Clinical Study Protocol

Omega-3 bioavailability from vegetable-omega-3 enriched products

Full Project Title: Bioavailability of long-chain omega-3 polyunsaturated fatty acids from foods enriched with vegetable-encapsulated omega-3 oils

Protocol Number:	OBP001
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Table of Contents

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Site Signatures

PROTOCOL TITLE:

PROTOCOL NUMBER: OBP001
DATE OF PROTOCOL: 13 October 2020

Confidentiality and Good Clinical Practice (GCP) Compliance Statement:

I, the undersigned, have reviewed this protocol, including the appendices and I will conduct the study as described in compliance with this protocol, GCP and relevant International Council on Harmonisation (ICH) guidelines.

Once the protocol has been approved by the Human Research Ethics Committee (HREC), I will not modify this protocol without obtaining prior agreement and approval from CSIRO/A*STAR Principal Investigator (PI) and the HREC. I will submit the protocol modification and/or any Informed Consent Form (ICF) modifications to the CSIRO/A*STAR PI and HREC and approval will be obtained before any modifications are implemented.

I understand that all the information obtained during the conduct of the study with regard to the participants' state of health will be regarded as confidential. No participants' names will be disclosed. All Participants will be identified by assigned numbers on all case report forms (CRF), laboratory samples or source documents. Clinical information may be reviewed by regulatory agencies. Agreement must be obtained from the participant before disclosure of participant information to a third party.

INVESTIGATOR SIGNATURE:

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COMPANY/POSITION: CSIRO

SIGNATURE: 

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Study Synopsis

Protocol Title:	Bioavailability of omega-3 long-chain polyunsaturated fatty acids (LCPUFA) from foods enriched with vegetable-encapsulated omega-3 oils
Aim:	To compare the bioavailability of omega-3 LCPUFA from two test food products containing vegetable-encapsulated omega-3 algal oil against a control test product (algal oil gel capsules) across two ethnicities (Australian European vs. Chinese Singaporean).
Study Design:	Multi-centre, randomised, controlled, acute, 3-way cross-over study design. Participants will consume in random order, at least 1-week apart, three test products after an overnight fast. Blood samples will be collected at 0, 2, 4, 6, 8 and 24 hours after consuming the products. Plasma fatty acid concentrations (µg/mL) will be analysed by gas chromatography and bioavailability of omega-3 LCPUFA determined by calculating the incremental area under the curve ($iAUC_{0\text{-}24h}$), time to maximal value (Tmax) and maximal value (Cmax).
Study sites:	CSIRO Nutrition and Health Research Clinic, Adelaide A*STAR Clinical Nutrition Research Centre, Singapore
Number of Participants:	N=24 (n=12 in Australia and n=12 in Singapore) Accounts for 20% attrition.
Inclusion/Exclusion Criteria:	**INCLUSION CRITERIA:** 1. Healthy men, 21-50-year old 2. BMI 18-27.5 kg/m^2 3. Consuming <2 meals of fatty fish/week and no fish oil supplements 4. Ethnicity ○ If Australian site: Australian European OR ○ If Singapore site: Chinese Singaporean **EXCLUSION CRITERIA:** 1. Self-reported history of chronic disease - cancer, type 2 diabetes, cardiovascular disease, liver disease or any condition that may, in the opinion of the principle investigator, influence the study outcomes. 2. Self-reported history of gastrointestinal disease, pancreatic insufficiency, conditions resulting in fat malabsorption - chronic pancreatitis, cystic fibrosis, coeliac disease, Crohns disease, gastric bypass surgery, small bowel resection, abnormal thyroid function 3. Bleeding disorders, currently taking anticoagulants or has received anticoagulants within 28 days of Day 1 of the trial, with the exception of low dose aspirin up to 150 mg daily* 4. Any medical procedures deemed by the principal investigator to affect study outcomes

	5. Known food allergies, hypersensitivity, dietary avoidance or intolerance to the study foods 6. Taking medications/supplements known to influence lipid metabolism and gastric emptying 7. On any weight-loss program 8. History of smoking during the 6 months prior to the study 9. Persons considered by the investigator to be unwilling, unlikely or unable to comprehend or comply with the study protocol. 10. Self-reported history of drug abuse or alcoholism 11. Participation in another research study within 30 days preceding the start of this study
Treatment:	Standardised breakfast plus: Test food 1: Semi-solid food – Soup (commercially available packet-soup) containing cauliflower encapsulated HiDHA® algal oil providing 400 mg DHA Test food 2: Solid food – Extruded snack (savory snack) containing cauliflower encapsulated HiDHA® algal oil providing 400 mg DHA Control: 2x Algal oil gel capsules containing HiDHA® algal oil providing 400 mg DHA
Study Duration:	~1 month (3 test products at least 1-week apart)
Study Population:	Healthy men
Primary Outcome:	Plasma DHA and total omega-3 LCPUFA $iAUC_{0\text{-}24h}$

