TUFTS MEDICAL CENTER/TUFTS UNIVERSITY RESEARCH PROTOCOL

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Study Title: Role of EPA and DHA in fish oil on inflammation and lipoprotein metabolism

I. Aim and Hypotheses

Chronic low-grade inflammation plays an important role in the pathogenesis of several diseases, including cardiovascular disease (CVD), placing substantial burden on patients and society. Some epidemiological and clinical evidence indicates that consumption of fish and fish oil containing the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduces the risk or severity of these diseases (1-3). This evidence has contributed to the Dietary Guidelines for Americans recommendation to consume >250 g/day of EPA and DHA (4). In addition, several other government and health organizations have advocated an increased consumption of fish or fish oil in the general population and in subjects with chronic inflammatory conditions (5-8). *However, recent findings of large randomized clinical trials (9-11) and more recent meta-analyses (12-14) have indicated that supplementation with fish oil does not reduce the risk of CVD, generating substantial controversy regarding the use of fish oil in the prevention or treatment of CVD.* It has been suggested that differences in the ratio of EPA to DHA in different fish oil preparations may contribute to the variability in findings among studies (15). While it has been generally assumed that EPA and DHA have similar effects on inflammation and on plasma lipid levels, recent evidence from animal and cell studies have suggested that EPA and DHA have some distinctive anti-inflammatory effects (16). In addition, some human studies have indicated that, while EPA and DHA are similarly effective in lowering plasma triglyceride (TG) levels, DHA, contrary to EPA, increases plasma low-density lipoprotein cholesterol (LDL-C) levels (17-19). *No comprehensive information on the common and differential effects of EPA and DHA on systemic inflammation and lipid metabolism is currently available in humans.* Due to the controversy on the effects of fish oil on CVD prevention and treatment, it is critical to assess the independent and common effects of EPA and DHA in the modulation of systemic inflammation and plasma lipid levels.

<u>Aim 1.</u> To characterize the effects of EPA alone and DHA alone, relative to each other and to placebo, on plasma biomarkers of inflammation and on inflammatory cell activation and gene expression in subjects with metabolic syndrome.

<u>Aiim 2.</u> To characterize the effects of EPA alone and DHA alone, relative to each other and to placebo, on plasma lipid, lipoprotein levels, and lipoprotein metabolism in subjects with metabolic syndrome.

II. Background and Rationale

A. Background: Polyunsaturated fatty acids of the omega-3 type in fish oil, EPA and DHA, effectively reduce plasma TG levels and have anti-inflammatory effects (20, 21), resulting in reduced cardiovascular disease, as shown in epidemiological studies and both primary and secondary CHD prevention trials (2, 3). While both EPA and DHA appear to have anti-inflammatory effects, most studies suggest a greater ant inflammatory effect of EPA (15). In addition, while both EPA and DHA reduce plasma levels of triglycerides by reducing the

secretion of very low density lipoproteins (VLDL), DHA, but not EPA, increases plasma levels of the atherogenic low density lipoproteins (LDL)(22, 23).
To date, no comprehensive study has been carried out to investigate the individual effects of EPA and DHA on inflammation and lipoprotein metabolism. Available data lead to the hypothesis that DHA, but not EPA, may increase LDL-C levels through an increased conversion of VLDL to LDL, possibly mediated by lipolytic enzymes.

B: **Rationale:** In vitro and in vivo (both human and animal) studies suggest differential effects of EPA and DHA (16). This study represents the first systematic effort to characterize the differential effects of EPA and DHA on inflammation and lipid metabolism in humans. The central hypothesis of this proposal is that EPA and DHA, in addition to common effects, have distinct and separate effects on systemic inflammation and lipid metabolism. The objective of the proposed study is to provide critical information regarding both common and distinctive roles of EPA and DHA in systemic inflammation and lipid metabolism. The current gaps in knowledge on the differential effects of EPA and DHA undermine the ability to provide optimal guidance for the use of EPA or DHA in specific conditions and in population-wide recommendations aimed at improving public health. Efforts in understanding the distinctive effects of EPA and DHA on inflammation and lipoprotein metabolism can have remarkable implications for the prevention and treatment of inflammatory states, cardiovascular disease, dyslipidemia, and other disorders, and ultimately lead to healthcare cost savings and improvement in quality of life.

