

Title of Study Protocol: **Fish oils and Adipose Inflammation Reduction (FAIR Study)**

Principal Investigator: **Rachana Shah, MD, MsTR**

Sponsors of Protocol: **National Institutes of Health**

Grants: **1K23DK095913-01A1 (NIDDK): Omega-3 PUFA suppress adipochemokines (PI:Shah)**

IRB Protocol Number: **818359**

Date of Document: **November 4, 2014**

Synopsis

Title	Fish oils and Adipose Inflammation Reduction (FAIR Study)
Short Title	FAIR Study
Protocol Number	818359
Study Design	Parallel arm, double blind, placebo-controlled trial
Study Center(s)	University of Pennsylvania Medical Center Philadelphia, PA USA
Objective	To examine the effect of omega-3 fish oil supplementation
Study Population, Diagnosis And Main Inclusion Criteria	Obese (BMI>30), non-diabetic men and non-pregnant/non-lactating women aged 18-50 not currently consuming significant amounts of omega 3 fatty acids.
Study Product, Dose, Route, Regimen, Duration of administration	Participants will be randomized to placebo of Lovaza 4 grams/day orally. Subjects will be treated for 8 weeks.
Reference therapy	Placebo
Pharmacodynamic Evaluations	Inflammatory markers (cytokines, chemokines, acute phase proteins), metabolic markers, oral microbiome composition, monocyte function and lipoproteins
Safety Evaluations	Clinical and laboratory measurements will be taken at screening and upon completion of the study.
Statistical Methodology	Analysis will compare within group and between group differences to determine effect of omega 3 fatty acids vs placebo on all measures indicated

1.0 ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the University of Pennsylvania's clinical research standards that meet regulations relating to Good Clinical Practice (GCP).

These standards adhere to the following guidelines:

- Good Clinical Practice: ICH Consolidated Guideline (International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).
- United States (US) Code of Federal Regulations (21 CFR) dealing with human subject protection and conduct of investigational clinical studies (21 CFR parts 50, 54, 56, 312, and 314).
- Declaration of Helsinki, concerning medical research in humans ("Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects," Helsinki 1964, amend Tokyo 1975, Venice 1983, Hong Kong 1989, revised version of Somerset West, Republic of South Africa, October, 1996 and Scotland, 2000).

This protocol and any amendments will be submitted to University of Pennsylvania institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

2.0 INTRODUCTION AND BACKGROUND

Adipose inflammation, induced by inflammatory leukocyte infiltration, is a crucial link between obesity and its metabolic complications^{1-3 2-8 9-12}, including insulin resistance, type 2 diabetes, and metabolic syndrome. Chemokine pathways including monocyte chemoattractant protein (CCL2) and fractalkine (CX3CL1) and their receptors CCR2 and CX3CR1 may modulate insulin resistance by regulating adipose macrophage recruitment and activation^{4,13-15}. Variable expression of CCR2 and CX3CR1 is a critical determinant of monocyte survival and inflammatory state and delineates two populations: pro-inflammatory CX3CR1^{lo}CCR2⁺ monocytes associated with classically-activated, inflammatory (M1) adipose macrophages vs. less inflammatory CX3CR1^{hi}CCR2⁻ thought to give rise to alternatively-activated (M2) adipose macrophages¹⁻³. Metabolic and inflammatory stimuli associated with obesity induce release of chemokines that promote disproportionate migration and adhesion of pro-inflammatory CX3CR1^{lo}CCR2⁺ monocytes and/or shift of M2 to M1 macrophages¹⁶.