List of Abbreviations

Abbreviation	Description
AE	Adverse Event
AUC	Area under the curve
BMI	Body mass index
CHD	Coronary heart disease
CRF	Case report form
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EW	Early Withdraw
FRAT	Food risk assessment team
HREC	Human Research Ethics Committee
ICF	Informed Consent Form
ICH-GCP	International Council for Harmonisation Good Clinical Practice
LCPUFA	Long-chain polyunsaturated fatty acids
NCD	Non-communicable disease
SAE	Serious adverse events

1. Introduction

Non-communicable chronic diseases (NCD) in both Australia and Singapore account for significant proportions of deaths, disease burden and hospitalizations, with poor quality diet as the major contributor to the NCD burden (1, 2). Marine derived omega-3 long-chain polyunsaturated fatty acids (LCPUFA) (particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are associated with numerous health benefits throughout the life cycle. Omega-3 Index (O3I) (EPA+DHA as % of red blood cell fatty acids – a biomarker of EPA+DHA status (3)) levels of 8% vs. 4% were related to a 30% reduced risk of fatal coronary heart disease (CHD) (4). More recently O3I levels at 8% have also been linked to increased longevity, reduced all-cause mortality, improved depressive symptoms, improved arthritis symptoms (5) and improved cognitive function (6). Despite the well-established health benefits of omega-3 LCPUFA, intakes in Western and most Asian diets are below recommendations (7, 8), and low levels of consumption have been associated with an increased risk of cardiovascular mortality in a Chinese population (7). Consumption of ~2-3 serves/week of fish, including oily fish, is recommended to achieve ~250 – 500 mg/day of combined EPA+DHA, and up to 1g/day for those with CHD (9-11). Achieving these intakes through dietary means is challenging, particularly for individuals who do not consume fish or seafood (major dietary sources of omega-3 LCPUFA) (5). Vegans and vegetarians are particularly at risk. Several studies have shown that vegan diets are devoid of DHA and vegetarian diets that include dairy food and eggs only provide about 0.02 g DHA/day. These low intakes were accompanied by substantially lower levels of DHA in plasma, serum, red blood cells (RBC) and plasma phospholipids (PL) in vegans and vegetarians compared to omnivores (12). Furthermore, a high degree of inter-individual variability exists in how omega-3 intake affects O3I levels (5). Dose, baseline omega-3 status and chemical form of supplements have been shown to affect O3I levels in response to intake (13). Other factors such as characteristics of food products and individuals' ethnicity have potential to impact O3I but have not been formally investigated previously. A better understanding of factors affecting response to increased omega-3 LCPUFA intakes will assist in the development of personalized approaches to assist consumers in achieving omega-3 status that is optimal for health. Furthermore, despite convincing evidence that diets rich in vegetables are associated with reduced risk of all-cause mortality and particularly cardiovascular mortality (14), populations globally fail to meet recommended intakes (15). Hence, strategies empowering consumers to achieve omega-3 LCPUFA and vegetable intake targets have potential to significantly impact health outcomes.

Some of the challenges in the production of foods fortified with omega-3 fatty acids include their undesirable odour and taste and their susceptibility to oxidative degradation (16). CSIRO has developed a novel vegetable-based encapsulation system for delivery of omega-3 LCPUFA which provides superior protection against oxidation of omega-3 fatty acids compared to other commercially available microencapsulation techniques (17), while at the same time delivering the nutritional benefits of vegetable consumption. Vegetable encapsulated omega-3 fortified food has the potential to deliver daily recommended dosage of omega-3 fatty acids (~250-500 mg/serve) in addition to a serving of vegetables. In addition, if a vegetarian omega-3 LCPUFA oil is used (e.g. algal oil), these products will also be suitable for vegans and vegetarians. One of the challenges in incorporating vegetable encapsulated omega-3 LCPUFA into fortified foods is the potential effect of the vegetable carrier matrix (e.g. fibre and other bioactives) on release and bioavailability of omega-3 LCPUFA *in vivo.* Furthermore, there is a possibility of inter-ethnic differences in omega-3 bioavailability. Heritability and anthropometric phenotypes, previously associated with omega-3 status (18), differ between Asian and Caucasian populations. The current project aims to address these challenges and to use the data generated as the basis for development of personalised formulations tailored to improve omega-3 status and vegetable intake and consequently health outcomes of consumers in Australia and Singapore.

