Closed to accrual: May 2, 2019

CLINICAL RESEARCH PROJECT

Protocol Title: Effect of Fish Oil Enriched In Omega-11 Fatty Acid On Lipoprotein Metabolism In Adults

Abbreviated Title: Omega-11 oil supplements and lipoprotein metabolism

Identifying Words: **Fish oils, Long chain monounsaturated fatty acids,** lipoproteins, cardiovascular disease

Principal Investigator:
*Marcelo J. Amar, M.D. (E)
Cardiovascular and Pulmonary Branch (CPB)
Building10, Room 5D03, NHLBI/NIH
Bethesda, MD 20892
Phone: 301-402-0521
E-mail: ma90x@nih.gov

Accountable Investigator:
Alan T. Remaley, M.D., Ph.D., CPB/ NHLBI (E) 301-402-9796, Bldg. 10, 2C433

Associate Investigators:
Alan T. Remaley, M.D., Ph.D., CPB/ NHLBI (E) 301-402-9796, Bldg. 10, 2C433
Amber Courville, Ph.D., RDN, CSSD (E) 301-594-8051, Bldg. 10/B2-2426
James Troendle, Ph.D., NHLBI, Biostatistician 301-435-0421, RKL2/ Rm 9196
Emma Staller, RN, TVMB/NHLBI (C)* 301-827-5321, Bldg. 10/6-3140

Research Contact:
*Marcelo J. Amar, M.D. 301-402-0521, Bldg. 10, 8N228

*The * indicates investigators allowed to obtain informed consent for this protocol.*

Subjects in study:

Number	Sex	Age Range
100 (goal is 20* evaluable subjects)	M/F	≥18

* evaluable subjects are subjects that have completed the full time line of the study (Figure 2), have collected stool specimens and thru the analysis of red cell membrane omega-3 fatty acids (RCM) are found to have taken and absorbed the supplement.

Product Uses Ionizing Radiation: No
Project Uses IND/IDE: Yes

Name of Supplement: LCMUFA-rich saury oil and Control Fish Oil
IND #: 133134
IND Sponsor: Marcelo Amar, M.D.

Project Uses "Durable Power of Attorney": No
Off Site: No
Multi-Institutional Project: No

Précis

Serum cholesterol is transported by lipoproteins, such as VLDL, LDL and HDL, which vary in their relationship to cardiovascular disease risk. LDL, for example, is proatherogenic, whereas HDL is cardio-protective. Long-chain monounsaturated fatty acids (LCMUFA), fatty acids over 18 carbons in length with a single double bond, have been shown in mice to decrease proatherogenic lipoproteins, such as LDL, and reduce atherosclerosis. This study will test the hypothesis that LCMUFA supplementation in humans will favorably alter the lipoprotein lipid profile in regard to cardiovascular disease risk. In addition, we will assess other parameters related to lipoprotein composition and function, as well as other biomarkers related to coagulation and inflammation, which have previously been shown to be affected by supplementation with omega-3 fatty acids.

This clinical research project is designed as a pilot, randomized, double-blinded, crossover study that will investigate the effect of a fish oil enriched with LCMUFA on lipoprotein metabolism. Subjects will receive control fish oil enriched in oleic acid, a monounsaturated fatty acid (C18:1), or a fish oil supplement produced from Saury fish (rich in LCMUFA) for approximately 8-10 weeks, with a wash out period of 8-10 weeks between the two arms of the study. The study consists of 4 outpatient visits when laboratory or research samples and CAVI tests will be performed. A 7-day food diary, pill count, and red cell membrane fatty acid levels will be monitored to assess compliance.

Table of Content

1. Objectives

The overall objective is to elucidate LCMUFA-induced changes on lipoproteins and lipoprotein metabolism. In particular, we will focus on the effect of LCMUFA supplementation on the level of pro-atherogenic cardiovascular biomarkers, such as LDL-C and triglycerides, as well as on the level of anti-atherogenic biomarkers, such as HDL-C. This will be first accomplished by performing a small pilot study to determine the effect size of LCMUFA supplementation on lipoprotein parameters to assist in the design of a possible future larger clinical trial.

2. Introduction

Cardiovascular disease (CVD) remains the leading cause of death, disability, and healthcare expense in the United States and is also a major medical problem worldwide. It is well known that elevated plasma levels of cholesterol and triglyceride on lipoproteins are major risk factors for CVD. Dietary lipids by modulating lipoprotein metabolism can have a profound effect on CVD risk. Unlike pro-atherogenic saturated fatty acids, numerous animal and human studies have shown that regular consumption of unsaturated fish oils have favorable effects on serum lipids, as well as on endothelial function, inflammation, thrombosis and arrhythmia. Most of these beneficial effects have been attributed to omega-3 fatty acids, such as the long chain polyunsaturated fatty acids eicosapentanoic (EPA, C20:4) and docosaehexanoic (DHA, C22:4)[1,2]. Fish oils, however, also contain varying amounts of other unusual types of fatty acids that are not commonly found in other food sources. For example, oils derived from saury[3], pollock[4], capelin[5], and sprats[6], as well as from marine mammals, such as seals and whales[7], are all enriched in long-chain monounsaturated fatty acids (LCMUFA; i.e., C20:1 and C22:1 isomers combined). LCMUFA are defined as fatty acids that are at least 20 carbons in length and contain only one unsaturated carbon double bond. Food is the primary source of LCMUFA, because fatty acids longer than 18 carbons are inefficiently synthesized by most mammals, including man. Interestingly, data from the original epidemiologic studies of Eskimos that first established a link between omega-3 fatty acid consumption and CVD protection also showed a possible beneficial role for LCMUFA in promoting cardiovascular health[8,9]. Studies on the association between red blood cell fatty acid levels, a marker of fatty acid dietary consumption, and incident coronary artery disease in the Physician's Health Study also showed a strong inverse association between LCMUFA consumption and CVD, even after adjusting for omega-3 fatty acid levels[10]. Recently, we showed in various mouse models[11,12,13,14] that ingestion of LCMUFA lowers total cholesterol and reduces atherosclerosis (Table 1).

Table 1. Influence of LCMUFA-rich oil on plasma cholesterol

Ref.	Animal models	Dietary oils incorporated into the diet	Dose of LCMUFA	Feeding term	TC (mg/dL) (13~40%↓)
[11]	Diet-induced obese mice C57BL/6J	Control: lard 32% Fish oil: lard 22% + saury oil 10%	~3 g/kg (n=10)	6 wk	Con:134±4 Fish: 107±5**
[11]	Type 2 diabetic mice KKAy	Control: soybean 10% Fish oil: saury oil 10%	~3 g/kg (n=10)	4 wk	Con:109±4 Fish: 66±3***
[12]	Diet-induced obese mice C57BL/6J	Control: lard 32% Fish oil: lard 22% + pollock oil 10%	~3 g/kg (n=10)	6 wk	Con:162±4 Fish: 113±3**
[13]	Diet-induced obese mice C57BL/6J	Control: lard 32% Fish oil: lard 27% + LCMUFA concentrate 5%	~3 g/kg (n=10)	6 wk	Con:176±24 Fish: 110±8**
[14]	Type 2 diabetic mice KKAy	Control: soybean oil 7% Fish oil: soybean 3% + LCMUFA concentrate 4%	~3 g/kg (n=10)	8 wk	Con:180±7 Fish: 156±8*

Saury fish (Cololabis adocetus) are widespread in the surface waters of the Eastern Pacific and are considered to be a pelagic, tropical oceanodromous fish. It is widely consumed in Asian countries, and unlike more commonly eaten fish, such as sardines, which contain large amount of EPA (Table 2), Saury fish contains a very high level of LCMUFA, particularly 20:1 n-11 (cis-9-eicosenoic acid or gadoleic acid) and 22:1 n-11 (cis-9-cetoleic acid). Except for one past study, which examined the effect of Saury fish consumption on post-prandial lipids and glucose15, no previous studies have investigated the effect of long-term supplementation of fish oil produced from Saury fish on plasma lipoproteins or other cardiovascular risk markers.