Omega-3 polyunsaturated fatty acids: Murine LC n-3 PUFA, from fish and fish oil supplements, include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and are used clinically to treat dyslipidemia. Decreased triglycerides with increased HDL is seen and adiponectin levels are increased in mice and humans on LC n-3 PUFA^{17,18}. Data assessing beneficial effect of LC n-3 PUFA on incidence of cardiovascular disease is conflicting^{19,20}. Several large scale studies have reported benefits of LC n-3 PUFA in primary^{21, 22, 23} and secondary^{24, 25} prevention of adverse cardiovascular outcomes; however, the recently completed ORIGIN trial showed no reduction in cardiovascular death in a large cohort of dysglycemic subjects receiving 1 gram LC n-3 PUFA vs. placebo²⁶. Dose, treatment duration, and underlying disease burden may underlie this discrepancy²⁷.

LC n-3 PUFA reduce obesity-related inflammation: LC n-3 PUFA themselves or anti-inflammatory metabolites they produce exert pleiotropic anti-inflammatory effects in human and animal models²⁸. In patients with chronic inflammatory diseases, LC n-3 PUFA reduce systemic inflammation²⁹⁻³²; remarkably, decreases in inflammatory markers are also suggested with LC n-3 PUFA in the low-grade inflammatory condition of obesity³³. In a trial of 25 adolescents with metabolic syndrome, 3 weeks of LC n-3 PUFA supplementation decreased circulating lymphocytes and monocytes as well as levels of cytokines TNF α , IL-1 β , and IL6 despite no improvement in weight or insulin sensitivity³⁴.

LC n-3 PUFA reduce monocyte inflammatory state and ATM infiltration: Dietary studies in humans suggest that LC n-3 PUFA reduce monocyte inflammatory mediators^{35,36}, monocyte migration and adhesion, and receptor expression (CD44 variants)^{37,38}. Human THP-1 macrophages treated with LC n-3 PUFA *in vitro* had decreased LPS-stimulated cytokine expression (TNF α , IL-1 β , IL6) and inflammatory transcription factor nuclear factor kappa beta (NF κ B) activity³⁹. In mice, adding LC n-3 PUFA to diet reduced circulating Ly6C^{hi} inflammatory monocytes⁴⁰. In rodents fed a high fat diet (HFD), addition of LC n-3 PUFA prevents inflammatory ATM infiltration, adiponectin decrease, and inflammatory gene expression (including chemokines CCL2 and CCL5)^{41, 42 43,44 45}.

LC n-3 PUFA may attenuate NF κ B signaling: Though multiple mechanistic models are proposed, the NF κ B pathway is consistently implicated in reduced inflammation by LC n-3 PUFA²⁸. NF κ B signaling is downstream of TLR4 and cytokine receptor activation⁴⁶. *In vitro* LC n-3 PUFA is shown to inhibit LPS-induced activation of NF κ B and I κ B phosphorylation in macrophages, with associated reduction in inflammatory cytokines⁴⁷ and increased production of anti-inflammatory marker IL10⁴⁸.

GPR120 is a LC n-3 PUFA receptor and may inhibit NFκB signaling: Recent work suggests the effects of LC n-3 PUFA on obesity-related inflammation are mediated via interactions GPR120, a G-protein-coupled receptor (GPR), which is highly expressed on adipocytes and macrophages⁴⁹. GPR120 is part of the G α q-linked GPR family and stimulation results in increased intracellular Ca⁺⁺ and ERK activation⁵⁰. Compared to wild-type mice GPR120 deficient mice fed a HFD have increased weight, insulin resistance, circulating and adipose inflammatory markers—including the chemokine CCL2, and ATMs⁵¹. Remarkably, humans with a dominant-negative loss-of-function variant in *GPR120* (R270H) have increased incidence of obesity⁵¹. In 3T3-L1 adipocytes, siRNA knockdown of GPR120 reduced expression of insulin signaling genes⁵². Highlighting its role as an LC n-3 PUFA receptor, GPR120 deficiency blocks the benefits of LC n-3 PUFA supplementation to HFD in mice, agonists to GPR120 mimic effects of DHA to block LPS-induced NFκB activation in macrophages, and knockdown of GPR120 abolishes DHA effects⁴⁹. Whether GPR120 deficiency modulates EPA effects is not reported, notably anti-inflammatory effects of EPA occur via distinct changes in plasma cell membranes in immune cells⁵³.

