of this study or any their collaborators will be eligible for recruitment. For QMUL student recruitment, permission from the Deanery will also be sought. Adverts will also be posted on social media such as Facebook and Twitter as well as newspapers. A PDF copy of the poster will be added to the William Harvey website.

Potential participants will be asked to contact the research team via email or over the phone. Then they will be invited to attend screening thereafter.

All potential participants will be given the Participant Information Sheet (PIS) by a member of the study team which they can take away for consideration. The participant will be given at least 48 hours to decide whether or not to take part in the study following being given the PIS.

All participants who demonstrate interest in enrolling will be invited to attend the William Harvey Clinical Research Centre (CRC) one week later where they will have the opportunity pose any questions to the research team and then will have the opportunity to formally enrol.

Up to a maximum payment of £400 will be organised for participation in the study to reimburse London travel expenses within Greater London.

8.2 Screening and Consent

The consenting procedure will follow the William Harvey Heart Centre, Clinical Research Centre's SOP 011.

The doctors obtaining informed consent will have current GCP training. They will be familiar with the study, the fish oil supplement and will use the current version of the Patient Information Sheet (PIS) and Consent Form, which has been approved by ethics.

The doctors obtaining consent will ensure that the participant is able to understand the information given and will explain the nature, purpose, procedures, risks and benefits of the study to the participant. The doctor will answer any questions the participant may have to the participant's satisfaction. The participant will be given at least 48 hours to decide whether or not to take part in the study following being given the PIS. The date the PIS is given to the participant will be documented to ensure that sufficient time is given for consideration.

The doctor will explain the consent form and obtain the participant's initials and dated signature on the consent form. They will then countersign and date the consent form after the participant has signed it. The original signed consent and PIS will be kept in the subject record in the CRC, a copy will be filed in the Site Investigator File and the participant given a copy.

Volunteers will also be asked, on the patient information sheet, if they are willing to have their RNA extracted and stored for subsequent analysis, on the understanding that they will not be informed of any genetic information, and that the team dealing with them in the trials unit will also never be made aware of any genotyping information. These samples will be stored for a maximum of 2 years. These samples will be used to investigate the expression of SPM biosynthetic enzymes, the SPM receptors and pathways that these molecules may activate.

Basic screening will performed, which will include- clinical history taking, vital signs- Blood pressure, pulse rate, ECG, physical examination and blood tests; full blood count, biochemistry, liver function tests, glucose and C reactive protein. The identity of healthy volunteers will be documented by taking a photocopy of an original photo ID card (passport, driving license, TFL photocard), and archived with the study records. Before each dosing the identity of the volunteers will be confirmed against the identity records on file.

8.3 Intervention

After determining eligibility participants will be randomly assigned to one of the eight groups outlined in the table below where each participant will receive all three doses and the placebo but in a different sequence to minimise any interaction effects.
In order to ensure adequate recruitment into all eight cycles prior to interim analysis group allocation will be divided into two blocks with 20 volunteers per block. Each of the supplement capsule contains 1.5g of essential fatty acids. So administration of 1 supplement capsule and 2 placebo capsules will deliver 1.5g of omega-3 fatty acids, administration of 2 supplement capsules and 1 placebo capsule will deliver 3 g of omega-3 essential fatty acids and administration of 3 supplement capsule will deliver 4.5g essential fatty acids.

Group	**Treatment**			
	Cycle 1	**Cycle 2**	**Cycle 3**	**Cycle 4**
Group 1	3 capsules Placebo	1 capsules Supplement & 2 capsules Placebo	2 capsules Supplement & 1 capsules Placebo	3 capsules Supplement
Group 2	1 capsules Supplement & 2 capsules Placebo	2 capsules Supplement & 1 capsules Placebo	3 capsules Supplement	3 capsules Placebo
Group 3	2 capsules Supplement & 1 doses Placebo	3 capsules Supplement	3 capsules Placebo	1 capsules Supplement & 2 capsules Placebo
Group 4	3 capsules Supplement	3 capsules Placebo	1 capsules Supplement & 2 capsules Placebo	2 capsules Supplement & 1 capsules Placebo
Group 5	3 capsules Supplement	2 capsules Supplement & 1 capsules Placebo	1 capsules IMP & 2 capsules Placebo	3 capsules Placebo
Group 6	2 capsules Supplement & 1 capsules Placebo	1 capsules Supplement & 2 capsules Placebo	3 capsules Placebo	3 capsules Supplement
Group 7	1 capsules Supplement & 2 capsules Placebo	3 capsules Placebo	3 capsules Supplement	2 capsules Supplement & 1 capsules Placebo
Group 8	3 capsules Placebo	3 capsules Supplement	2 capsules Supplement & 1 capsules Placebo	1 capsules Supplement & 2 capsules Placebo

This above protocol will be followed until all forty participants are recruited (unless halted after the interim analysis).