A. Experimental design: randomized, placebo-controlled, double-blind, cross-over study (Figure 1). Subjects will be randomized to one of two sequences of supplementation [EPA followed by DHA (n=12), or DHA followed by EPA (n=12)] using a computer-generated randomization schedule. To reduce patient scheduling issues and increase compliance, subjects enrolled in to the study will be allowed short breaks (usually less than 3 weeks) between phases. EPA and DHA capsules will be identical and will be provided in a blind fashion by the manufacturer (Prevention Pharmaceuticals). The study physician will be the only member of the research team with knowledge of the supplement assignment. Code will be broken at the end of the study.

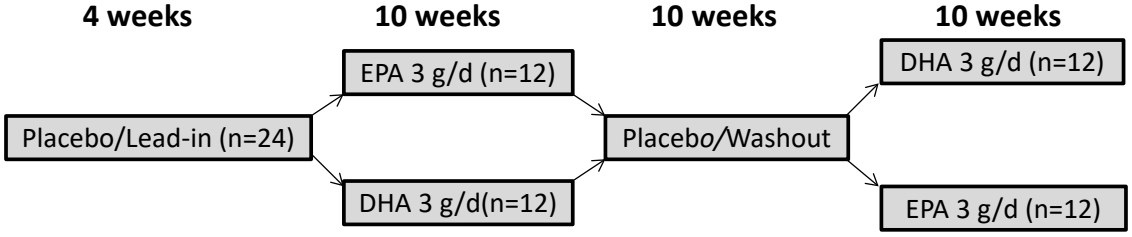

Figure 1. Study design.

B. Sample size and statistical analyses): Sample size calculation with TNF-α, IL-6 and LDL-C as primary outcomes were conducted using published data (23-25). Under the assumption that EPA has the full effect and DHA ¼ of the anti-inflammatory effect on TNF and IL-6 and that DHA has the full effect on LDL-C raising and EPA no effect, 24 subjects are needed to achieve 80% power using a two-sided 0.05 significance level (enroll 32 to insure completion of 24 subjects). Significant differences in mean between phases will be assessed by mixed model analysis (PROC MIXED) with or without adjustment for treatment sequence (Tukey-Kramer) and paired t-tests.

24 subjects (enroll 420 to insure completion of 24 subjects) will be required to complete the study. CTSI provided statistical support for sample size calculations. Briefly, primary outcome is TNF-alpha. No studies comparing directly EPA to DHA are available; based on our assumption

that EPA will have the full effect of fish oil and DHA ¼ of the effect and using the mean estimate of the true ratio of 0.79 and a mean coefficient of variation of 0.28 for TNF-alpha, as based on the literature (Moertl et al, Am Heart J 2011;161:911-919 and Zhao et al J Int Med Res 2009;37:1831-1841), we find that 24 subjects will be required to achieve 80% power to infer that the post-treatment ratio of TNF-α is different from 1.0 in such a 2x2 cross-over design using a two-sided 0.05 significance level. CTSI will consult on statistical analyses.

C. Subject Characteristics

1. Subject criteria: Men and women, age ≥50 years and <75 years; women will be postmenopausal. Postmenopausal status will be self-reported and defined as absence of menstrual period for at least 1 year or surgical menopause (bilateral oophorectomy).

a) Inclusion criteria:

- fasting plasma TG levels between 90 and 500 mg/dL
- C-reactive protein (CRP) levels ≥2 µg/mL
- at least one of the following criteria for the definition of metabolic syndrome: abdominal obesity (waist circumference >40 inches in men and ≥34 inches in women), hypertension (blood pressure ≥130/≥80 mmHg or use of anti-hypertensive medications), and fasting glucose ≥100 mg/dL.

b) Exclusion criteria:

- high-fish diets (>2 fish meals/week)
- taking fish oil supplements or supplements containing EPA or DHA
- allergy to sardines
- allergy to sunflower oil
- regular use of anti-inflammatory medications (NSAID, COX inhibitors, corticosteroids)
- anticoagulant therapy
- alcohol consumption >7 drinks/week
- uncontrolled thyroid dysfunction
- insulin-dependent type 2 diabetes mellitus
- kidney or liver disease
- smoking
- alterations in coagulation (prolonged partial thromboplastin time, PTT) as measured at the screening visit
- Use of lipid-lowering medications or medications known to alter lipoprotein metabolism (fibrates, niacin, sex hormones, hormonal replacement therapy, etc.). *
- Any major acute or chronic diseases that may interfere with the study outcomes
- Gastrointestinal diseases affecting absorption including gastric bypass, ulcerative colitis, Crohn's disease, or chronic diarrhea
- unwillingness to adhere to study protocol
- non-English speaking
- no Social Security number

* If, however, interruption of lipid lowering medication is deemed safe and his/her primary care physician agrees to taking off the potential study subject from the lipid-lowering medication, such subject may be enrolled into the study after a 4-week washout phase.

c) Withdrawal/Termination criteria: low compliance, defined as taking less than 80% of study capsules, or inability to comply with the study schedule. Also subjects may not participate in another study while participating in this study.

D. **Risk/benefit assessment**: No major risks or hazards are expected.

1. **Physical risk**: No known risks are associated with the ingestion of EPA or DHA capsules. In subjects with prolonged coagulation time, there may be a risk of further increase in coagulation time. Therefore, subjects will be screened for coagulation

problems before enrollment into the study, specifically during the preadmission screening visit.
Blood draw: There is a small amount of bruising and discomfort, and rarely infection, associated with the drawing of blood samples. Every effort will be made to minimize discomfort to volunteers.
There is small risk of bleeding associated with the injection of heparin (50 IU/kg body weight) which is performed for the measurement of lipoprotein lipase and hepatic lipase.

One risk is the possible loss of confidentiality. Stored samples will be labeled with a unique number (code) that will not contain any private information such as name or date of birth to ensure the samples cannot be identified. There is no intention for allowing anyone other than the principal investigator to have access to these codes. If Dr. Lamon-Fava turns this study over to a new principal investigator, she will give the key code to the new principal investigator. Every possible precaution will be taken to keep volunteer information confidential. However, despite strict measures, possible loss of confidentiality remains as a risk of participation in this research study.

2. **Psychological risk**: No psychological risks are expected

3. **Social risk**: No social risks are expected

4. **Economic risk**: No economic risks are expected

5. **Benefit of participating in the study**:
   There will be no direct benefits to the volunteer for study participation. There is a possibility of benefit from reduction in inflammation and TG levels during the active treatment phases of the study. Knowledge may be gained by their participation, which may benefit the health of others.

E. **Specific methods and techniques used throughout the study**:

1. **Laboratory tests**:
   During the last week of the placebo/lead-in phase and each of the two supplementation phases a total of 160 ml of blood (32.5 teaspoons) will be drawn (120 ml [24.4 teaspoons] in visit 1 and 40 ml [8.1 teaspoons] in visit 2). The following will be measured:
   - Clinical Chemistry Profile (glucose, albumin, ALP, SGPT, SGOT, blood urea nitrogen, creatine phosphokinase, creatinine, lactate dehydrogenase, total protein, bilirubin, globulin, albumin/globulin ratio, uric acid, calcium, phosphorus, magnesium, sodium, potassium, chloride, total and LDL-cholesterol, triglyceride)
   - PT/PTT
   - CRP; immunoassay
   - Plasma cytokines (TNF-α, IL-6, and MCP-1); multiplex immunoassay
   - Monocyte isolation and response to LPS: cell culture
   - Monocyte transcriptomics: GeneChip® Human Genome U133 Plus 2.0 Array (Affimetrix) and real time PCR
   - Monocyte lysates: AMPK phosphorylation, TAK1 phosphorylation, NF-kB by Western blotting
   - Plasma lipid (TC, TG, LDL-C, HDL-C): automated assays
   - Enzyme concentration (CETP, LCAT, LPL and HL): ELISA
   - CETP, LPL and HL activity: enzymatic release of 13-C labeled fatty acids, assessed by liquid scintillation counter

2. **Study Procedures**: Study subjects will be recruited as described below. Selection for a screening visit will occur via telephone interviews. Selected subjects will then report to

the MRU for the screening visit 1, after a 12-hour fast, for the measurement of plasma lipid, CRP, and glucose levels, and for the assessment of blood pressure and abdominal adiposity. Subjects that meet the inclusion criteria for the study based on screening visit 1 results will be invited for a second screening visit following a 12-hour. During screening visit 2, blood will be drawn for CBC, chemistries, lipid profile, TSH and PT/PTT. The subjects meeting all the inclusion and exclusion criteria will be enrolled into the study at visit 0 (enrollment visit). At visit 0 subjects will sign the Informed Consent Form and meet with the study dietician who will provide instructions on how to follow a Therapeutic Lifestyle Changes diet (25-35% of calories as total fat, <7% as saturated fat, and <200 mg/day cholesterol) throughout the study.