LC n-3 PUFA may affect the oral microbiome

Periodontal disease has been associated with increased risk of cardiometabolic diseases^{54,55}. There is data that suggests that dietary fat intake, including omega-3 fatty acids affect the composition of the intestinal and oral microbiome^{56,57}. The direct effect of LC n-3 PUFA supplementation on oral microbiome composition and the relationship between inflammatory chemokine pathways and the microbiome have not previously been evaluated.

Overall LC n-3 PUFA modulate chemokine signaling including adipocyte and macrophage inflammation, ATM infiltration, and monocyte activation; these actions may be mediated, at least partially, via GPR120 and NFκB. However, no human studies examining the effect of LC n-3 PUFA on the CCL2 and CX3CL1 adipochemokine pathways have been reported and the role of GPR120 and NFκB on these pathways has not been explored. Insight into LC n-3 PUFA action on these functional pathways may clarify mechanisms and guide therapeutic developments in human.

We propose a clinical trial to assess the validity of LC n-3 PUFA as an adjuvant treatment in prevention of obesity-related adipose inflammation and metabolic disturbance. While clinical studies of LC n-3 PUFA show decreased systemic inflammation, and animal models demonstrate decreased adipose inflammation, the effect of LC n-3 PUFA on *human* adipose inflammation chemokine signaling and GPR120/NFκB activity in obesity have not been explored. We will utilize a double-blind placebo-controlled trial of the LC n-3 PUFA fish oil Lovaza from GlaxoSmithKline (omega-3-acid ethyl esters; a combination of ethyl esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) in obese non-diabetic adults, to determine if LC n-3 PUFA decreases adipose CCL2-CCR2 and CX3CL1-CX3CR1, alters circulating monocyte and ATM phenotype, and stimulates GPR120 signaling and NFκB attenuation. We will explore the relationship between the oral microbiome and metabolic and inflammatory parameters at baseline, as well as the effect of LC n-3 PUFA supplementation on the oral microbiome composition.

We have chosen Lovaza at 4 grams/day (1860 mg EPA and 1500 mg DHA) for 8 weeks based upon anti-inflammatory effects noted in our preliminary data. A relatively young and healthy population was chosen to remove risk of chronic disease and medication use, yet subjects will be obese to enrich for low grade chronic adipose and systemic inflammation where LC n-3 PUFA are expected to have greater impact. Our previous work shows elevated CX3CL1 protein levels in obese adipose¹⁵, while elevated CCL2 and macrophage infiltration with obesity is established^{13-15,58}.

3.0 STUDY OBJECTIVES

Hypotheses

Primary

1. Omega-3 fatty acid supplementation for eight weeks reduces systemic chemokine signaling (plasma levels of CX3CL1).

Secondary

1. Omega-3 fatty acid supplementation will attenuate systemic and adipose tissue inflammation and leukocyte activation.
 - a. Additional circulating inflammatory chemokines and cytokines levels e.g., IL-6, MCP-1, CCL5, TNFalpha
 - b. Circulating monocyte subpopulations (ratio of inflammatory CX3CR1^{low}CCR2⁺ to less inflammatory CX3CR1^{hi}CCR2⁻)
 - c. Protein levels and mRNA expression of adipose tissue inflammatory chemokines and cytokines (CX3CL1, MCP-1, IL6, TNFalpha, CCL5)
 - d. Adipose tissue macrophage phenotype (ratio of inflammatory M1 to regulatory M2 macrophages, relative T-cell quantification)
2. Changes in chemokine signaling will correlate with improvements in metabolic parameters
 - a. Decreases in plasma lipids
 - b. Decreases in fasting glucose, insulin and homeostasis model assessment for IR (HOMA-IR), a marker of insulin resistance