2. Aim, Objectives and Hypotheses

Aim:

To evaluate the bioavailability of omega-3 LCPUFA from foods enriched with vegetable-encapsulated omega-3 oils across two ethnicities.

Objective/s:

- To compare the bioavailability of omega-3 LCPUFA from two test food products containing vegetable-encapsulated omega-3 algal oil against a control test product (algal oil gel capsules).
- To evaluate whether ethnic background (Australian European vs. Chinese Singaporean) affects the bioavailability of omega-3 LCPUFA when consuming vegetable-encapsulated omega-3 products relative to a control test product.

Hypotheses:

H0: Delivery of omega-3 LCPUFA with vegetables as carriers does not impact bioavailability.

H0: Bioavailability of omega-3 LCPUFA is not influenced by inter-ethnic differences.

Outcomes:

- Primary outcome: Incremental area under the plasma DHA and total omega-3 LCPUFA concentration (µg/mL) curves ($iAUC_{0\text{-}24h}$)
- Secondary outcomes: Time to maximal value (Tmax) and maximal value (Cmax) on the plasma DHA and total omega-3 LCPUFA concentration (µg/mL) curves.

3. Study Population

N=24 (N=12 in Australia; N=12 in Singapore) healthy men. Men are chosen as study population to ensure a homogenous population.

4.1 Inclusion and Exclusion criteria

Inclusion Criteria

1. Healthy men*, 21-50-year old**
2. BMI 18-27.5 kg/m^2
3. Consume <2 meals of fatty fish/week
4. Not Consume fish oil supplements over the past 3 months
5. Ethnicity
   a. If Australian site: Australian European
   OR
   b. If Singapore site: Chinese Singaporean

* Only males will be recruited to ensure a metabolically homogenous sample

** The lower-end age criteria has been increased from the typical 18 to 21 to provide consistency with Singaporean recruitment criteria (Participants under the age of 21 require guardian approval in Singapore).

Exclusion Criteria

1. History of chronic disease - cancer, type 2 diabetes, cardiovascular disease, liver disease or any condition that may, in the opinion of the principle investigator, influence the study outcomes*
2. History of gastrointestinal disease, pancreatic insufficiency, conditions resulting in fat malabsorption - chronic pancreatitis, cystic fibrosis, coeliac disease, Crohns disease, gastric bypass surgery, small bowel resection, abnormal thyroid function*
3. Bleeding disorders, currently taking anticoagulants or has received anticoagulants within 28 days of Day 1 of the trial, with the exception of low dose aspirin up to150 mg daily*
4. Any medical procedures deemed by the principal investigator to affect study outcomes
5. Known food allergies, hypersensitivity, dietary avoidance or intolerance to the study foods
6. Taking medications/supplements known to influence lipid metabolism and gastric emptying
7. On any weight-loss program
8. History of smoking during the 6 months prior to the study*
9. Persons considered by the investigator to be unwilling, unlikely or unable to comprehend or comply with the study protocol
10. History of drug abuse or alcoholism*
11. Participation in another research study within 30 days preceding the start of this study

***Self-reported, no clinical testing will be performed.**

4. Study Design and procedures

A multi-centre, randomised, controlled, acute, 3-way cross-over single-blind study design will be used. The trial will be performed at CSIRO's Nutrition and Health Research Clinic, Adelaide (n=12) and A*STAR's Clinical Nutrition Research Centre in Singapore (n=12).

Participants will attend a screening visit to confirm their eligibility for inclusion into the trial (Figure 1). If eligible, participants will remain at the clinic where they will be enrolled into the study and participate in the first intervention on that day (Screening will be conducted at V1). After enrolment participants will attend the research clinic for test procedures on 3 occasions at least 1-week apart during which they will consume one of three test products at each occasion in random order. Participants will attend the clinic on 6 occasions in total (to include returning for 24hr blood sample) – visits 2, 4 and 6 (Figure 1). A 1-week washout period is sufficient for plasma sample to return to baseline (19). Participants will be randomly assigned by computer sequence generation (http://www.randomisation.com) to one of three treatment orders (Figure 1). The random allocation sequence will be generated by a staff member not involved with entering participants into the trial to ensure allocation concealment.