8.4 Randomisation Process

Patients will be block randomised on a 1:1:1:1:1:1:1:1 basis to receive either one of the three doses of fish oil or placebo in one of 8 study groups, using a binary random number sequence (www.random.org).

8.5 Blinding Process

The study treatment will be prepared and labelled by the WHRI Heart Centre Pharmacy, according to the randomisation schedule. Active and placebo treatments will be labelled such that it is not possible to distinguish between them. Each subject's treatment will be given a unique code number, that is traceable to study group and batch numbers of placebo and supplement.
Investigators and research team including CI, PI, trial coordinator, research nurses and lab staff, will be blinded to the treatment being provided. A sealed copy of the randomisation code will be kept in a locked file in the WHRI Heart Centre Dispensary. The investigator will be supplied with sealed envelopes, each one containing the treatment allocation for the subject whose number appears on the outside of the envelope. Those envelopes will be kept in the study master file, readily accessible to clinical staff. Emergency procedures for revealing treatment codes are specified in Section 7.6 below. The investigator, sponsor's medical expert and clinical monitor will remain blinded throughout the study, unless safety concerns necessitate unblinding.

8.6 Un-blinding Process

In the event of an emergency PI or medically qualified delegate can request unblinding from the dispensing team.

The a member of the research team will notify the CI in writing as soon as possible following the code break detailing the necessity of the code break. Given that the nature of the placebo and supplement, where the supplement is a commercially available supplement that will be taken within the recommended daily doses and that the participants will all be healthy volunteers it is not anticipated that any emergencies will arise as a direct consequence of consumption of either the placebo or the fish oil supplement. Therefore 24h unblinding is not required.

The PI must document the breaking of the code and the reasons for doing so on the CRF/data collection tool in the site file and medical notes. All unblinding will also be documented at the end of the study in any final study report.

At the end of the trial once all the analysis has been conducted the codes will be broken by the CI.

8.7 Experimental Methods

After collection samples will be processed William Harvey Centre for Biochemical Pharmacology/Clinical Pharmacology Laboratories within one hour of following standard operating procedures listed in the *Lipid Mediator Unit Laboratory Manual*.

White Blood cell phagocytosis of Escherichia coli:

- 20 µl of blood will be incubated with $2x10^7$ C.F.U. of fluorescently labeled E. coli

- Cells will be incubated at 37°C for 1h
- Cells will then be processed for flow cytometry using established protocols
- The extent of phagocytosis will be assessed using flow cytometry

Peripheral blood cell activation

- 20µl of whole blood will be incubated with or without 100µM of PAF for 1h at 37°C
- Cells will the be washed 2x with PBS containing 5% fetal calf serum
- Cells will then be incubated with the following fluorescently conjugated antibodies for 15 min at room temperature
- Cells will then be processed for flow cytometry
- Adhesion molecule expression as well as platelet-leukocyte aggregates will be assessed using flow cytometry

Lipid mediator profiling

- Plasma (2ml) will be placed in 8 ml of ice-cold methanol containing deuterium labeled internal standards
- Samples will be stored at temperatures of -80°C
- Samples will be processed in batches of 20-24.
- Here samples will be centrifuged at 3000xg for 10 min at 4°C
- Supernatants will be collected
- Lipid mediators (LM) will be extracted using solid phase extraction techniques
- Isolated LM will be profiled using liquid chromatography tandem mass spectrometry (see ref 17)
- At the end of the study any excess or unused samples will be disposed of unless written consent is obtained from participant for longer term storage. Sample disposal will be conducted following standard operating procedures as outlined in the Biochemical Pharmacology laboratory manual.

RNA storage

If participant consent, blood will also collected and stored in RNA stabilizing reagent for later processing. These samples will be used to assess the expression of genes involved in the initiation and resolution of inflammation. This sample collection will be conducted with the understanding that the participants will not be informed of any genetic information, and that the team dealing with them in the trials unit will also never be made aware of any genotyping information (a one-way donation). These samples will be stored for a maximum of 2 years. RNA will be used to investigate the expression of SPM biosynthetic enzymes, the SPM receptors and pathways that these molecules may activate.