Exploratory

1. Omega-3 fatty acid supplementation will decrease indirect markers of GPR120 and NFκB action. Adipose protein levels of IκB and total/phospho-TAK1 will be assessed as well as GPR120 mRNA expression and protein levels will also be quantified,

2. Any effects seen on GPR120 and NF κ B action will be correlated to serum and urine DHA and EPA levels.
3. Omega-3 fatty acid supplementation will cause changes in oral microbiome (bacterial DNA composition).
4. Baseline composition and treatment-related change in oral microbiome composition will be correlated to dietary history (as recorded in dietary recall and food frequency questionnaire) as well as to metabolic parameters
5. Subject's genotype at GPR120 and other loci involved in omega-3 action, inflammatory signaling, and nutrient sensing will affect response to treatment.

Objectives: Compared to placebo, we will examine the effect of omega-3 fish oil supplementation in obese subjects on:

Primary:

- 1) Plasma levels of CX3CL1

Secondary:

- 2) Additional plasma cytokines and chemokines
- 3) Circulating monocyte subpopulations
- 4) Adipose tissue cytokine and chemokine levels
- 5) Adipose tissue macrophage subpopulations
- 6) Plasma lipids, insulin, glucose, and HOMA-IR

Exploratory:

- 7) Adipose protein levels of I κ B and total/phospho-TAK1
- 8) GPR120 mRNA expression and protein levels
- 9) serum and urine DHA and EPA levels
- 10) Oral microbiome bacterial DNA composition
- 11) GPR120 genotype

4.0 STUDY DESIGN AND RATIONALE

We propose to conduct a parallel arm, double blind, placebo-controlled trial to understand the anti-inflammatory and metabolic effects of marine derived omega-3 fatty acids (EPA + DHA) on obesity-related inflammation and chemokine signaling in humans. Obese (BMI > 30 kg/m²) but non-diabetic young males and females (n ~ 44; 25 to 50 years of age) taking no omega-3 fatty acid (EPA + DHA) supplements and consuming a low fish diet (defined as usual intake of high omega-3 fish (tuna and other non-fried fish) < 3 to 4 servings per month) will be included. A low habitual fish intake is important to control EPA + DHA intake during the supplement period (see exclusion criteria below). Subjects also will be required to maintain their current fish intake throughout the treatment period.

There will be two treatment groups; (1) placebo and (2) omega-3 fatty acids 4 grams/day. The trial will be randomized, double blind, placebo-controlled, with parallel treatment groups; treatment with omega-3 fatty acids 8 weeks. We will enroll participants until at least 22 subjects per study-arm complete all study visits (i.e., at least 44

participants).

4.1 Drug, Dose Selection and Duration

Prescription Lovaza (omega-3-acid ethyl esters) from GlaxoSmithKline at 4 grams day (2 1-gram capsules twice daily, with food) will be used. We expect this dose to be safe because it is a dose that is recommended, and routinely used, for management of moderate or worse hypertriglyceridemia (levels ≥ 400 mg/dL). We will use matching placebo capsules so that all participants are taking 2 capsules twice daily – with food as recommended in clinical practice.

The study duration was chosen in order to provide adequate time for potential anti-inflammatory effects of treatments without requiring maximum lipid modifying effects while also reducing the potential for drop-out and loss to follow up that is associated with longer study durations.

5.0 SUBJECT POPULATION

5.1 Number of Subjects

We will enroll 22 subjects per arm for a total of 44 subjects completing. Based on prior experience with similar studies we anticipate a drop-out/failure to adhere rate of $\leq 20\%$. Therefore, we estimate that we will randomize ~52 (~26 per arm) subjects to reach our desired sample size.

5.2 Source of Subjects

Participants will be recruited from the Preventive Cardiology Research databases, comprised of healthy subjects who have participated in previous research studies and who have asked to be contacted for future studies. Potential participants will also be recruited via local IRB-approved flyers, internet, and newspaper advertisements (see flyer and advertisement attached) targeting the Delaware Valley region.