Table 2. Main fatty acid composition of different types of fish oil and olive oil

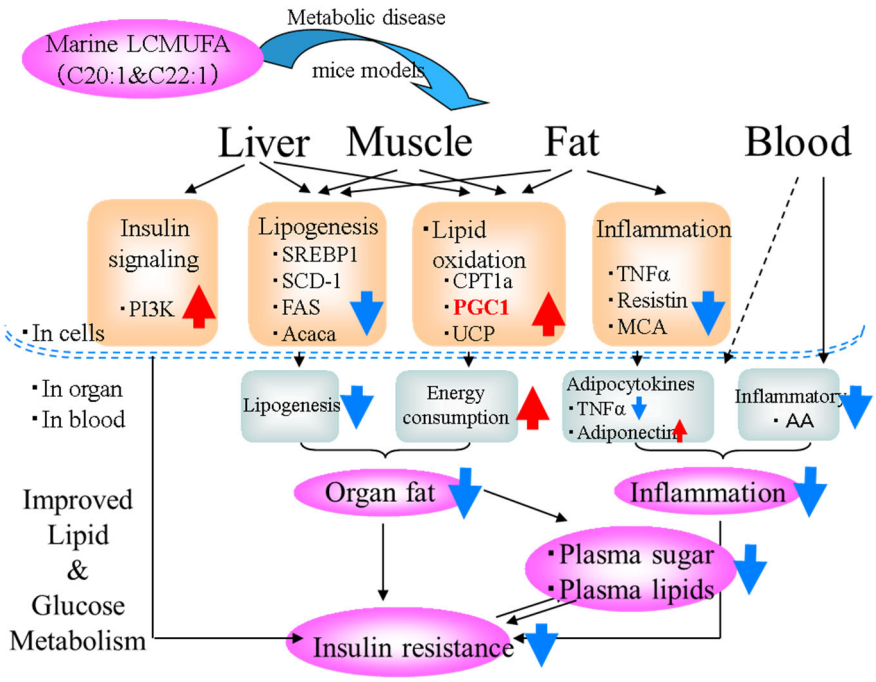
Fatty acids (%)	Sardine oil	Saury oil	Olive oil
C16:0	6.7	11.4	14.8
C16:1	8.9	2.7	-
C18:0	0.7	1.7	2.7
C18:1	5.8	5.3	70
C18:2 n-6	1.1	1.7	12.3
C18:3 n-3	0.9	1.5	-
C20:1	0.8	13.1	-
C20:4 n-6	1.2	0.6	-
C20:5 n-3 (EPA)	28.5	6.2	-
C22:1	0.1	15.7	-
C22:5 n-3	2.7	1.2	-
C22:6 n-3 (DHA)	11.8	11.3	-

Although LCMUFA is not abundant in most diets, monounsaturated fatty acids (MUFA), which are fatty acids 18 carbons or less in length with a single carbon double bond, are relatively abundant. The most common MUFA in daily nutrition is oleic acid (C18:1 n-9),

and olive oil is one of the major sources of oleic acid. From the NHANES study, the average daily consumption of oleic acid in the US is nearly 30 grams per day[16]. Several studies indicated an increase of HDL-cholesterol and a corresponding decrease in triglyceride, following an oleic acid-rich diet[17, 18]. The consumption of diets rich in MUFA, particularly oleic acid, has been linked to a lower prevalence of atherosclerosis and has been shown to have beneficial effects on lipoprotein metabolism[19]. In type 2 diabetic subjects, oleic acid-rich diet exerted a hypoglycemic effect and reduced glycosylated hemoglobin in the long term[20]. In fact, the U.S. Food and Drug Administration have approved a Qualified Health Claim for olive oil stating the following: "Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil has to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day." [21].

The mechanisms involved in physiological effects of beneficial fatty acids, such as EPA, oleic acid and LCMUFA are only partially understood. For example, Omega-3 fatty acids (EPA) are known to be natural ligands of several nuclear receptors that regulate gene expression, including PPAR, hepatic nuclear factor, liver X receptors, and retinoid X receptors. They also alter expression of transcription factors, such as sterol regulatory element binding-protein and carbohydrate response element binding-protein (ChREBP) [22], which may contribute to the physiologic effects of omega-3 fatty acids on lipid metabolism and inflammation. Omega-9 fatty acid, such as oleic acid, decrease cholesterol ester transfer protein (CETP) levels, a protein mediates the transfer of cholesteryl esters from HDL to apolipoprotein B-containing lipoproteins[23]. The isoenergetic replacement of a high-saturated fatty acid diet by a MUFA or a high-carbohydrate low-fat diet has been shown to decrease the CETP concentration in young, healthy, normolipidemic subjects[24]. In animal studies, LCMUFA-rich diet improved the adipocytokine profile, and resulted in favorable changes in triglycerides and LDL-C and in the expression of several genes involved in glucose/lipid metabolism and inflammation (Fig. 1)[11, 13, 14]. The mechanism for the similar and differential effect of EPA, oleic acid and LCMUFA on LDL-C and other lipoprotein changes, however, is not fully understood nor whether these differences in lipoprotein metabolism relate to the pathogenesis of cardiovascular disease.

Fig. 1 Possible mechanism involved in the observed improvement of cardiovascular disease risk following dietary LCMUFA-rich oil supplement



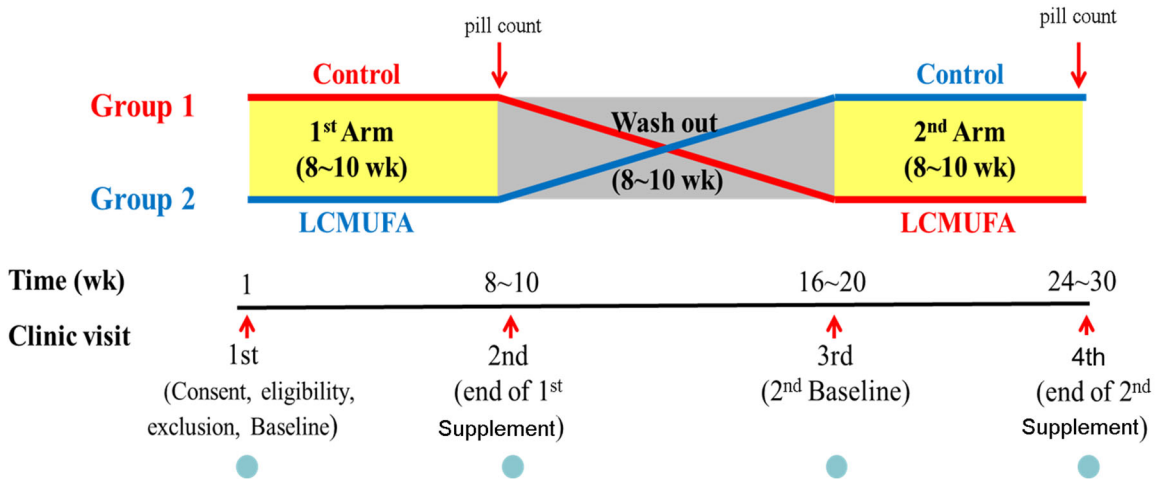
In this pilot study, we plan to examine the effect of LCMUFA-rich fish oil on lipid and lipoprotein metabolism. A fish oil supplement containing a mixture of sardine oil and olive oil that contains the same amount of Omega-3 fatty acids as the LCMUFA-rich fish oil will be used as a control. Given the widespread use of fish oils in the general population and the recent concern about their possible lack of effectiveness for cardiovascular disease protection[25, 26], a more detailed understanding on the effect of various fat on lipoprotein metabolism is needed. Results from this study may lead to new insights into the optimum mixture of beneficial fatty acids in fish oil supplements for cardiovascular health protection.