8.8 Clinical Protocol

At least 24h prior to consent, participants will be provided an information sheet by an appropriate method including email, post or by attending the clinic, detailing the rationale and hypothesis of the study and on attending the screening visit the study physician will address any questions that the participants may have. They will be asked to sign the informed consent and then their eligibility criteria will be assessed based on the inclusion criteria set out for the study. Basic observations, blood pressure, pulse, resting supine ECG, Blood samples for full blood count, biochemistry and glucose and CRP, then a physical examination will be performed.

Volunteers passing screening will then:

- Will give a baseline blood sample (12ml)
- Be given one of the randomly allocated three doses of fish oil supplement, or a matching placebo [this will be on a 1:1:1:1 ratio].
- Give blood (12ml/time interval) will be collected again at 2h, 4h, 6h
- Be allowed to leave and they will be asked to return the following day for the collection of the 24h blood sample.
- Be asked to return after a minimum of 48h but no later then 2 weeks
- Identity will be verified
- Blood will be obtained (12ml)
- Be given the next supplement dose/placebo that is listed on their assigned study group and the injection witnessed by a member of the study team.
- Blood will be obtained after 2h, 4h and 6h.
- Be free to leave and will be asked to return after 23h from supplementation
- The 24h blood sample will be collected and the participants will be asked to return again after a minimum of 48h and no later then 2 weeks for their next supplement dose/placebo cycle
- Identity will be verified
- Blood will be collected (12ml)
- Supplement/placebo will be administered and ingestion witnessed.
- Blood will be collected at the same intervals outlined above
- Participants will be asked to return for the last supplement dose/placebo listed on their study group at least 48h after the last blood donation (24h blood sample) but no later then 2 weeks.
- Essentially the same protocol outlined above will be followed for the last cycle.
- A first follow-up phone-call to the volunteer will be made to check well-being at 2 weeks (+/- 1 week) after the end of the volunteers last cycle
- A Second follow-up phone-call to the volunteer will be made to check well-being at 4 weeks (+/- 1 week) after the end of the volunteers last cycle

Participants can withdraw from the study at any time.

8.9 Schedule of Assessment (in Diagrammatic Format)

Assessment	Screening	Study Visit 1 (Week 1)	Study Visit 2 (Week 1+24h)	Study Visit 3 (Week 2)	Study Visit 4 (Week 2+24h)	Study Visit 1 (Week 3)	Study Visit 2 (Week 3+24h)	Study Visit 3 (Week 4)	Study Visit 4 (Week 4+24h)	Follow up telephone call	End of Study telephone call
Informed written consent Inclusion/Exclusion criteria	x										
ID Asessment using Photo id	x	x	x	x	x	x	x	x	x		
Weight	x										
Height	x										
Screening bloods*	x										
Pregnancy test	x										
Resting ECG	x										
Physical examination	x										
Assess adverse events		x	x	x	x	x	x	x	x	x	x
Dosing		x		x		x		x			
Document any medication	x	x		x		x		x			
Blood draw** @ 0hrs		x		x		x		x			
Blood draw @ 2hrs		x		x		x		x			
Blood draw @ 4hrs		x		x		x		x			
Blood draw @ 6hrs		x		x		x		x			
Blood draw @ 24hrs			x		x		x		x		

8.10 End of Study Definition

The CI is delegated the responsibility of submitting the EOT notification to REC once reviewed by sponsor. The EOT notification must be received by REC and within 90 days of the end of the trial.
If the study is ended prematurely, the Chief Investigator will notify the Sponsor & REC including the reasons for the premature termination (within 15 days).

The trial will end after the last healthy volunteer's last visit and the analysis of all sample analysis has been completed.

8.11 Subject Withdrawal

PI may/must withdraw participants from the study if during any point they intake any medication, alcohol, other supplements or oily fish prior to the last blood draw (24h). If participants are not able to make their next visit within the stipulate window (2 weeks + 48h from the supplement/placebo dose) they will be re-screened prior to re-enrolling.

If blood cannot be obtained at the 0h, 2h, 4h or 6h time intervals then the participant will be withdrawn from the primary analysis. If the blood cannot be withdrawn at the 24h interval the participant can still be included for primary and secondary endpoint analysis.

If a participant is withdrawn from the study they may re-enroll in the study after being >14 days free of medication.