5.3 Inclusion Criteria

To be eligible for enrollment in this study, participants must meet all of the following criteria:

1. Men and non-pregnant/lactating women between the ages of 18 and 50.
2. BMI ≥ 30 kg/m²
3. Participants who are able to give written informed consent and willing to comply with all study-related procedures.

5.4 Exclusion criteria

1. Diabetes Mellitus (glucose fasting >126, or random >200, or use of any anti-diabetic agent)
2. Self-reported fish or shellfish allergy
3. Planned usage of any prescription or non-prescription anti-inflammatory, antibiotic, glucose-lowering, lipid modulating or otherwise relevant medication during the study period.
4. Recent (within 6 months) use of LC n-3 PUFA or fish oil supplements or self-reported dietary intake of >3 servings of fish/month
5. Blood pressure >140/90
6. Recent (within 6 months) use of statins, niacin, or fenofibrates
7. Current or planned pregnancy/lactation. Pre-menopausal women unwilling to prevent pregnancy by use of the following approved contraceptive strategies: diaphragm, cervical cap, condom with spermicide, surgical sterility, birth control pills, Depo-Provera injection, IUD, progestin implant, or abstinence.
8. History of liver disease or abnormal LFTs (AST, ALT, Alk. Phos; bilirubin > 2x ULN) at Screening Visit
9. Men who are unwilling to limit alcohol consumption to < 14 alcoholic drinks per week or < 4 alcoholic drinks per occasion (AMA / NIAAA criteria for “at risk” usage levels) while participating in the study
10. Women who are unwilling to limit alcohol consumption to < 7 alcoholic drinks per week or < 3 alcoholic drinks per occasion (AMA / NIAAA criteria for “at risk” usage levels) while participating in the study.
11. Hemoglobin less than 11.0 g/dL
12. Any reported arrhythmia, usage of anti-arrhythmic therapy, or abnormal screening electrocardiogram
13. Known bleeding disorder or coagulopathy
14. Any major active rheumatologic, pulmonary, hematologic or dermatologic disease or inflammatory condition or minor active infection
15. Self-reported history of HIV positive
16. Patients who have undergone any organ transplant

17. Individuals who currently use tobacco products or have done so in the previous 30 days
18. Treatment with aspirin, NSAIDs, COX-2 inhibitors, steroids or any immunomodulatory therapy 2 weeks prior to the Screening Visit
19. Participants who are unwilling to eliminate omega-3 fatty acid (EPA + DHA) supplements and/or fortified food, or have their usual intake of high omega-3 fish (tuna and other non-fried fish) be > 3 to 4 servings per month as assessed by a simple screening questionnaire
20. Recent (within 6 months) treatment with coumarin-type anticoagulants
21. Positive urine pregnancy test result.
22. Self-reported history of injected recreational drug use.
23. Any medical condition or abnormal laboratory value that is judged clinically significant by an investigator

6.0 STUDY PROCEDURES

6.1 Informed Consent

Before study initiation, the clinical protocol, the Informed Consent Form (ICF), and any advertisements for subject recruitment will be submitted for review and approval to the Institutional Review Board (IRB). The investigator, or his designated research staff, will obtain written informed consent from each subject enrolled in the study, in accordance with the U.S. Food and Drug Administration (FDA) regulations 21 CFR parts 50.20-50.27. It is the responsibility of the investigator to ensure that informed consent is obtained from the subject or his/her guardian or legal representative before any activity or treatment is undertaken which is not part of routine care.

6.2 Subject Identification Code

All subjects who sign an ICF will be assigned a subject code. The code assigned will not contain any identifying information. The subject code will be used to identify the subject for the rest of the study.

6.3 Safety Assessments

Safety will be evaluated by the incidence, severity, and relationship to treatment agent of adverse events.