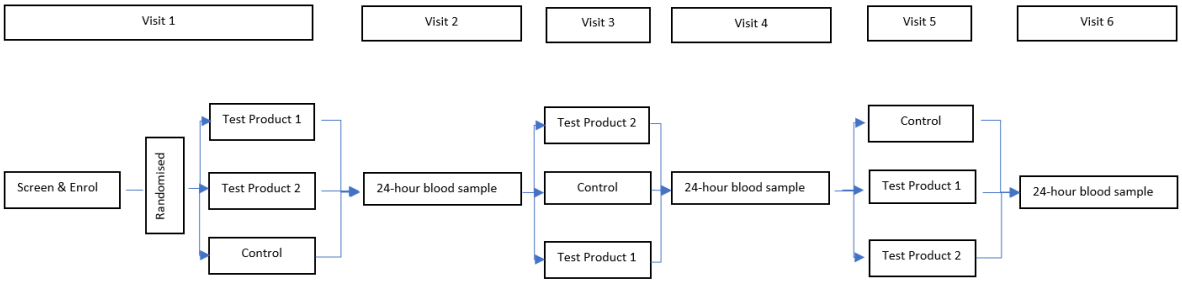

Figure 1. Study design

Participants will visit the research clinic on six occasions (Figure 1).

- Occasion 1: Screening + 1st intervention (randomised) involving in-clinic assessments (~9 hours).
- Occasion 2: Participants will return to the clinic for a short visit (30 minutes) where a 24-hour blood sample will be collected (Figure 2).
- Occasion 3: (1 week following Occasion 1) 2nd intervention (randomised) involving in-clinic assessments (~8.5 hours).
- Occasion 4: Participants return to the clinic for a short visit (30 minutes) where a 24-hour blood sample will be collected.
- Occasion 5: (1 week following Occasion 3) 3rd intervention (randomised) involving in-clinic assessments (~8.5 hours).
- Occasion 6: Participants return to the clinic for a short visit (30 minutes) where a 24-hour blood sample will be collected.

Screening occasion: Participants will be pre-screened using a screening questionnaire and those determined to be potentially eligible will be invited to attend an in-clinic screening visit. After they have given signed informed consent (see details below) their eligibility will be confirmed by reviewing their medical history, demographic information and fatty fish intake obtained from the screening questionnaire, measuring height and weight and calculating BMI, and measuring vital signs (blood pressure and temperature). Eligible participants will be enrolled into the study and remain at the clinic for further 8.5 hours and complete their first intervention

Intervention occasions (Figure 2): Participants will arrive at the research clinic after an overnight fast (>10h)). Weight, percentage body fat mass and vital signs (blood pressure and temperature) will be measured, an intravenous cannula will be inserted into the participants arm for a duration of 8 hours during which the participant will remain at the research clinic.

A fasting blood sample (4 mL) will be collected after which participants will consume a standardised breakfast with their test product (see section 5 for more detail) within 15 minutes. Subsequent blood samples will be collected after 2, 4, 6 and 8 hours. The cannula will be removed, and participants will leave the research clinic. Participants will return to the research clinic the next morning after an overnight fast for collection of the 24-hour sample. The period of 24 hours was chosen as it represents an intake interval of daily intake of omega-3 supplements (19). Throughout the 9-hour day at the clinic participants will have access to low-fat snacks and a lunch meal devoid of omega-3 LCPUFA that they can consume *ad libitum* (see section 5 for more detail). Participants will leave the clinic with a take-home low-fat dinner meal.

Apart from the study specific test products, participants will be requested to maintain their habitual lifestyle patterns throughout the duration of the study and to avoid fatty fish and omega-3 LCPUFA containing supplements. Participants will be provided with a checklist to record any non-compliance or accidental consumption of these foods and return the checklist at their subsequent visit.

Staff responsible for sample and statistical analysis will be blinded to which treatment was consumed when, but participants and staff administrating treatments will not be blinded.

Plasma fatty acid concentrations (µg/mL) will be analysed by gas chromatography and bioavailability determined by calculating the incremental area under the curve ($iAUC_{0\text{-}24h}$) for plasma DHA and total omega-3 LCPUFA (Primary outcome), time to maximal value (Tmax) and maximal value (Cmax).