3. Study Design and Methods

3.1 Overview

The study will be a prospective double-blinded randomized crossover study, with four clinical visits (Fig. 2). During the first visit, subjects will be screened by an exclusion/inclusion questionnaire and baseline clinical laboratory testing. If not excluded, subjects may also be assessed for vascular compliance by Cardio-ankle vascular index (CAVI) testing[27, 28]. Exercise and diet changes will be followed by a nutritionist, using a 7-day food diary*.

Fig. 2. Study design



Eligible subjects will be randomized to take Control Fish Oil capsules (CFO - a mixture of sardine oil and oleic acid-rich olive oil) or LCMUFA-rich fish oil capsules produced from Saury fish (LCMUFA) as described in Table 3.

Table 3 Amount of fatty acids contained in each capsule

mg/capsule(1g)	Control oil (1g) (sardine oil + olive oil)	LCMUFA-rich Saury oil (1g)
EPA+DHA	178	151
LCMUFA	7	252
C18:1	390	51
saturated	124	198
omega-6	82	36
omega-3	222	237
MUFA	438	336

Each fish oil supplementation period will last a minimum of 8 weeks and subjects receiving CFO capsules will receive a daily approximate dose of 3g of total omega-3 fatty acids and approximately 5g of total MUFA (Table 4). Subjects will return for a second visit at the end of this study arm and will discontinue any study related supplements. After a washout period of 8-10 weeks, they will return for a third visit to start the second arm of the study for another 8 weeks minimum. In case of scheduling problems subjects will continue on supplementation up to 2 extra weeks. A fourth and last visit will take place at the end of this second study arm and subjects will discontinue any study related supplements. Blood samples, as well as stool samples (optional), will be collected on each visit for analysis. CAVI analysis may also take

place. The participant compliance will be monitored by pill counts at the second and fourth visits. In addition, fatty acid composition of red blood cells will be assessed by GC analysis[29]. Subjects may be contacted by phone or NIH medical secure email system at least once during each period to monitor compliance and any adverse events. Subjects will also be consented to be contacted for possible future studies. After completion of both arms of the study, results from each participant will be unblinded for possible data analysis.

Table 4. Total amount of fatty acid contained in 12 capsules (12g) consumed per day during trial

g/12g/day	Control oil (12g) [sardine oil + olive oil]	LCMUFA-rich Saury oil (12g)
EPA+DHA	2.1	1.8
LCMUFA	0.8	3.0
Oleic acid (C18:1)	4.7	0.6
Total saturated FA	1.5	2.4
Total omega-6	0.98	0.43
Total omega-3	2.7	2.8
Total MUFA	5.3	4.1

3.2 Visits

There will be a total of 4 outpatient visits for this study. The return visits can be delayed or anticipated up to two weeks, if there are scheduling problems (but will be a minimum of 8 weeks apart). The first study day will be the screening and baseline measurements, followed by three additional visits coinciding with study landmarks. The study will be discussed in detail with interested subjects. Any procedures needed to assess eligibility (i.e. blood laboratory tests, pregnancy test) will be performed after obtaining a signed informed consent. Prior to the first visit a telephone call or email exchange through NIH medical secure email system may be used to give general information about the study, to explain the exclusion/inclusion criteria and describe the tests that will be performed but no personally identifiable information will be collected. This will not be considered a screening. In addition, phone calls/email may be used during the study to clarify any questions and to support compliance to the study. Any adverse event will be collected and reported as per item 12 of this proposal.

Visit 1: At the first visit (screening and baseline), the subjects will be consented and will be screened by an exclusion/inclusion questionnaire, baseline laboratory tests, electrocardiogram (EKG) and a pregnancy test for females. A stool sample may be obtained,

but it's optional. In addition, 10 mL of blood will be stored for research tests if the subject is eligible for the study, otherwise the blood/stool sample will be discarded. The subject will have vital signs and Body Mass Index measured, and will undergo a history and physical examination. If not excluded, they will be interviewed by a nutritionist and will receive a diet and exercise assessment to determine their nutritional status (using a 7-day food diary* or any other form of nutritional assessment). In addition, a brief physical examination will take place and a Cardio-Ankle Vascular Index (CAVI) test may be performed. Once eligibility is confirmed, subjects will be randomized, will receive a 10-week supply (at once or divided in refills) of the first dietary supplement (CFO or LCMUFA capsules), will be instructed to take 4 capsules, 3 times a day, (total of 12 per day) after meals, for 8 (or up to 10 weeks, if delayed) and will be scheduled to return for a second visit.

Re-screening Visit as applicable: Repeat laboratory values, including baseline research blood, may be needed to reassess eligibility within 40 days of a screening failure. If a subject is found ineligible, during or after the first visit, the 10 mL of research blood will be discarded.

Visit 2: Eight weeks after initiating the fish oil supplement (+ 2 weeks if needed) the subjects will return for their second visit. They will receive laboratory tests and pregnancy testing for females. A stool sample may be obtained, but it's optional. In addition, 10 mL of blood will be stored for research tests. The subject will have vital signs and Body Mass Index measured, will receive a diet and exercise assessment to determine their nutritional status, and a Cardio-Ankle Vascular Index (CAVI) test may be performed. If necessary, a brief physical examination may be performed, a pill count will also be performed to evaluate compliance. At the end of the 2nd visit, the subjects will be instructed to discontinue any fish oil supplementation for 8 weeks (+ 2 weeks, if necessary), during the wash-out period.

Visit 3: The third visit will occur 8 weeks (+ 2 weeks if needed) after visit #2 for a 2nd baseline measurement. At this visit, the subjects will receive laboratory tests and pregnancy testing for females. A stool sample may be obtained, but it's optional. In addition, 10 mL of blood will be stored for research tests. The subject will have vital signs and Body Mass Index measured may receive a diet and exercise assessment to determine their nutritional status, and a Cardio-Ankle Vascular Index (CAVI) test may be performed. If necessary, a brief physical examination may be performed. At the end of the 3rd visit, subjects will receive a 10 week supply of the second dietary supplement (CFO or LCMUFA capsules) and will be instructed to take 4 capsules, 3 times a day, after meals (total of 12 capsules a day), for 8 weeks (+2 weeks, if needed).

Visit 4: The fourth and final visit will occur 8-10 weeks after starting the second fish oil supplementation. Subjects will receive laboratory tests and a pregnancy testing for females. A

stool sample may be obtained, but it's optional. In addition, 10 mL of blood will be stored for research tests. The subject will have vital signs and Body Mass Index measured, may receive a diet and exercise assessment to determine their nutritional status, and a Cardio-Ankle Vascular Index (CAVI) test may be performed. If necessary, a brief physical examination may be performed. A pill count will also be performed to evaluate compliance. At the end of the 4th visit, the subjects will be instructed to discontinue fish oil supplementation.