The table below illustrates the measurements to be performed at each study visit after enrollment into the study. At the screening visit, subjects will undergo medical history, weight and waist circumference measurement, blood pressure and vital signs assessment, and a blood draw for the assessment of clinical chemistry profile and hs-CRP.

At visit 0, subjects will receive the supply of supplements for Phase 1 of the study, at visit 2 they will receive the Phase 2 study supplements, at visit 4 they will receive the Phase 3 study supplements and at visit 5 will receive the Phase 4 study supplements.

Outpatient visit	Measurement	ml of blood
Phase 1, visit 1	Weight, waist circumference, blood pressure, vital signs Clinical chemistries, hs-CRP, plasma cytokines and lipids Blood monocyte isolation and response to LPS.	120
Phase 1, visit 2	Weight, waist circumference, blood pressure, vital signs Dietary counseling and supplement distribution Plasma cytokines and lipids, enzyme (CETP, HL, LPL, LCAT) concentration and activity	40
Phase 2, visit 3	Weight, waist circumference, blood pressure, vital signs Clinical chemistries, hs-CRP, plasma cytokines and lipids Blood monocyte isolation and response to LPS.	120
Phase 2, visit 4	Weight, waist circumference, blood pressure, vital signs Supplement distribution Plasma cytokines and lipids, enzyme (CETP, HL, LPL, LCAT) concentration and activity	40
Phase 3, visit 5	Weight, waist circumference, blood pressure, vital signs Dietary counseling and supplement distribution plasma cytokines and lipids	30
Phase 4, visit 6	Weight, waist circumference, blood pressure, vital signs Clinical chemistries, hs-CRP, plasma cytokines	

	and lipids Blood monocyte isolation and response to LPS.	120
Phase 4, visit 7	Weight, waist circumference, blood pressure, vital signs Plasma cytokines and lipids, enzyme (CETP, HL, LPL, LCAT) concentration and activity	40

Due to the study inclusion criteria, the majority of the subjects will likely already follow this diet. At the admission visit, all subjects enrolled into the study will receive nutritional counseling from a registered dietician to help them achieve and maintain the goals of the Therapeutic Lifestyle Changes diet. Prevention Pharmaceuticals will provide identical study capsules as 750 mg EPA and 750 mg DHA. Placebo capsules will contain high oleic acid sunflower oil. Capsules will be provided by Prevention Pharmaceuticals in a blinded fashion and will be dispensed according to each individual randomization number which will be computer-generated. Subjects will be instructed to take two capsules in the morning and two capsules in the evening with meals every day during both the placebo and supplementation phases. Every effort will be made to encourage subjects to adhere to this regimen. Participants will be contacted by the Principal Investigator by phone every four weeks during the course of the study to ensure adherence to both study treatment and study procedures. Study subjects will be instructed to return any excess capsules at the end of each phase. Returned capsules and those at the end of the study will be returned to the manufacturing company at the end of the study. A capsule count will be performed during each visit, with 80% set as minimum compliance for continuation in the trial. If a participant withdraws or is excluded from the study prior to completion, his/her randomization sequence will be assigned to the next subject enrolled. Subjects will begin taking the active supplements, according to the pre-set randomization schedule, after completing the placebo/lead-in phase and will report to the MRU at the end of each phase.
EPA and DHA capsules will be manufactured by Prevention Pharmaceuticals through fish oil extracting procedures from farmed sardines and will be delivered to the Principal Investigator in a blinded fashion (marked A and B, the investigator will not know the content of these). Placebo capsules will be prepared by Prevention Pharmaceuticals using high-oleic acid sunflower oil (commercially available from J Edwards International) and will be delivered to the Principal Investigator marked as C (PI will know the identity of placebo capsules to allow their administration in the lead-in and washout phases). Capsules will be stored in the Principal Investigator Office, in a locked cabinet. Capsules will be stored at room temperature and instructions will be provided to subjects both orally, at the time of supplement distribution to study subjects, and in writing on the supplement bottle label.