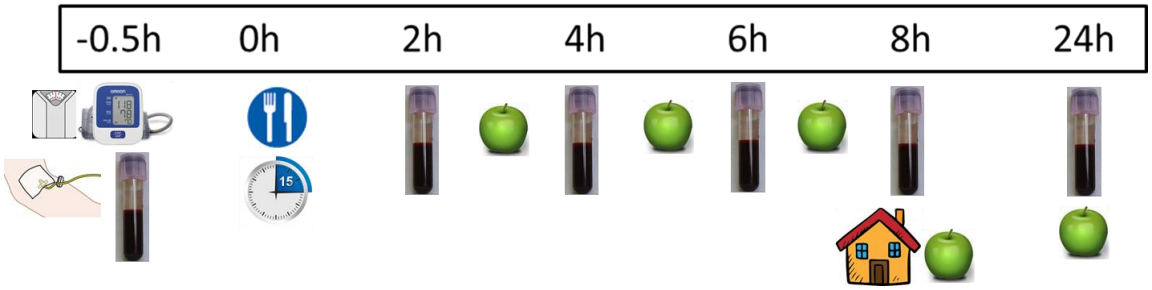

Figure 2. Test day procedures

Informed Consent

The Principal Investigator or designee will ensure that all participants must sign freely given written informed consent, approved by HREC, before undergoing any in-clinic screening activity or procedure.

The Principal Investigator or designee will inform the individual of the objectives, methods, anticipated benefits, and potential risks and inconveniences of the study. The individual will be given every opportunity to ask for clarification of any points he or she does not understand and, if necessary, to ask for more information. All questions about the study should be answered to the satisfaction of the individual. The participant will be given time to consider participation in the study.

Participants will be required to sign and personally date the informed consent form (ICF) in the presence of the designee and the designee who conducted the informed consent discussion must also sign and personally date the ICF. After signatures are obtained a copy of the signed ICF will be provided to the participant for their reference and the original document filed in the Investigator Site File.

Withdrawal of Participants

Participants are free to withdraw from the study at any time without providing reason(s) for withdrawal and without prejudice or penalty/loss of benefits to which they are otherwise entitled. Participant can be withdrawn for any of the following reasons:

- Participant request; withdrawal of informed consent
- Any adverse event (AE), intercurrent illness, medical condition or any situation where in continued participation in the study Is not in the best interest of participant as decided by PI
- Participant non-compliance with protocol at the discretion of the Principal Investigator or designee or study is terminated

The reason/s for withdrawal will be documented in the source documents. Participants who refuse to give their consent will not be included in the study, and participants who withdraw their consent will not continue in the study.

The Principal investigator or designee may withdraw a participant from the study at any time. In this event, the clinic will ensure the reasons have been clearly explained to the individual. Adverse event and concomitant medications will be reviewed at the time of withdrawal. A two-week follow-up will be performed for any unresolved AE at the time of withdrawal.

5. Test meals

Test meals:

1. Semi-solid food – 200 g serve Soup (commercially available packet-soup) containing cauliflower encapsulated HiDHA® algal oil providing 400 mg DHA + standardised breakfast
2. Solid food – 50 g serve extruded snack (savoury snack) containing cauliflower encapsulated HiDHA® algal oil providing 400 mg DHA + standardised breakfast
3. Control: 2 x algal oil gel capsule containing HiDHA® algal oil providing 400 mg DHA + standardised breakfast

HiDHA® algal oil will be supplied by NuMega Ingredients Pty Ltd.

The standardised breakfast will consist of a small carton of Milo (Nestle: 200 ml), an apple (200 g), one muesli bar (Uncle Toby's: 24 g) (total energy = ~1600 kJ) and the test products/control.

Throughout the 8-hour day at the clinic participants will have access to low-fat snacks and a lunch meal devoid of omega-3 LCPUFA that they can consume *ad libitum.* Low-fat is defined as ≤3 g fat/100 g solid food or ≤1.5g fat/100 ml for liquid food (20, 21). Snacks may include rice crackers, fruit, low-fat dairy products (yoghurt, chocolate milk), fruit juice, water, etc. Lunch and dinner meals will be low-fat frozen meals selected from the Lean Cuisine Steam range available in both Australia and Singapore https://www.vescofoods.com.au/brands/lean-cuisine). Breakfast (selection of cereal) will be provided to participants after collection of the 24-hour sample.