*Subjects will be encouraged to complete the 7-day food record prior to the return visits, in order to facilitate the nutritional and exercise assessment. However, if the subject does not complete, or only partially completed the seven day food record, the nutritional and exercise assessment will still take place and any dietary or life-style changes will be noted.

The procedures at each visit are summarized in Table below:

Procedure at each visit	Visit 1 (screening and baseline)	Visit 2 (end of the 1st arm)	Visit 1 (the 2nd baseline)	Visit 2 (end of the 2nd arm)
Exclusion/inclusion questionnaire	✓	N.A.*	N.A.	N.A.
Vital signs and Body Mass Index measurement	✓	✓	✓	✓
Pregnancy test for female	✓	✓	✓	✓
Cardio-Ankle Vascular Index (CAVI) test	✓	✓	✓	✓
Physical examination	✓	If necessary	If necessary	If necessary
Clinical laboratory tests	✓	✓	✓	✓
Additional 10 mL blood collecting for research test	✓	✓	✓	✓
Seven-day food diary	✓	✓	✓	✓
Pill counting	N.A.	✓	N.A.	✓

*N.A.: not applicable

4. Subject Accrual

This is a single site study and subjects will be enrolled at the NIH Clinical Center. Participants will be recruited via flyer and/or recruitment advertisement placed in the NIH Record, the NHLBI Recruitment website, the Clinical Center News and by email or listserv.

4.1 Inclusion Criteria

- Male and female participants 18 years of age or above.
- Subject must be healthy, with no known history of cardiovascular disease.

- Pre-menopausal or women of childbearing potential must be non-lactating and using an effective form of birth control during the course of the study.
- Subject understands protocol and provides written, informed consent in addition to a willingness to comply with specified follow-up evaluations.

4.2 Exclusion Criteria

- Pregnancy, planned pregnancy (within the study period) or women currently breastfeeding.
- Subjects with weight changes greater than 20% over the past 3 months.
- Subjects planning a significant change in diet or exercise levels.
- Subjects already consuming more than 1.5 g per day of EPA/DHA in any form.
- Known sensitivity or allergy to fish, shellfish or omega-3 fatty acids supplements
- Subjects with known bleeding disorders (for example, Hemophilia)
- Subjects previously diagnosed with atrial fibrillation
- Subjects with clinically diagnosed hepatic disease (including but not limited to auto immune disease, hepatitis and cirrhosis)
- Subjects with chronic diarrhea, gastric bypass or lap-band procedures, ostomies, bowel motility problems, or other conditions that could affect intestinal fat absorption
- Subjects with any acute and life-threatening condition, such as prior sudden cardiac arrest, acute myocardial infarction (last six months), stroke, embolism
- Liver enzymes (AST or ALT) levels above 3x upper limit of normal
- Subjects with a TSH greater than 1.5xULN or clinical evidence of hypo or hyperthyroidism
- Subjects taking supplements or medications that affect lipoproteins for at least the past 8 weeks, such as fish oil supplements, bile-acid sequestrants, plant sterol supplements, fibrates, statins or Niacin.
- Subjects with hemoglobin <10g/dL
- Subject with platelet counts <60x103/microliter
- Subjects with uncontrolled hypertension (resting blood pressure > 160 mmHg systolic
- and /or > 100 mm Hg diastolic)
- Subject with uncontrolled diabetes (HbA1c ≥10)
- Subjects who consume excessive alcohol (binge drinking on 5 or more days in the past month)
- Subject participating in other clinical studies and/or receiving other investigational drug products prior to randomization
- Subject taking PCSK9 inhibitors within 8 weeks prior to enrollment
- Subjects being treated with tamoxifen, estrogens, or progestins that have not been stable for >4 weeks.
- Subjects initiating new medications or patients on multiple medications may also be excluded according to investigator discretion
- Anticipated surgery during the study period

- Blood donation in the last 2 weeks or planned blood donation during the study
- Subjects requiring regular transfusions for any reason
- Subjects may also be excluded for any reason that may compromise their safety or the accuracy of research data.

5. Supplements

Supplements will be stored, dispensed and disposed (remaining capsules) by the NIH CC pharmacy. The fish oil supplements that will be studied will be provided by Nippon Suisan Kaisha, Ltd. The supplements will be supplied as a liquid-filled gel capsule for oral administration. Each capsule of the control fish oil (CFO) contains at minimum 220 mg of omega-3 fatty acids, 400 mg of total MUFA, and 380 mg of oleic acid from the mixture of sardine fish oil and olive oil. Each capsule of LCMUFA-rich fish oil (LCMUFA) contains at minimum 220 mg of omega-3 fatty acid, 330 mg of total MUFA, and 240 mg of LCMUFA from saury fish oil.

The participants will be instructed to take the supplements equivalent to a total of approximately 3g per day of omega-3 fatty acid, and 5g of total MUFA in three divided doses (4 capsules, 3 times a day after meals in a total of 12 capsules a day). The dosing is comparable with earlier clinical trials on fish oils[21-25]. They will also be advised to take the supplements after meals, as they are better absorbed with fat.

Product Name: CFO, control fish oil, (a blend of EPA sardine- fish oil and oleic acid-rich olive oil) and LCMUFA-rich saury oil

Supply: Nippon Suisan Kaisha Ltd (for EPA- and LCMUFA-rich fish oils) and DSP Gokyo Food & Chemical Co., Ltd. (for oleic acid-rich olive oil)

Product Description: Obtained from Nippon Suisan Kaisha, Ltd. and DSP Gokyo Food & Chemical Co., Ltd.

Storage: Room temperature at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F)

Route of Administration: Oral

Preparation: Manufactured by:
Nippon Suisan Kaisha, Ltd. (Nishi-Shimbashi Square, 1-3-1, Nishi-Shimbashi, Minato-ku, Tokyo 105-8676, Japan)

Ingredients:

For EPA-rich sardine fish oil- and LCMUFA-rich saury fish oils:

Active: GMP produced and packaged omega-3 and omega-11 –fatty acid triglyceride, EPA, DHA, and LCMUFA.

Inactive: tocopherol, processed starch, glycerolglycerin, carrageenan, gelatin, and vitamin E.

For oleic acid-rich olive oil:
Active: Certified organic refined olive oil that enriched in omega-9 –fatty acid triglyceride, oleic acid.

Inactive: processed starch, glycerolglycerin, carrageenan, gelatin, and vitamin E.

Toxicities: None Known. The toxicity of sardine oil, LCMUFA or oleic acid-rich oil supplement has not been studied in patients.

Drug Interactions: Clinical studies have not been done to thoroughly examine the effect of sardine oil- or LCMUFA-rich fish oil supplement with anticoagulants. Patients receiving these fish oil supplements and an anticoagulant or other drug affecting coagulation should be monitored periodically (e.g., aspirin, NSAIDS, warfarin, coumarin).

Stability*:* LCMUFA or oleic acid-rich fish oil supplement will be maintained and distributed as directed by the supplement manufacturer to maximize maintenance and the quality of active and inactive ingredients in the supplement. Capsules will be initially assigned a 3 year expiration from time of manufacture. Ongoing stability studies will determine if this may be extended. Capsules are not to be frozen.

6. Laboratory Methods

Up to 40 mL of fasting blood will be collected at each visit to perform clinical or research tests. Clinical tests that may be used are listed below and will be performed at the NIH Clinical Center Department of Laboratory Medicine on the second floor of building 10 in the Clinical Center.