If the participant withdraws before the completion of the 4th visit and dosing of supplement/placebo the samples and information will be still be analysed, but the study analysis will be primarily by per-protocol (complete treatment cycles only), although all data will also be analysed for Intention-to-treat as a secondary analysis.

Withdrawn subjects may be replaced at the discretion of the investigator.

9. STATISTICAL CONSIDERATIONS

9.1 Primary Endpoint

The Primary endpoint of the study will be an increase in peripheral blood SPM levels that will be measured using established liquid chromatography tandem mass spectrometry based approach.

9.2 Secondary Endpoints

The secondary endpoints of this study are

1) an increase in *ex vivo* phagocytosis of *Escherichia coli* by peripheral white blood cells
2) a decrease in white blood cell activation when cells are incubated with an inflammatory stimulus (e.g. Platelet activation factor). This will be achieved by assessing the expression of adhesion molecules on peripheral blood cells using flow cytometry and fluorescently conjugated antibodies.

9.3 Sample Size

Power calculations were conducted using data obtained from published studies where healthy volunteers were given 1 mg essentially (from a different source to that being investigated in the present study) and peripheral blood lipid mediator levels were evaluated pre-essential fatty acid and 4 hours after supplement intake (Colas et al Reference 17). Using pre and post values of 10 mediators values from these studies and Wilcoxon matched-pairs signed rank test a sample size of 33 participants is required to detect a threefold odds of an increase in post value when compared with the pre value with a power of 80% at the 5% level of statistical significance using the formulae from reference 18. Fifty mediators will be examined and it is recognized that at the 5% level of statistical significance around 2-3 mediators will falsely be identified as having significant changes. However the functional effect of all significant mediators will be subsequently examined by assessing white blood cell function, including the ability of peripheral white blood cells to phagocytose bacteria and the response of peripheral white blood cells and platelets to an inflammatory stimulus. If we do not observe increases in the ability of peripheral white blood cells to phagocytose bacteria and/or decreases in the activation of peripheral white blood cells and platelets then these mediators will be assumed to be false positive results. For these analysis the white blood cell function and peripheral blood lipid mediator levels at the 2h and 4h intervals post essential fatty acid/placebo administration will be compared to pre-supplementation values. The time point and dose that give the greatest change in all the mediators and the greatest regulation of peripheral white blood responses will be judged to be the main outcome measure for future trials.

9.4 Statistical Analysis Plan

Wilcoxon matched-pairs signed rank test will be used to analyse the pre and post values of 10 mediators as well as white blood cell function (at 2 and 4h). The time point corresponding to the greatest number of the mediators being statistically significantly different from their pre-test values at the 5% level of statistical significance will be identified as the time point of interest. At the 5% level of significance testing 50 mediators will result in at least 2-3 associations being falsely statistically significant. Thus, the functional effect of all significant mediators will be subsequently examined by assessing white blood cell function, including the ability of peripheral white blood cells to phagocytose bacteria and the response of peripheral white blood cells and platelets to an inflammatory stimulus to determine if the identified associations are likely to be true changes. If we do not observe increases in the ability of peripheral white blood cells to phagocytose bacteria and/or decreases in the activation of peripheral white blood cells and platelets then these mediators will be assumed to be false positive results. Values at different times will not be compared just the value at each time point will be compared to the same person's pre test values.

10. ETHICS

This protocol and any subsequent amendments, along with any accompanying material provided to the patient in addition to any advertising material will be submitted by the Investigator to an independent Research Ethics Committee (REC). Written Approval from the Committee must be obtained and subsequently submitted to the JRMO to obtain Final Sponsorship approval. According to the algorithm on the MHRA website, the proposed study is a non-clinical trial of an investigational medicinal product (non-CTIMP), and does not require prior approval by the MHRA (https://www.gov.uk/guidance/clinical-trials-for-medicines-apply-for-authorisation-in-the-uk#clinical-trial-phases). The study will be conducted at the WHRI Institute, in compliance with ICH GCP, the principles of good manufacturing practice and the SOPs issued by NRES for RECs in the UK.
All subjects must give written consent to participate in this study. Consent for screening evaluations may be obtained using the information and consent form that will also be submitted by the Investigator to REC prior to the start of the study. The study-specific information and consent form will be signed by the participant before any screening evaluation. Before giving consent, participants must read the information sheet about the study. They must also read the consent form. They will then discuss the study with the investigator or his deputy and be given the opportunity to ask questions. The study-specific information sheet and the consent form must be approved by the REC.