3. **Subject Timeline**: The study is approximately 34-week long and includes a 4-week placebo lead-in phase, followed by a 10-week EPA or DHA supplementation phase, a 10-week placebo/washout phase and then a cross-over to a 10-week DHA or EPA supplementation phase. The length of the study may be longer if a participant takes a break between phases. A detailed description of the schedule of vital sign assessments, blood draws, and other study procedures is provided in Table 1. Subjects will be allowed to change the schedule of their visits by 5 days (before or after the scheduled appointment) to allow for unexpected events (weather, illness, etc.).

F. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Definition of Serious Adverse Event (SAE) and Adverse Event (AE) for this study:

An **Adverse Event (AE)** is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or laboratory finding, symptom, or disease, temporally associated with a subject's participation in the research.

A **Serious Adverse Event (SAE)** is any Adverse Event (AE) that:
1. Results in death, or
2. Is life-threatening, or
3. Results in hospitalization or prolongation of existing hospitalization, or
4. Results in a persistent or significant disability/incapacitation, or
5. Results in a congenital anomaly/birth defect, or
6. May jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above.

2. Reporting timeframe for SAEs and AEs:

In case of an unanticipated problem or adverse event the nursing staff will immediately notify the principal investigator and the study physician. Upon notification, the principal investigator, Dr. Lamon-Fava, will take immediate corrective action to eliminate or minimize risk to enrolled subjects. The MRU medical director, also study physician, Dr. Lisa Ceglia, will assess the severity of the unanticipated problem or adverse event and determine its relation to the study protocol. The unanticipated problems and adverse events will be reported by the principal investigator to the Tufts Medical Center/TUHS IRB following the timelines detailed in Table 1. Reports will be submitted to the IRB for each event occurring for each subject individually using the Tufts MC/TUHS IRB Event Reporting Form. All supporting documentation will also be attached to the Event Reporting Form.

Table 1: Reporting Unanticipated Problems and Adverse Events

Unanticipated Problem: *Internal* or *External*	Immediate reporting. *Event Reporting Form* with supporting documents will be submitted to IRB within 5 business days
SAE: *Internal*, *Related* or *Probably Related, Unexpected*	If the SAE meets criteria for an ***Unanticipated Problem*** it will be reported as such
SAE: *Internal*, all other situations	*Event Reporting Form* will be completed and submitted to IRB within 15 business days
Non-Serious AE: *Internal*, all situations not considered an *Unanticipated Problem*	Clinically significant *AEs* may be summarized at the time of Continuing Review, or study termination if before the next scheduled Continuing Review

3. Accountability procedures as they relate to drugs, devices, and data: Because this is a low-risk study, it does not have a Data and Safety Monitoring Board. The principal investigator in conjunction with the IRB will ensure the integrity of the data and the safety of the volunteers. The Principal Investigator will conduct the research following the principles of respect for persons, justice, and beneficence. The IRB, through initial and then annual reviews, will ensure continued protection of the welfare of human subjects and compliance with relevant regulations.

G. Subject Participation

1. **Recruitment**: Participants will be recruited from the Greater Boston area using several recruitment strategies such as advertisements in newspapers, local newsletters and flyers posted on university websites (Tufts University, Boston University). Additionally, the Metabolic Research Unit (MRU) resources include a roster of >20,000 names of subjects from which potentially qualifying women and men can be identified and contacted through the use of direct mailings. Although employees will not be targeted for recruitment and enrollment, employees of Tufts University and/or the HNRCA (employee-subjects) who voluntarily want to participate in the study will be eligible for screening and enrollment. In order to qualify for the study, employee-subjects must respond to IRB-approval advertisement of their own accord and will not be directly approached by any person seeking to recruit them for participation in the study. Members of the research team, as direct-report subordinates of the PI and anyone who is direct-report subordinate to any of the research team members in any other capacity will not be eligible to participate in the study. If employee-subjects qualify to participate in the study, they cannot participate as volunteers during hours in which they are being compensated by Tufts University for their regular work. This includes use of vacation, personal days and sick time.