Clinical laboratory tests:

- Pregnancy testing
- Liver function test (ALT, AST, bilirubin)
- Uric acid
- Creatinine
- Creatinine kinase (CK)
- Insulin
- Fasting glucose
- Fibrinogen
- PT, PTT
- Whole blood aggregation

• CBC
• hs-CRP
• HbA1C
• TSH
• CBC
• EKG

Clinical lipid and lipoprotein related tests:
• Lipid Panel (Total cholesterol, LDL-C, HDL-C, Triglyceride)
• Lipoprotein Profile (NMR)
• ApoA-I
• ApoB

In addition, we may also conduct some or all of the following research tests: PHA stimulation whole blood, LC-MS-based proteomics, GC-MS-based lipidomics, specific proteins/enzymes related to lipoprotein metabolism (CETP, PLTP, PON, LCAT, LPL), ApoC-II, apoC-III, apoA-V, PCSK9, cholesterol efflux studies, ex-vivo lipolysis study, fatty acid analysis of red blood cells, leptin, adiponectin, blood cell RNA expression, flow cytometry phenotyping of white blood cells, plasma cytokines.

Standard of care laboratory tests and procedures not listed above may be requested and will not be used for research purposes but will only be used to evaluate the patients' health.

7. Data and Biospecimen Management Plan

7.1 *Data and Biospecimen Management*

Data Management and Access:

The PI will be responsible for overseeing entry of data into a password-protected electronic system that complies with NIH security standards and NHLBI DIR policy, and for ensuring data accuracy, consistency and timeliness of entry. The PI, associate investigators/research nurses and/or a contracted data manager may assist with the data management efforts. All human subjects personally identifiable information (PII), as defined in accordance to the Health Insurance Portability and Accountability Act (HIPAA), eligibility and consent verification will be recorded in conformity with DIR policy. Primary and final analyzed data will have unique codes so that research data can be attributed to an individual human subject participant. Coded data may be sent to associate investigators and collaborators outside of the NIH with IRB approval; local institutional approval may be required. Data with subject personal identifiers may be sent to associate investigators and collaborators outside of the NIH only after approvals of both NHLBI and local IRBs or executed reliance agreement with NHLBI's IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

Biospecimen Management:

Blood samples will be coded and stored in conformity with DIR Policy (e.g., BSI). Coded biospecimens may only be sent to collaborators outside of the NIH after, IRB approval in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement.

End of Study Procedures:

Data retained by the NHLBI will be stored in a password-protected database in conformity with NHLBI DIR policy until they are no longer of scientific value, as determined by the PI.

Breach of Confidentiality:

PI will report any breach of subject confidentiality or trial data to the clinical director and IRB per NIH policy, including NIH HRPP SOP 16 - Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.

7.2 Data Sharing and Future Use of Data

Data Sharing Plans:

Research data will not be shared with non-approved researchers. If data sharing becomes necessary it may occur after IRB review and approval or a determination from the NIH OHSRP.

Future Use of Biospecimens:

Following the termination of the study, biospecimens will be destroyed. If any future research on biospecimens is intended, an IRB review and approval will be submitted.

8. Monitoring of Subjects and Criteria for Withdrawal of Subjects

8.1 Stopping Rules for Subjects

- A single episode of severe gastrointestinal discomfort that in consultation with the physician is severe enough to discontinue the protocol
- Moderate gastrointestinal symptoms that persists for more than 5 consecutive days relating to the supplementation and as determined by contact with the investigator
- Pregnancy and breastfeeding
- Anaphylactic reaction due to consumption of the fish oil supplement

- New onset atrial fibrillation/flutter
- Major bleeding episode (CTCAE 4.0 grade III or IV)
- Subjects taking irregular use of fish oil capsules that in the opinion of the PI may compromise the data integrity.
- Any other severe symptoms related to the study and as determined by contact with the physician.

Subjects who are found to be pregnant or wish to breastfeed during the study will automatically be withdrawn. A pregnancy screening will be done for all female volunteers at the first visit and subjects will be excluded from the study if pregnant. It is not known whether the oils used in this study are secreted in breast milk. It is therefore not advisable for any breastfeeding woman to participate in this study. However, if a subject becomes pregnant during this study, monitoring of the pregnancy will continue until conclusion of the pregnancy.

Unlikely but possible adverse effects of omega-3 supplements may include atrial fibrillation (risk less than one in a hundred persons). Patients that develop symptoms of atrial fibrillation (palpitations, rapid heart rate, fainting, shortness of breath, etc.) will be instructed to visit the emergency room and seek treatment immediately. In order to prevent any future events, participants with atrial fibrillation will be withdrawn from the study. Individuals that develop an allergy to fish or shellfish may experience an anaphylactic reaction from the supplement and will also be instructed to visit the emergency room immediately and will also be withdrawn from the study (risk believed to be less than one in a thousand persons not known to have fish or shellfish allergy).

9. Analysis of the Study

The primary outcome measures of this study will be differences between randomized groups in differences between treatment periods in changes from baseline to end of period in the plasma total cholesterol. Secondary outcome measurements will be changes in proteomics, lipoprotein particle number, HDL functional test (i.e. efflux study) composition and size. Each subject will serve as their own control.

9.1 Sample Size Determination

Sample Size Determination and Methods

The study is designed to be a pilot trial to determine the effect size of the LCMUFA-rich fish oil supplement for a possible future larger clinical trial. The sample size requested in this study is based on our experience on other studies, such as the Omega-3 Fish Oil study. We are requesting to enroll up to 100 subjects to obtain 20* evaluable subjects that complete the whole study, and will perform an interim analysis after 10 subjects have completed both arms in the study. The proteomic analysis makes this study practical and innovative. Based on the information gathered from other studies and on the NHLBI proteomics core experience, the analysis of ten subjects is a realistic number to meet our scientific goals. The interim analysis

will be performed only for proteomic changes, and the magnitude of proteomic change will result in the decision of study continuation. This will allow us to gather sufficient data to estimate changes that could justify a larger study.

* evaluable subjects are subjects that have completed the full time-line of the study (Figure 2), have collected stool specimens and thru the analysis of red cell membrane omega-3 fatty acids (RCM) are found to have taken and absorbed the supplement.*

9.2 Statistical Analysis

Descriptive statistics will be calculated for all variables. A student's t-test will be used to compare the difference in mean changes between the baseline and the end of each arm period while accounting for period and group effects. Data of proteomics will be analyzed by paired student's t-test to identify proteins with statistically significant ($p < 0.05$) changes in normalized spectral counts, and an at least 1.3~1.5-fold will be considered as a physiologically meaningful change to continue the study. The study will be stopped if the changes are not statistically significant or the changes is smaller than 1.3-fold.

10. Human Subject Protection

10.1 Rationale for Subject Selection

Subjects of all genders will be considered for inclusion in this study. There will be no racial, ethnic, or gender discrimination. Cognitively impaired and institutionalized persons will not participate in this study. Criteria for exclusion or withdrawal from the study are based on the presence of other disease processes that may interfere with the interpretation of our results or situations that may be harmful to the health of subjects.

10.2 Rationale for the Exclusion of Children

Participant older than 18 will be considered for inclusion in this study. There is not sufficient supporting data to establish normal levels, an upper limit or safe intake of fish oil/omega-3 fatty acids in children.