Participants are free to withdraw from the study at any time, without providing a reason.

The CI will ensure that the REC is informed promptly of any serious adverse event that occurs during this study and that is both related and unexpected (see section 13), in line with NRES SOPs, and will provide the REC with annual progress reports of the study, if it lasts longer than a year.

11. SAFETY CONSIDERATIONS:

Very high (< 10mg) omega-3 doses have been described to lead to impaired blood clotting. However this should not be of concern in the present study since the highest

dose to be employed will be of 4.5 g. In addition, the highest does being employed in the present study is also below the Generally Recognized as Safe (GRAS) status as well as the recommendations of the European Food and Safety Authority of 5 g per day

12. DATA HANDLING AND RECORD KEEPING:

12.1 Confidentiality The Principal Investigator has a responsibility to ensure that participant anonymity is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval. All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) and all subsequent amendments as defined in the JRMO SOP 20 Archiving.

The Chief Investigator and the study team will adhere to these parameters to ensure that the Participant's identity is protected at every stage of their participation within the study. To ensure this is done accordingly, at time of consent each participant will be allocated a unique screening number by either the PI or a member of the study team before undergoing any screening procedures.

Personal information will be collected upon enrolment, this will be stored in accordance with QMUL Data Protection regulations.

In order to maintain confidentiality, participants will be identified by a unique trial identifier only. This number will be randomly generated and will be assigned to each participant. In addition to relating to the participant's dataset the code will also be associated with the specific study cycle that the participant is enrolled to.

12.2 Record Retention and Archiving

Data will be managed with reference to the Barts CTU SOP 'Data Management' Reference: SOP CPTU GEN TM 06, Version 2.0.

Data will be recorded using dedicated software and computers for each of the analyses conducted. The data will then be kept anonymised by investigators. Samples will be stored and coded using the coding system in 14.3 and the key to the code will be held by the PI.

During the course of research, all records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the Research Governance Framework and Trust Policy that the records are kept for a further 20 years.

Destruction of essential documents will require authorisation from the Sponsor.

The documents will be archived in accordance with the Sponsor's SOP (JRMO SOP). Both electronic and paper documentation will be retained for archiving. These will be stored at Barts Health corporate records.

13. LABORATORIES (if applicable)

13.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with study activities.

13.2 Notification and reporting Adverse Events or Reactions

If the AE is not defined as serious, the AE is recorded in the study file and the participant is followed up by the research team. The AE is documented in the participants' medical notes (where appropriate) and the CRF.

13.3 Serious Adverse Event (SAE)

A serious adverse event (SAE) is defined as an untoward occurrence that:
(a) results in death;
(b) is life-threatening;
(c) requires hospitalisation or prolongation of existing hospitalisation;
(d) results in persistent or significant disability or incapacity;
(e) consists of a congenital anomaly or birth defect; or
(f) is otherwise considered medically significant by the investigator.

An SAE occurring to a research participant should be reported to the Sponsor and main REC where in the opinion of the Chief Investigator the event was:

• Related – that is, it resulted from administration of any of the research procedures, and
• Unexpected – that is, the type of event is not listed in the protocol as an expected occurrence.

13.4 Notification and Reporting of Serious Adverse Events

Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' will be recorded in the participants' notes, the CRF, the sponsor SAE form and reported to the JRO within 24 hours of research staff being notified and to the Main REC within 15 days in line with require the timeframe. The co-investigators listed in this protocol will be authorized to sign the SAE forms in the absence of the PI.

Since this study will be blinded, the treatment code for the patient will be broken in the reporting of an 'unexpected and related' SAE. This will be performed by the Barts CTU, who is independent of the study and will allow the rest of the research team to remain blinded. The unblinding of single cases by the PI in the course of this study will only be performed if necessary for the safety of the trial subject.

13.5 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial participants from any immediate hazard to their health and safety, the measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor and Research Ethics Committee (via telephone) of this event immediately.

The CI has an obligation to inform the REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office [JRMO])

must be sent a copy of the correspondence with regards to this matter.

13.6 Annual Safety Reporting

The CI will send the Annual Progress Report to the REC using the NRES template (the anniversary date is the date on the REC "favourable opinion" letter from the MREC) and to the sponsor.