2. **Registration**: This study will be registered on clinicaltrials.com.

3. **Screening Interview/questionnaire**:
   Prescreening
   After potential volunteers have been identified they will complete a 20 minute telephone interview (Prescreening Phone Questionnaire). The interviewer will explain the study design and ask questions to assess whether a potential volunteer meets the inclusion criteria.

   Screening
   If, on the basis of the prescreening questionnaire, the interviewer determines that the potential volunteer meets the study inclusion criteria, s/he will be asked to attend Screening visit 1 in the fasted state (12 hrs) to determine study eligibility. During that visit potential volunteers will have blood pressure, height and body weight recorded, BMI calculated, 5 ml (1 teaspoon) of blood drawn, and a medical history administered by a nurse. A Registered Dietitian will obtain information on the potential volunteer's dietary habits with specific emphasis on the habitual consumption of fish. If eligible based on results of screening visit 1, subjects will be asked to return to the MRU for screening visit 2 for a 21.5 mL blood draw (4.3 teaspoons).

4. **Transportation**: The volunteers will be expected to provide their own transportation to the facility. The facility is accessible by public transportation. Additionally, volunteers who choose to drive to the HNRC can obtain parking validation at a reduced rate.

5. **Informed consent process and timing of obtaining of consent**: Nursing staff or the Principal Investigator will administer both the Screening Informed Consent Form as well as the Study Informed Consent Form from each potential volunteer. The consent interview will be administered by the Principal Investigator in a private room in the MRU at the HNRCA. The Screening Informed Consent Form will be obtained on the day of the volunteers screening visit. Subjects will be given the time each of them requests to consider participation in the study. The Study Informed Consent Form will be obtained from each volunteer on the first day of the study after they have had the purpose of the study explained to them and given the opportunity to read the form and ask questions. Before consent, each potential subject will be asked to describe the study back to the investigator to make sure he/she has a good understanding of the purpose of the study and the level of commitment required. After all the questions have been answered, and the volunteer fully understands the study procedures, the nursing staff or study coordinator will obtain the signed informed consent from the volunteer. The volunteers

will also be given the option to sign an optional informed consent form to bank specimen samples. Left-over samples will be stored only from those subjects who consent to specimen banking and future use. The decision to bank will not affect volunteer's participation in the study. A copy of the signed consent forms will be provided to the volunteer.
Informed consents, as well as other study records, will be stored in the Principal Investigator office, in a locked cabinet. A copy of the signed consent form will also be given to the study participant.

a. **If non-English speaking persons will be enrolled, state the informed consent process for enrolling the subjects, including who will conduct the consent interview, use of interpreters, translated documents, etc.:** Non-English speaking persons will not be recruited or enrolled in this study because of difficulty with standardizing dietary counseling across multiple languages and different ethnic dietary patterns.

(NOTE: Exclusion of non-English speaking subjects from research requires ethical and scientific justification. This justification must be stated elsewhere in the protocol.)

6. **Location where study will be performed**:
Metabolic Research Unit
and Cardiovascular Nutrition Laboratory
Jean Mayer USDA Human Nutrition Research Center on Aging
Tufts University
711 Washington Street
Boston, MA 02111

7. **Personnel who will conduct the study, including**:
   a. **Present during study procedure(s) and their proximity during the study**:
   Study nurse
   Study dietitian
   Principal Investigator
   Study Coordinator

   b. **Primary responsibility for the following activities**:

      i. **Obtaining informed consent**:
      Study nurse
      Principal Investigator

      ii. **Providing on-going information to the study sponsor and the IRB**:
      Principal Investigator
      Co-Investigators
      Study Physician

      iii. **Maintaining participant's research records**:
      Study nurse
      Study dietitian
      Principal Investigator
      MRU admission's staff
      Study Coordinator

8. **Subject fees**:
Screening visit 1 stipend: $20
Screening visit 2 stipend: $25

Completion of study stipend: $980 for completing all study phases ($160 for lead-in phase, $320 each for EPA and DHA phases, and $180 for completing the placebo/washout phase).
Stipend prorated for drop-outs, $20 per week

9. **Procedures to protect subject confidentiality**: The identity of the volunteers, medical records and data relating to this research will be kept confidential, except as required by law and except for inspections by the USDA or the Tufts Medical Center Institutional Review Board. The medical records will be filed in the medical record room only available to designated research staff. Upon entry into the research study, the volunteer will be assigned a unique code number. For storage, blood samples will be identified with this unique code number (not related to medical record number), date, laboratory generated login number and sample description (i.e. plasma, serum).