6.3.1 Monitoring for Adverse Events: Details of all AEs that occur after the first dose of study drug will be collected as indicated in Section 12. Any illness or injury that occurs

before the first dose of a study drug will be recorded under Medical History and evaluated to determine if the occurrence affects the patient's eligibility to participate in the study. Subjects will be asked the following standard questions by the designated clinical evaluator:

At clinic check-ins:

1. "Have you had any medical problems since your last visit?"
2. "Have any medical problems present at your last visit changed, i.e., stopped, worsened, or improved?"
3. "Have you taken any medicines, other than study drug, since your last visit?"

Any spontaneous AE information provided by the patient will be recorded.

NOTE: An AE may be a new disease(s), any untoward event(s), or an exacerbation of a pre-existing condition. See Section 12.0 for complete details.

6.4 Adipose biopsy:

Approximately 500-1000 mg of subcutaneous adipose tissue will be biopsied from the gluteal region, processed, weighed and frozen until analyzed. Adipose tissue will be obtained through a small (~5mm) incision under local anesthesia (2% Lidocaine without epinephrine), as has been previously described¹³⁻¹⁵. A portion (200 mg) of the samples will be flash frozen and analyzed for gene expression and protein assays. Another portion (500-800 mg) will be placed in sterile media and undergo digestion with collagenase, filtration, and centrifugation for separation of the stromal vascular fraction (SVF). SVF cells will be stained with antibodies for flow cytometer analysis. Finally, remaining tissue will be fixed in paraffin for immunohistochemistry.

6.5 Subgingival analysis

Saliva samples will be collected using the OMNIgene DISCOVER (OM-505) kit (DNA Genotek), an all-in-one kit which collects and stabilizes both microbial DNA and RNA in saliva. Samples will be stored at -80C prior to extraction. Nucleic acids will be extracted using the QIAGEN QIAamp MinElute Virus Spin Kit (Qiagen). Subgingival plaque samples will be obtained from 3 oral cavity regions in each subject (2 samples per region) using a soft-bristled microbrush (DenTek). Specimens will be collected from the same anatomical location in all subjects. DNA will be isolated using the PSP Spin Stool DNA kit (Invitex). DNA samples will be amplified using V1-V2 region primers targeting bacterial 16S genes and sequenced using IlluminaHiSeq or 454 Pyrosequencing technology.

6.6 Specific Study Procedures (by visit)

A detailed study time-table and list of all procedures to be performed at each visit is included in Appendix A.

6.6.1 Screening (Visit 1)

Potential participants will be screened first by a telephone or e-mail interview with the

research coordinator (see Appendix B for email interview form; for telephone interviews this information will be verbally exchanged). Participants who meet initial study requirements will be invited for a screening visit (visit 1) at the CTRC. Prior to this visit, participants will receive a detailed health questionnaire (see Appendix C) that has been used in numerous previous studies that addresses questions regarding past medical history, current use of medications and dietary supplements, exercise and social habits. In addition, participants will be asked about fish consumption in order to ensure recruitment of subjects with lower EPA + DHA intake during the study period. Participants will be asked to complete these questionnaires at home and bring the completed forms with them to their screening visit. During the screening visit, they will meet with research staff to review and sign an IRB-approved consent form. Research staff will review the health questionnaire with the participant as well as measure weight, height, waist and hip circumference, sitting blood pressure and heart rate. A brief physical exam will be performed as well as an electrocardiogram (ECG). In addition, participants will have blood drawn (after a 12 hour fast) for evaluating fasting lipids, glucose, serum chemistries and liver function and a complete blood count. Female participants will also be asked to provide a urine sample for a pregnancy test. These laboratory parameters are included to assure the participant meets eligibility criteria and that he/she is generally in good health. Once all clinical data has been reviewed, study personnel will contact each study volunteer to let them know if they are eligible.