The test products will be manufactured in facilities certified for production of food for human consumption in accordance with an approved HACCP (Hazard Analysis Critical Control Point) plan. Only food grade ingredients will be used in the production process. All the equipment that will be used in the production process will be sanitised, swabbed for microbial contamination and recleaned until it becomes suitable for food contact. All the processing steps, from the purchase of the ingredients to the processing and handling conditions at each processing stage will be recorded in a process log sheet. The microbial and physicochemical quality of the final products will be tested in accordance with the approved HACCP plan. The products will be released as fit for human consumption after the Food Risk Assessment Team (FRAT) team examined the process log sheet and the results of the microbial and physicochemical analyses.

7. Assessments

Medical history and demographic information:

Medical and surgical history, medication and supplement use and demographic information will be obtained by questionnaire and interview of participants. This information will be used to screen participants.

Assessment of fatty fish intake:

In order to assess participant's eligibility to inclusion criteria 3 - "Consuming <2 meals of fatty fish/week over the past 3 months", potential participants will be asked to record the frequency and type of fish they habitually consume. E.g. "How often do you consume fish?" Frequency options will include <1x/month, 1-3x/month, 1x/week, 2x/week, 3-4x/week, 1x/day, >1x/day. "What type of fish to you usually consume". The categories of fish according to fatty fish / medium- or low-fat fish sources are summarised in the Appendix and will be used to determine participant's eligibility accordingly.

Anthropometric measurements:

Height and weight will be measured at the screening visit and BMI calculated using the formula: weight (kg)/height (m) ^{2}. Weight will also be collected at each clinic visit. Height will be measured using a stadiometer and body weight will be measured using calibrated electronic digital scales.

Bioelectrical Impedance Analysis (BIA) will be used to assess % body fat mass using a multi-frequency BIA with 8 tactile electrodes InBody 230, Biospace Co. Ltd, Seoul) in Australia and Tanita BC-418, Tokyo in Singapore. Measurements will be obtained after voiding. Participants will stand upright, positioning their bare feet on the footpads and their hands on the handles. A small electrical current is passed through the body, resistance is measured, and total body water and the corresponding body composition measures are calculated by the inbuilt software.

Vital signs:

Resting blood pressure will be measured using an automated blood pressure monitor (InBody BPBIO750 Blood Pressure Monitor). In a seated position, an initial mock measurement will be performed at the start of the visit (measurement not recorded). Following 5 minutes of rest in a quiet room, a further measurement will be performed. If measurement is out of clinical range, a subsequent measurement will be performed after 5 to 15 minutes rest.

. Body temperature will be measured using a digital tympanic thermometer.

Vital signs will be assessed at each clinic visit.

Blood sample collection and preparation:

Fasted venous blood samples will be collected into vacutainers containing EDTA (purple top, 4mL) for the preparation of plasma. Plasma will be prepared by centrifugation at 2851g for 15 min at 4°C. The resulting plasma will be aliquoted (500 µL) and stored at -70°C until analysis. Samples from each subject will be analysed within the same analytic run to reduce variation.

Fatty acid analysis of plasma and test products

Plasma fatty acid concentrations (µg/mL) will be analysed using a gas chromatography–mass spectrometry (GCMS) technique. A 100 µL plasma sample will be treated with 1 mL 0.2M KOH/MeOH (0.005% BHT) and 50 µL of a 1 mg/mL solution of C17:0 (internal standard; 26.44 mg triheptadecanoin [C17:0 triglyceride, Sigma No. T-2151 99] in 25ml chloroform). This will be followed by 10 min of

heating at 90 °C; addition of 2 mL methanol; acidification with 200 µL of acetyl chloride and heating at 90°C for 1 hr while vortexing approximately every 10min after which the samples will be returned to room temperature. Methyl esters will be extracted into 1.5 mL hexane and 1.0 µl aliquot injected onto a gas chromatographic column (DB – FastFAME, 20m x 0.18 mm from Agilent Technologies), using a PerkinElmer Clarus 690 gas chromatograph / Clarus SQ 8 T Mass Spectrometer with a split 50:1 injector. Fatty acids will be identified by comparison with authentic Sepelco 37 component FAME mix (Sigma-Aldrich, Australia) and verified using the NIST MS search database software. Peaks will be measured using TurboMass software and fatty acids will be calculated against the internal standard.