10. **Confidentiality**:

    a. **Certificate of Confidentiality**: None

    b. **How data will be coded, recorded, and stored to protect confidentiality**: All samples and data generated from the research study will be labeled and/or recorded with a unique code number and no other identifier. The information that will allow the unique code number to be linked to the volunteer's name will be stored as a password-protected computer file. Samples will be stored until all analyses are complete. If blood samples collected during the course of this research study are sent outside the HNRCA for analysis, they will be labeled in a manner that contains no information that can make it possible to trace the sample back to the volunteer as an individual. At the conclusion of the study, the information file that would allow linking of the volunteer's identification information with the sample labels will be destroyed. Therefore, after the research study is completed, the identity of subjects will not be traceable to the data and samples stored. Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure, HIPAA and 21 CFR 11 compliant web-based application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. data types and range checks), complete audit history, restricted access to PHI and a de-identified export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap is hosted by the Tufts University Clinical and Translational Science Institute (CTSI) and is made freely available to the Tufts CTSI research community. Vanderbilt University was responsible for leading the development of the system and along with a consortium of over 1600 member institutions provides a significant amount of online training material to support researchers developing studies using this platform. Tufts CTSI compliments this resource with a series of classes to further support, train and empower the researcher in their endeavors. In addition, the Biostatistics and Data Management Core Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging provides planning assistance and support for development of REDCap data collection projects thorough study-specific data dictionaries defined by members of the research team.
    The Tufts CTSI – REDCap deployment employs a MySQL 5.x database and a Microsoft IIS web server located within Tufts Medical centers' DMZ and is accessible via a secure SSL connection.

    Confidentiality of Employee-subjects: The identity of the employee-subjects, their medical records, and the data relating to this research will be kept confidential, except as required by law. Under no circumstances will the data related to this

research, or their medical records be linked to the employee ID number. Upon entry into the research study the employee-subject will be assigned a unique code number. For storage, blood samples will be identified with this unique code number (not related to medical record number, or employee ID number), date, laboratory generated login number and sample description (i.e. plasma, serum). All samples and data generated from the research study will be labeled and/or recorded with that number and no other identifier. Therefore, after the research study is completed, the identity of subjects will not be traceable to the data and samples stored.

Record Retention Plan: Volunteers' records will be filed in the Metabolic Research Unit's Medical Records File Room located on the 11th Floor of the Human Nutrition Research Center. Research records will be stored in a locked file cabinet in the Principal Investigator's office. Digital data records, in the form of excel spreadsheets, will be stored in the Principal Investigators' password-protected computer. Research records, including volunteer records, will be retained for a period of 7 years after completion of the study, in agreement with Tufts MC / TUHS IRB record retention policies and requirements.

c. **Parties who will have access to the data, including the key to the identity code**: Dr. Lamon-Fava (Principal Investigator) will have access to the key to identity code. Dr. Lamon-Fava will have access to the de-identified data. Laboratory personnel responsible for analyzing the samples will have access only to the de-identified data. The study statistician (to be identified) will have access only to the de-identified data.

d. Parties who will have access to research records: PI and co-investigators will have access to the research records.

11. **Alternatives**: The volunteer can choose not to participate in this research study. S/he is free to withdraw from this study at any time for any reason.

H. **Outcome**: Determine the common and distinct effects of EPA versus DHA on inflammation and lipid metabolism. This information will lead to targeted and personalized use of these fatty acids.

I. **Tissue banking considerations**: Volunteers will be given the option to sign the optional informed consent form to bank samples. Left-over samples will be stored only from those subjects who consent to specimen banking and future use. The decision to bank will not affect volunteers' participation in the study.

VULNERABLE POPULATIONS: No vulnerable populations will be included in this study.

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