6.5.2 Randomization visit (Visit 2)

One to four weeks after the screening Visit 1, eligible participants will return to the CTRC after a 12 hour fast for a 2 hour visit. Participants will be asked to brush their teeth after their last meal the day before the visit, or at the start of the 12-hour fasting period, and to refrain from additional brushing or use of mouthwash until after the study visit. Participants will meet with a research nurse / coordinator who will assess any change in medical history and medication use, as well as review any adverse events. Blood pressure, heart rate, height and weight will be measured, blood and urine will be collected for pre-randomization baseline efficacy measures and a urine pregnancy test performed for all females. The simple fish consumption questionnaire will be administered to assess any changes to the level of omega-3 intake in the diet.

They will also be asked to record all food and beverages consumed on dietary records on 3 days prior to the visit, as well as completing a digestive health questionnaire and diet history questionnaire. All nutrient data collected from 3-day dietary records will be analyzed by Food Processor 8.1 (06/03, Escha). Participants will be asked to complete these questionnaires at home and bring the completed forms with them to their study visit. If a subject is unable to print out or complete the forms at home, they will be completed at the visit.

Saliva and subgingival plaque samples will be taken for assessment of oral microbiome. Saliva samples will be collected using a standardized kit. Under the supervision of study personnel, subjects will be given the kit, which consists of a funnel attached to a tube, and instructed to spit into the funnel until the amount of liquid reaches a fill line located on the outside of the attached tube. Once the proper amount has been collected, the lid of the funnel will be closed and the funnel removed from the tube. The tube is then

capped and shaken for about 10 seconds. Subgingival plaque samples will be collected by swabbing several regions of the buccal mucosa using microbrushes. A separate brush will be used for each swab and two samples will be collected from each region.

In order to establish effects of treatments on adipose inflammatory parameters, we will obtain gluteal region subcutaneous adipose tissue biopsies under local anesthesia (2% Lidocaine). A detailed description of the adipose collection procedures is given in Section 6.4. Participants will then be randomized to drug or placebo group. Supplies of study drug will be provided to last 8 weeks (4 pills/day of either Lovaza or placebo).

Participants will be provided verbal and written instruction to take the study drugs as follows; 2 fish oil/placebo with food in the morning, 2 fish oil/placebo with food in the evening. A pill diary will be given to all subjects.

6.5.3 Telephone / E-mail Follow up (Visit 3)

Participants will be contacted by telephone or e-mail approximately four weeks (visit 3) post-randomization to review medication compliance and pill diary, adverse events and any changes in fish consumption. Participants with <80% apparent adherence to study medication will be counseled to increase compliance so that their adherence at 8 weeks will be $\geq 80\%$.

6.5.4 Completion Visit (Visit 4)

Participants will come to the CTCRC after a 12 hour fast. Participants will meet with a study coordinator who will assess changes to the medical history and medication use. They will also be asked to record all food and beverages consumed on dietary records on 3 days prior to the visit. Any adverse events will be recorded, pill diary reviewed, and study medications will be counted (pill count) Weight and height will be recorded and BMI calculated. ECG will be performed. Blood will be drawn for post-drug screening of liver functions and chemistries as well as for all outcome-related measures (lipids, insulin/glucose, inflammatory markers). Urine for efficacy measures will also be collected. Urine pregnancy test will be performed in all females. Saliva and subgingival plaque samples will be taken to assess for any change in oral microbiome by the treatment. An adipose biopsy will be performed as per Section 6.4.

6.6 Study Discontinuation/Completion

A subject may be withdrawn from the study at any time at either the investigator's discretion or the subject's request. An effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study. The primary reason for discontinuing participation in the study must be stated and may include, but is not limited to, one of the following:

- ◆ Development of any abnormality in blood counts, blood chemistries, or urinalysis because of which the investigator deems continued participation in the study to be ill-advised
- ◆ Use of unapproved concomitant medications