13.7 Overview of the Safety Reporting responsibilities

The CI has the overall pharmacovigilance oversight responsibility. The CI has a duty to ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

13.7.1 Pharmacovigilance responsibilities

All treatment will be carried out at The William Harvey Research Institute & SAE's will be reported by staff to the JRMO as per their SOPs. The Trial Master File and Site file will be kept at The William Harvey Research Institute.

14. PRODUCTS, DEVICES, TECHNIQUES AND TOOLS

14.1 Summary Monitoring Plan

The trial will be monitored in accordance with sponsor SOPs and is open to both monitoring and/or audit from the sponsor. Study teams will be initiated and monitored in accordance with the sponsor SOPs led by the lead center study team/coordinator.

14.2 Audit and Inspection

Quarterly meetings will be held with by the research team to review the progress of the study and discuss and issues that may arise.

14.3 Serious Breaches in GCP or the Trial Protocol

The sponsor of the Clinical Trial is responsible for notifying the licensing authority in writing of any serious breach of:

· The conditions and principles of GCP in connection with that trial; or
· The protocol relating to the trial, within 7 days of becoming aware of that breach.

For the purposes of this protocol, a 'serious breach', is a breach which is likely to effect to a significant degree:

· The safety or physical or mental integrity of the participants of the trials; or
· The scientific value of the trial.

The CI is responsible for reporting any serious breaches to the sponsor (JRMO) **within 24 hours**. The sponsor will notify and report to REC within 7 working days of becoming aware of the serious breach.

These non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The sponsor will maintain a log of the non-compliances reported to them and the CI will maintained a site Deviation log. The sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the JRMO will agree an appropriate action, including an on-site audit

15. SAFETY REPORTING

Adverse Events (AE)

An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with study activities.

Notification and reporting Adverse Events or Reactions

If the AE is not defined as SERIOUS, the AE is recorded in the study file and the participant is followed up by the research team. The AE is documented in the participants' medical notes (where appropriate) and the CRF.

Serious Adverse Event (SAE)

In other research other than CTIMPs, a serious adverse event (SAE) is defined as an untoward occurrence that:

(a) results in death;
(b) is life-threatening;
(c) requires hospitalisation or prolongation of existing hospitalisation;
(d) results in persistent or significant disability or incapacity;
(e) consists of a congenital anomaly or birth defect; or
(f) is otherwise considered medically significant by the investigator.

An SAE occurring to a research participant should be reported to the main REC where in the opinion of the Chief Investigator the event was:
• Related – that is, it resulted from administration of any of the research procedures, and
• Unexpected – that is, the type of event is not listed in the protocol as an expected occurrence.

Notification and Reporting of Serious Adverse Events

Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' are to be reported to the sponsor within 24 hours of learning of the event and to the Main REC within 15 days in line with the required timeframe. For further guidance on this matter, please refer to NRES website and JRMO SOPs

Please note in the case of a blinded study, it is recommended the treatment code for the patient is broken in the reporting of an 'unexpected and related' SAE. Please seek advice on how this can be achieved whilst maintaining the team blind. The unblinding of single cases by the PI/CI in the course of a clinical trial should only be performed if necessary for the safety of the trial subject.

Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety,. The measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor and Main Research Ethics Committee (via telephone) of this event immediately.

The CI has an obligation to inform both the Main REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office [JRMO]) must be sent a copy of the correspondence with regards to this matter. For further guidance on this matter, please refer to NRES website and JRMO SOPs.

Annual Safety Reporting

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC "favourable opinion" letter from the MREC) and to the sponsor. Please see NRES website and JRMO SOP for further information

Overview of the Safety Reporting responsibilities

The CI/PI has the overall pharmacovigilance oversight responsibility. The CI/PI has a duty to ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

Please outline the process/organisation within the study team to ensure that all SAE reporting is conducted in accordance with the sponsor's timelines.

16. MONITORING &AUDITING

Quarterly meetings will be held with by the research team to review the progress of the study and discuss and issues that may arise.

17. TRIAL COMMITTEES

N/A.

18. FINANCE AND FUNDING

Funding for this study will be provided by Metagenics.

19. INDEMNITY

The sponsor is Queen Mary, University of London, who provide insurance for this study.

20. DISSEMINATION OF RESEARCH FINDINGS:

All relevant data from this study will be submitted to peer review journals for publication following the termination of the study in line with sponsor and trust publication policy.

21. REFERENCES

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lease use a standard referencing system.

22. APPENDICIES

Please do not include the Participant Information Sheet and Consent Form; these should be stand alone documents