8. Statistical analysis

A sample size of n=10 per site provides >80% power to detect a difference of 10% in AUC (22). N=12 per site will be recruited to account for a 20% attrition rate.

A statistical analysis plan will be developed before statistical analysis commences. In brief, data will be presented as means and SD/SEM. Plasma DHA and total omega-3 LCPUFA concentrations will be plotted into graphs. $iAUC_{0\text{-}24h}$ (primary outcome), will be calculated using the trapezoidal rule and corrected to baseline levels. Time to maximal value (Tmax) and the maximal value (Cmax) will be determined. Primary analysis will be pairwise comparisons between test foods vs. control using linear mixed models with fixed effects for treatment, time, ethnicity and assessing the interaction of treatment*time and treatment*time*ethnicity. Participant identifier will be fitted as a random effect, i.e. one intercept per participant. Potential covariates will be identified *a priori* and included in the model (e.g. BMI). Intention-to-treat analysis will be performed primarily and per protocol analysis as secondary analysis.

Statistical analysis will be performed using appropriate statistical packages e.g. SPSS software (IBM Corporation, New York, USA). Statistical significance will be determined at a *P*-value of <0.05. Statistical analysis will be performed blinded and treatments revealed after completion of the statistical analysis.

9. Assessment of Adverse Events

Following Informed consent, adverse events (AE) reported by participants for the duration of their time in the trial will be documented and followed up in accordance with International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

Adverse Event

Definition:
An AE is defined as any unfavourable and unintended change in the structure (signs), function (symptoms) or chemistry (laboratory data) of the body associated in time with the use of the study product whether or not considered related to the use of the product. Any worsening (e.g. any clinically significant adverse change in frequency or intensity) of a pre-existing condition which is temporally associated with the use of the product is also an AE. A serious adverse event (SAE) is defined as any AE that result in any of the following outcomes: death; a life-threatening AE; inpatient hospitalisation or prolongation of existing hospitalisation; a persistent or significant disability/incapacity; or a congenital anomaly/birth defect(23)(22)(22)(21)(22)(21)(21)(20)(20)(20).

Suspected Unexpected Serious Adverse reactions (SUSARs) are SAEs that are believed to be related to a study treatment and are both unexpected (i.e. the nature or severity is not expected from the information known on the product) and serious. SUSARs require expedited reporting to applicable regulatory authorities.

AE will be scored for its severity and likelihood of the symptoms being related to the trial product as follows:

Assessment of Severity:
"1" Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities
"2" Moderate: An event that is sufficiently discomforting to interfere with normal activities
"3" Severe: An event which is incapacitating and prevents normal everyday activities

Assessment of Causality:
The causality of an AE (i.e. their relationship to study product) will be assessed by the Principal Investigator or designee at the study site. Assessing causality requires considering whether there was a reasonable possibility that the event may have been caused by the study treatment.

The relationship of the AE to the study product will be specified by the investigator using the following definitions:

Not Related: The adverse event is clearly explained by another cause not related to the study product.
Probably Not Related: A potential relationship between study product and the adverse event could exist (i.e. the possibility cannot be excluded), but the adverse event is most likely explained by causes other than the study agent.
Possibly Related: The adverse event and administration of study product are reasonably related in time, and the adverse event can be explained equally well by causes other than the study product.
Probably Related: The adverse event and use of study product are reasonably related in time, and the adverse event is more likely explained by study product than other causes.
Definitely Related: The adverse event and use of study product are related in time, and a direct association can be demonstrated.

Assessment of Outcome:
For each recorded AE or SAE an assessment of outcome at the time of last observation. The outcome of AEs or SAEs will be documented as follows:
• Fatal: The participant died
• Resolved: The AE or SAE has ended
• Resolved with Sequelae: The AE or SAE has ended but changes are noted from baseline
• Unresolved: The AE has not ended. The AE outcome can only be categorised as unresolved if the AE is:
o Ongoing at the final safety or early withdrawal visit and the Medical Investigator deems that no further follow-up is required
o Lost to follow-up after repeated unsuccessful attempts to contact the participant
o Ongoing and referred to the participant's physician or specialist

Reporting:
Details of the AE are documented by the relevant clinic staff member in the participant's Case Report Form (CRF) on the applicable form while the participant is present or at the time of contact. Once the AE has been documented, the information is reviewed by the trial coordinator. Follow up of the event should be conducted by the coordinator or the person conducting the next consecutive visit unless

the AE is reported at the end of a study then the coordinator will follow up. All unresolved AEs at the completion of the study will be followed after two weeks by phone call. At this follow-up call, any stable unresolved AEs of mild intensity would stay unresolved and would not have further follow-ups. All other unstable, unresolved AEs with moderate to severe intensity will be actioned as per PI's discretion. If the participant has been lost to contact, it must be demonstrated that three phone calls were made on different days and times followed by a final email to contact the person to follow up the AE.