10.3 Rationale for the Exclusion of Pregnant Women

Subjects must not be pregnant or actively seeking pregnancy in order to participate in this study. Pregnancy may introduce unpredictable effects on lipoprotein metabolism and influence the results of the study. The specific effects of pregnancy in this context may be the subject of a separate study. Some form of contraception must be used by subjects while enrolled. Contraception use will be determined by a questionnaire given to the subjects at time of enrollment.

10.4 Rationale for the Exclusion of Cognitively Impaired Subjects

Cognitively impaired and institutionalized persons will not participate in this study. Subjects must be able to provide informed consent, and understand and comply with the supplement plan and follow-up.

10.5 Inclusion of NIH Staff

NIH staff (employees, NIH contractors, special volunteers, guest researchers, and trainees) may voluntarily participate in this protocol.

- If the individual requesting to participate in the protocol is a co-worker, the consent from the NIH staff member (co-worker) will not be obtained by the staff member's direct supervisor but by another research staff member approved for obtaining informed consent who is not a co-worker.
- Recruitment, enrollment and compensation of NIH employee subjects will be consistent with the Guidelines for the Inclusion of Staff in NIH Intramural Research Studies (April 2016) (SOP 14F, Appendix C) and NIH Policy Manual Chapter 2300-630-3 and the Leave Policy for NIH Employees Participating in NIH Medical Research Studies.
- Neither participation nor refusal to participate as a subject in this protocol will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.
- The consenting staff member will make the NIH Information Sheet on Staff Research Participation available to staff members who are considering enrolling in research. (SOP 14F, Appendix C, Appendix A of the protocol)
- Employee subjects' privacy and confidentiality will be respected by protocol and consenting staff the same as for all subjects participating in research protocols. However, all subjects will be made aware that there are limits to these protections.

10.6 Evaluation of Benefits and Risks/Discomforts

Benefits:

There are no direct benefits to the patient. However, omega-3 supplements and omega-9 supplements have been shown to decrease pro-atherogenic lipid levels in several clinical trials. Routine clinical laboratory testing and disclosure of results will be available to all subjects. Subjects are also entitled to being notified of results from the clinical cardio-ankle vascular measurement, if requested.

Risks/Benefit Analysis:

As of May 2, 2019, this study is now closed to new subject accrual and continues in data analysis only and the level of risk is minimal.

Risk (45 CFR 46.102 (h)(i)): The research involves no more than minimal risk to subjects and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the condition or disorder under study.

Study Agent:

Sardine oil- or LCMUFA-rich fish oil, have minimum risk to subjects.
Generally, adverse effects of fish oil supplements may include atrial fibrillation of new onset and increased propensity for bleeding. Drug interactions may exist between individuals receiving Aspirin or clopidogrel (Plavix).
The most common side effects of fish oil include taste perversion, and eructation (belching). Participants will be notified of these effects in the informed consent document. Any subject exhibiting symptoms such as palpitations, rapid heart rate, or shortness of breath will instructed to go to the emergency room immediately and be withdrawn from the study. Individuals with an unknown allergy to fish or shellfish not disclosed at screening may experience an anaphylactic reaction, which will also be instructed to go immediately to an emergency room and will require withdrawal from the study. In rare cases, fish oil supplement can trigger allergic reactions. Symptoms may include difficulty breathing, swelling of the face, fever, or flu-like symptoms and a skin rash. Some studies reported consumption of omega-3-fatty acids is associated with a prolongation of bleeding time. However, the prolongation of bleeding time has not been shown to surpass normal limits and did not result in clinically significant bleeding episodes[33,34]. Any patient currently treated with an anticoagulant will be excluded from this study.

Oleic acid-rich olive oil, commercially available olive oil supplements have minimum risk to subjects.

Blood Draw: Subjects may feel lightheaded or dizzy after having blood drawn. There may be pain at the vein puncture site and a slight risk of bruising. To minimize this risk, the routine blood-drawing protocol will be followed and pressure will be applied to the area.

The protocol will follow the NIH Clinical Center MAS policy M95-9 guidelines for limits of blood drawn for research purpose in the Clinical Center. For adults, no more than 10.5 mL/kg or 550 mL, whichever is smaller, will be drawn for research purposes over any 8-week period.

Cardio-Ankle Vascular Index (CAVI): Inflation of blood pressure cuffs may cause transient discomfort. Subjects with fragile skin may suffer minor trauma (as per ABIs, related to usual BP measurements). This procedure involves the use of a VaSera VS-1500N system; an FDA approved vascular screening system.

EKG: Up to 12 self-adhesive electrodes will be attached to the subject's arms, legs and/or chest. Some skin irritation can occur where the electrodes are placed. Once the electrodes are placed, the test will begin. After the test, the electrodes are removed. The subject may have minor discomfort, similar to removing a bandage, when the electrodes are removed. Rarely, a reaction to the electrode adhesive may cause redness or swelling where the patches were placed.

10.7 Protocol Consent Processes and Documents

Each subject will receive an oral and written explanation of the goals, procedures, and risks of this study. The original, signed informed consent document will be placed in the medical record, and the subject will receive a signed copy of the informed consent document. A copy will also be placed in the shadow chart. A member of the protocol team will be available to answer questions about the study to be performed.

If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form and Oral Consent Process as described in MAS Policy M77-2, NIH HRPP SOP 12, 45 CFR 46.117 (b) (2), (If a study with an IND or IDE, also cite 21 CFR50.27 (b) (a)). The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form and oral consent process for up to a maximum of 5 participants in a given language and will report to the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5 we will have that consent document translated into the given inherent language and, we will seek individual IRB approval for any additional short form needs pending the full translation of the IRB approved consent document.

Participation of NIH Staff

If the individual requesting to participate in the protocol is a co-worker, the consent from the NIH staff member (co-worker) will not be obtained by the research coordinator or the employee's direct supervisor but by another research staff member who is approved for obtaining informed consent, and who is also not a coworker.

10.8 Patient Advocate

A patient's rights representative is available to patients on this protocol. The representative can be reached at 301-496-2626 and is located in Building 10. Patients may ask any questions about the study and may withdraw their consent at any time.

11. Conflict of Interest

DSP Gokyo Food & Chemical Co., Ltd. Swanson Health Product Inc. and Nippon Suisan Kaisha, Ltd. are providing the supplements for this study to NIH without charge. No NIH investigator involved in this study receives any payment or other benefits from DSP Gokyo Food & Chemical Co., Ltd. Swanson Health Product Inc. or Nippon Suisan Kaisha, Ltd. The principal investigator assures that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. No members of the research team reported a potential conflict of interest. There are no conflicts of interest with any financial organization regarding the material mentioned in this protocol.

12. Adverse Events, Deviations, and Unanticipated Problem Reporting

12.1 Adverse events (AE)

Definition: Adverse events (AE) are defined by the FDA as any unfavorable or unintended diagnosis, symptom, sign (including abnormal laboratory finding), symptom or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. An unexpected adverse event is one that is not described in the investigator protocol, published literature or the informed consent document.

All AEs, regardless of severity, will be followed until satisfactory resolution. AEs should be reported by the subject up to 30 days following the last dose of study drug.

Adverse events will be attributed (unrelated, unlikely, possibly, probably or definitively) to study medication and/ or disease and AEs will be graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be downloaded at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Reporting: All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. All adverse events will be reported to the IRB at the time of continuing review.

Exceptions: The following anticipated non-UP adverse events will not be reported to the IRB unless they occur at a rate greater than (greater than 25% of the total n=70) and/or at a severity greater than (i.e. Grade III higher) that known to occur in healthy volunteers:

- Abdominal pain (Grade I & II)
- Diarrhea (Grade I & II)
- Eructation (Grade I & II)
- Taste perversion (Grade I & II)
- Acid-Reflux (Grade I & II)
- Cough (Grade I & II)
- Flatulence (Grade I & II)
- Abdominal discomfort (Grade I & II)
- Constipation (Grade I & II)
- Increased PT/PTT (Grade I & II)

An abnormal laboratory value or changes from an abnormal baseline value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention

- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Abnormal laboratory values not associated with clinical symptoms will be evaluated but will not be considered an AE.

The laboratory results will be monitored by healthcare professionals and documented if clinically significant by the MD or any AIs in the study.

12.2 Serious Adverse Events (SAE)

Definition: Serious adverse events (SAE) are defined by the FDA as any untoward medical occurrences that result in death, are life-threatening, require or prolong hospitalization, cause persistent or significant disability or incapacity, result in congenital anomalies or birth defects, or are other conditions which in the opinion of the investigator represent significant hazards. All SAEs will be reported to the Sponsor PI, Marcelo Amar, M.D.

Reporting: SAEs that do not meet the criteria of Unanticipated Problem (UP) must be reported to the IRB Chair and Clinical Director within 14 days of learning of the event using the NIH problem report form.

Pregnancy itself is not regarded as an SAE unless there is suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. Any SAEs associated with pregnancy (eg, congenital abnormalities/birth defects/spontaneous miscarriages or any other serious events) must additionally be reported as such using the SAE report form. If pregnancy occurs during the course of this study an unanticipated problem report will be submitted to the Clinical Director and the IRB.

Deaths possibly, probably, or definitely related to the study agent will be reported to the Clinical Director within 7 days after the PI first learns of the event.

Any and all serious adverse events relating to the acquisition of blood samples, such as laceration, dissection, thrombosis of an artery, especially if those events cause injury to the hand requiring surgical correction, will be reported verbally and in writing to the Clinical Director of both the NHLBI and the Clinical Center and the chair of the IRB. The verbal report will occur within 48 hours of the occurrence. The written report of the serious adverse event (e.g., death or life-threatening adverse event) will be reported within 7 days. For all

other serious adverse events relating to the acquisition of blood samples, the written report will be within 15 days.

IND #: Pending

IND Sponsor: Marcelo Amar, MD, NHLBI

The PI will report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The Sponsor (or designee) will determine the reportability of the event to the FDA and IND safety report will be submitted to the FDA as required as either an IND Safety Report or Annual report.

IND Annual Report

A summary of all SAEs, nonserious AEs, and other events will be recorded and submitted to the Sponsor and FDA in annual progress reports (21 CFR 312.64(b)). Annual progress reports will be submitted within 60 days after the anniversary date of the IND.

Events will be submitted to Dr. Marcelo Amar, IND Sponsor at:
Marcelo Amar, M.D.
9000 Rockville Pike, Building 10
Rm CRC 8N228, NHLBI
Bethesda, MD 20892

12.3 Protocol Deviations (PD)

Definition: Protocol Deviations are any change, divergence, or departure from the IRB approved research protocol.

A PD is serious if it meets the definition of a SAE (see section 12.2) or if it compromises the safety, welfare or rights of subjects or others.

Reporting: Serious PDs will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious PDs will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

12.4 Unanticipated Problem (UP)

Definition: An UP is any incident, experience, or outcome that meets all of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to

a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and

b. the characteristics of the subject population being studied; and

2. **related or possibly related** to participation in the research; and

3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug. This may be a protocol deviation(s) and/or non-compliance.

Reporting: Serious UPs will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious UPs will be reported to the IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event.

If pregnancy occurs during the course of this study an unanticipated problem report will be submitted to the Clinical Director and the IRB.

13. Data and Safety Monitoring Plan

Based on clinical trials, the omega-3 fish oil supplements have been well tolerated and are unlikely to increase morbidity and mortality in subjects meeting the inclusion criteria of the study.

Protocol Monitoring

As per ICH-GCP 5.18 and 21 CFR 312.50 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors employed by an independent firm and working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the

investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data

Safety Monitoring

Principal Investigator: Accrual and safety data will be monitored by the PI. The protocol will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over and above that anticipated from niacin.

NHLBI IRB

Accrual and safety data will be monitored and reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient informed consent document will be reviewed and approved by the properly constituted IRB operating according to the 45 CFR 46 Code of Federal regulations. This committee must approve all amendments to the protocol or informed consent document, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

FDA: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to the FDA in compliance with 21CFR 312.33

14. Compensation

Compensation will be provided to the subjects for their time and inconvenience of participating on this protocol based on the values listed below:

Procedures	Inconvenience Unit	$	Frequency	Total $$
Outpatient Visit (first hour)	2	$20	Up to 8	$160
Outpatient Visit (additional hours, up to 4 hours)	1(per hour)	$10	Up to 8	$320
Physical Examination	1	$10	Up to 8	$80
Screening Blood Draw	1	$10	Up to 3	$30
Research Blood Draw	1	$10	Up to 6	$60
Stool Sample (optional)	1	$10	Up to 4	$40
Diet & Exercise Assessment	1	$10	Up to 6	$60
CAVI	2	$20	Up to 6	$120
EKG (Electrocardiogram), General	1	$10	Up to 2	$20
Total potential compensation:				$890

Subjects will receive full compensation only after completing the entire study. Subjects that fail to complete the study for any reason will not receive compensation.

15. References

1. Calder PC, Yaqoob P. Understanding omega-3 polyunsaturated fatty acids. Postgrad Med 2009; 121:148–157.
2. Ruxton CH, Reed SC, Simpson MJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet 2004; 17:449–459
3. Ota T, Takagi T, Kosaka S. Changes in lipids of young and adult saury cololabis saira (Pisces). Mar Ecol Prog Ser 1980; 3:11–17
4. Bailey KM, Powers DM, Quattro JM, Villa G, Nishimura A, Traynor JJ, Walters G. Population Ecology and Structural Dynamics of Walleye Pollock (Theragra chalcogramma). In: Dynamics of the Bering Sea 1999; 581–614
5. Pascal JC, Ackman RG. Long-chain monoethylenic alcohol and acid isomers in lipids of copepods and capelin. Chem Phys Lipids 1976; 16:219–223
6. Hardy R, Mackie P. Seasonal variation in some of the lipid components of sprats (Sprattus sprattus). J Sci Food Agric 1969; 20:193–198
7. Ratnayake WN, Ackman RG Fatty aalcohols in capelin, herring and mackerel oils and muscle lipids: I fatty alcohol details linking dietary copepod fat with certain fish depot fats. Lipids 1979; 14:795–803
8. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. Am J Clin Nutr 1980, 33:2657–2661.
9. Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr 1975, 28:958–966.
10. Matsumoto C, Matthan NR, Lichtenstein AH, Gaziano JM, Djoussé L. Red blood cell MUFAs and risk of coronary artery disease in the Physicians' Health Study. Am J Clin Nutr 2013; 98:749-754.
11. Yang ZH, Miyahara H, Takemura S, Hatanaka A. Dietary saury oil reduces hyperglycemia and hyperlipidemia in diabetic KKAy mice and in diet-induced obese C57BL/6J mice by altering gene expression. Lipids 2011; 46:425-34
12. Yang ZH, Miyahara H, Takeo J, Hatanaka A, Katayama M. Pollock oil supplementation modulates hyperlipidemia and ameliorates hepatic steatosis in mice fed a high-fat diet. Lipids Health Dis 2011; 10:189
13. Yang ZH, Miyahara H, Mori T, Doisaki N, Hatanaka A. Beneficial effects of dietary fish-oil-derived monounsaturated fatty acids on metabolic syndrome risk factors and insulin resistance in mice. J Agric Food Chem 2011; 59:7482-7489
14. Yang ZH, Miyahara H, Iwasaki Y, Takeo J, Katayama M. Dietary supplementation with long-chain monounsaturated fatty acids attenuates obesity-related metabolic dysfunction and increases expression of PPAR gamma in adipose tissue in type 2 diabetic KK-Ay mice. Nutr Metab (Lond) 2013; 10:16
15. Yang ZH, Miyahara H, Takeo J, Katayama M. Ingestion of a single serving of saury alters postprandial levels of plasma n-3 polyunsaturated fatty acids and long-chain monounsaturated fatty acids in healthy human adults. Lipids Health Dis. 2012; 11:95.
16. Ervin RB, Wright JD, Wang CY, Kennedy-Stephenson J. Dietary intake of fats and fatty acids for the United States population: 1999-2000. Adv Data. 2004; 8:1-6.