All SAE, whether causally related to the treatment or not, and SUSAR must be immediately reported firstly to the Research Clinician, the relevant Human Research Ethics Committee (HREC) and to applicable regulatory authorities within 24 hrs.

10. Ethical Considerations

The study will be performed in accordance with the ethical principles that has their origin in the Declaration of Helsinki, and that are consistent with ICH-GCP and the applicable local ethical & regulatory requirements.

The PI is responsible to ensure the protocol, informed consent and all other related documents have been reviewed and approved by the (HREC prior to the commencement of the study. The PI is responsible for informing the HREC of any amendment to the protocol and will report promptly to the HREC new information that may adversely affect the safety of the Participants or the conduct if the study. Any necessary extensions or renewals of HREC approval will be obtained for changes to the study such as modification of the protocol, the informed consent forms and any information provided to participants. The PI or designee will submit written summaries and any study reports to HREC annually or as requested. On completion of the study, the PI (or designee) will notify the HREC that the study has concluded.

All personal data collected and processed for the purposes of this study will be managed by site staff with adequate precautions to ensure confidentiality of those data and in accordance with the applicable privacy regulations per the investigators' country/jurisdiction. Study data will be stored in a computer database and confidentiality maintained in accordance with national data legislation. Participants in this database will be identified by Participant number.

11. Data Handling, Record Keeping & Retention

All data collected from a participant will be recorded in source documents (paper and electronic). Study site will enter data from the participant's source documents into an electronic case report form (eCRF) designed in REDCap. Copies of all Source, eCRF data and study related documentation must be retained at the site for the duration of the study.

Data collected during this study will be handled, processed and managed as per study specific data management plan. Final datasets will be uploaded to the CSIRO Data Access Portal and shared in de-identified manner as per study specific data sharing plan.

Following completion of the study and publication of results, all study related documents will be archived by study sites and retained at least for 15 years.

13. Quality Control and Quality Assurance

Internal quality management of study conduct, data and biological specimen collection, documentation and completion in compliance with the protocol, ICH GCP and applicable regulatory requirements will be performed by study sites.

14. Direct Access to Source Data/Documents

Upon request or notification, the Investigator will ensure and permit direct access to source data and documents for trial related monitoring, audits, HREC review and regulatory inspections. In case of electronic source, a read-only assess will be provided.

15. Publication Policy

The results will be submitted for scientific publications including publication in scientific journals, conference abstract, and verbal or poster presentations in accordance with the publication policy outlined in the agreement between CSIRO and A*STAR.

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Appendix

Categories of fish according to fat content

Fatty fish sources (~>500 mg LCPUFA/serve) (serve = 150 g fish / 100g canned fish)	• Salmon (fresh or canned) • Mackerel • Sardines (fresh or canned) • Anchovies • Trout • Tuna (fresh or canned) • Mullet	• Silver perch • Morwong • Bream • Herring • Smoked/green/blue mussels (canned) • Smoked oysters (canned)
Medium- to low-fat fish sources (<500 mg LCPUFA/serve) (serve = 150 g fish / 100g canned fish)	• Yellowtail kingfish • Trevally • Snapper • Flounder • Mirror dory • Jack mackerel • Blue-spotted goatfish • Small-spotted herring • Blue-eye trevalla • Australian bass • King Dory • Wrasse • John Dory • Blue morwong • Coral trout • Blue grenadier (Hoki) • Golden perch • Ocean jacket • Leather jacket	• Murray cod • Hussar • Garfish • Barramundi • Dhufish • Mangrove Jack • King George whiting • Smoked cod • Flathead • Shark (Flake) • Jackass morwong • Squid/calamari (100g) • Oysters, raw (100g) • Scallops (100g) • Prawn (100g) • Octopus (10 • Crab (100g) • Lobster/crayfish (100g) • Moreton bay bug (100g) • Whiting

Source: Australian Heart Foundation