17. Mozaffarian D., Clarke R. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. Eur J Clin Nutr. 2009; 63:22–33.
18. Yu S., Derr J., Etherton T.D., Kris-Etherton P.M. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. Am J Clin Nutr. 1995; 61:1129–1139.
19. Mensink R.P., Zock P.L., Kester A.D., Katan M.B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. Am. J Clin Nutr. 2003; 77:1146–1155.
20. Franz M.J., Bantle J.P., Beebe C.A., Brunzell J.D., Chiasson J.L., Garg A., Holzmeister L.A., Hoogwerf B., Mayer-Davis E., Mooradian A.D., et al. Nutrition principles and recommendation in diabetes. Diabetes Care. 2004; 27:S36–S46.
21. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2004/ucm108368.htm
22. Pawar A, Jump DB. Unsaturated fatty acid regulation of peroxisome proliferator-activated receptor alpha activity in rat primary hepatocytes. J Biol Chem 2003; 278:35931-35939.
23. Kurushima H, Hayashi K, Shingu T, Kuga Y, Ohtani H, Okura Y, Tanaka K, Yasunobu Y, Nomura K, Kajiyama G. Opposite effects on cholesterol metabolism and their mechanisms induced by dietary oleic acid and palmitic acid in hamster. Biochim Biophys Acta. 1995; 1258:251–256.
24. Jansen S, López-Miranda J, Castro P, López-Segura F, Marín C, Ordovás JM, Paz E, Jiménez-Perepérez J, Fuentes F, Pérez-Jiménez F. Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester transfer protein concentrations in young, healthy, normolipemic men. Am J Clin Nutr. 2000; 72:36–41.
25. Schmidt S, Stahl F, Mutz KO, Scheper T, Hahn A, Schuchardt JP. Different gene expression profiles in normo- and dyslipidemic men after fish oil supplementation: results from a randomized controlled trial. Lipids Health Dis 2012; 11:105.
26. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002; 43:1363-1379.
27. Kubota et al. Cardio Ankle Vascular Index is A Predictor of Cardiovascular Events. Artery Res 2011; 5:91-96.
28. Ibata J, Sasaki H, Kakimoto T, et al. Cardio-ankle vascular index measures arterial wall stiffness independent of blood pressure. Diabetes Res Clin Pract 2008; 80:265-270.
29. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007; 86:74-81.
30. FDA Announces Qualified Health Claims for Omega-3 Fatty Acids; Available at: http://www.fda.gov/siteindex/ucm108351.htm

31. Hoy SM, Keating GM. Omega-3 ethylester concentrate: a review of its use in secondary prevention post-myocardial infarction and the treatment of hypertriglyceridaemia. Drugs 2009; 69:1077-1105.
32. J.M. McKenney, D. Sica. Prescription omega-3 fatty acids for the treatment of hypertriglyceridemia. Am J Health Syst Pharm 2007; 64:595-605.

16. APPENDIX A: NIH INFORMATION SHEET ON STAFF RESEARCH PARTICIPATION (April 2016)

As an NIH employee, contractor, Special Volunteer, Guest Researcher, or trainee, you may participate in intramural research studies unless it is prohibited by your Institute or Center (IC), or if you are excluded by the criteria of the protocol in which you want to enroll. The inclusion of NIH staff in a particular protocol must also be approved by the IRB. You may be motivated by altruism, a commitment to research in your own or related fields, or want access to clinical trials of potential direct therapeutic benefit. When deciding, you should make an informed decision about participation. This information sheet offers some points to consider for NIH staff who are considering research participation at NIH.

First, similar to any individual who is considering research participation, you should seek adequate information about the study purpose, what is required of you in terms of procedures, interventions and time, and the potential risks and benefits of participation. For more information, see the NIH Clinical Center's public website "Are Clinical Studies for You?" at http://www.cc.nih.gov/participate/studies.shtml.

When you are thinking about participation in a research study that is being conducted by your supervisor, or others with whom you work closely in your laboratory, branch, or unit, you should consider some additional factors:

A. **Possible bias**: Are you confident that you can be unbiased about reporting answers, side effects, or other information that could influence the study outcome or risk to you?

B. **Confidentiality**: Has the principal investigator (PI) spoken about what information will be collected from you as part of the study? Has the PI discussed what information will be available to those within, and outside, the study team? If applicable, are you comfortable sharing your medical history (including, for example, mental health history or STDs) and your social history (e.g. substance use) with study investigators who may be your coworkers, or with the possibility of them discovering something about your health during the study (e.g. pregnancy status or a new diagnosis)? Although every effort will be made to protect your information and keep it private and confidential, your information may, depending on the nature of the protocol, become available in medical records or to authorized users outside of the study team. Discuss any concerns with the PI.

C**. Pressure**: Do you perceive any pressure or expectations from your supervisor or colleagues regarding participation? Could that pressure influence your decision or make it difficult for you to choose whether or not to participate? Remember that it is your choice whether or not to participate and that your decision to participate or not should not have an effect, either beneficial or adverse, on your position at NIH.

D. **Time and Compensation**: Can you take time off from work to complete the study requirements or participate solely during non-duty hours? Can you receive compensation for your participation in this study? Will your supervisor give you permission to participate during work hours? See the NIH Policy Manual 2300630-3 *Leave Policy for NIH Employees Participating in NIH Medical Research Studies*.

E. **Consent Process:** Is the person obtaining your consent for the study your supervisor, a subordinate, or co-worker? If so, is there an independent person monitoring the consent process? If the study PI is a supervisor and intends to obtain consent from you, an independent person (e.g., through Bioethics or the NIMH Human Subjects Protections Unit [HSPU], or others as approved by the IRB) must monitor the consent process. If the person obtaining consent from you is a co-worker then an independent person (e.g., through Bioethics or the NIMH HSPU, or others as approved by the IRB) may be required to monitor the consent process, as determined by the IRB for the specific study.

If you are thinking of enrolling as a subject at the NIH Clinical Center and you have any questions or concerns, please contact the Office of Human Subjects Research Protections (OHSRP) at 301-402-3444 and/ or the Patient Representative if you are thinking of enrolling as a subject at the NIH Clinical Center on 301-496-2626. If you are at a NIH site outside the Clinical Center then please contact local site leadership.