- ◆ Occurrence of intolerable AEs
- ◆ Development of LFT abnormalities (>1.5 ULN), unexplained muscle aches/myopathy, kidney dysfunction (Cr>ULN), or significant bleeding (e.g. GI bleed, cerebral hemorrhage, major ENT bleed).
- ◆ Withdrawal of consent by patient
- ◆ Noncompliance with protocol, e.g., the patient fails to appear at one or more visits
- ◆ Non adherence with study medications – defined as failure to take >80% of study medications (pill count and diaries)
- ◆ Development of an intercurrent illness, injury, or medical condition likely to interfere with patient safety, the overall assessment, or the required administration of study medication
- ◆ Development of any condition for which the investigator feels treatment withdrawal is justified
- ◆ Termination of the study by the sponsor (NIH)

The procedure to discontinue a subject from the study is as follows:

- ◆ record any AEs since previous visit
- ◆ record any medications
- ◆ record vital signs
- ◆ perform a physical examination (if discontinuation is due to an AE)
- ◆ collect blood for clinical laboratory analysis

Patients who discontinue from the study because of an AE(s) should be treated and followed according to established medical practice.

The study may also be discontinued at the discretion of the Principal Investigator, if any safety concerns are identified during the course of the study.

7.0 SAFETY MEASURES

A detailed description of all safety related issues including the risks of study, participant monitoring, adverse event data collection and reporting is provided in a formal Safety Plan (Appendix D) developed also for our Data Safety and Monitoring Board (DSMB). Therefore, in this section we briefly describe the safety measures that we will record and measure during the execution of the study. Additional safety-related details are provided in the Safety Plan.

7.1 Lovaza-study related safety measures

The most common reported side effects with Lovaza are burping, infection, flu symptoms, upset stomach, change in sense of taste, back pain and skin rash. We will monitor for development of these side effects at follow-up Visit 3 and Visit 4 and volunteers will be encouraged to contact the study coordinator or PI if these side effects

occur or become bothersome. There is a risk of an increase in alanine aminotransferase (ALT) and/or low-density lipoprotein cholesterol (LDL-C) levels; therefore we will be monitoring participants' ALT and LDC-C levels at Visit 2 (baseline) and at study completion. There is a possible association between Lovaza and more frequent recurrences of symptomatic atrial fibrillation or flutter in patients with paroxysmal or persistent atrial fibrillation. Thus there will be an ECG at visit 1 and subjects with arrhythmias will be excluded. ECG will also be done at end of study to assess of any potential induction of arrhythmias by use of study drug. Subjects will be discontinued as described above in Section 6.6.

If any of the above responses occur, the specific AE will be assessed with the circumstance of the occurrence, and discussed with the DSMB, NIH, and University of Pennsylvania IRB in order to outline a response / plan that accounts for this occurrence. Once the AE has been assessed, dosing will resume according to protocol.

8.0 DATA COLLECTION AND ANALYSIS

8.1 Source Documents & Case Report Forms

All primary primary records, such as original copies of signed consent forms, will be stored in a locked filing cabinet and will only be available to study investigators. The documents will be stored in files labeled only with a unique research identification number. Electronic copies of these documents will be created and stored within the REDCap database. These primary records will not be available to any future recipients of registry-repository data without separate IRB approval.

8.2 Computer Systems

All electronic data will be managed and stored using the research-focused electronic data capture system REDCap, under an agreement with the software's development consortium, led by Vanderbilt University. REDCap supports two secure, web-based applications designed exclusively to support data capture for research studies. REDCap is a PHP web application served by Apache Tomcat over a 128 bit SSL connection using a signed certificate. The application relies on a study-specific data dictionary defined in an iterative self-documenting process that will be conducted by all members of the research team. The data dictionary is the foundation for custom case report form design and validated coding of variables. Authentication of research staff will be performed via LDAP using CHOP's enterprise Active Directory service. The application generates a complete audit trail of user activity, provides reporting, and has an automated export mechanism to common statistical packages (SAS, SPSS, Stata, R/S-Plus).

The REDCap MySQL database is replicated in real time to a completely redundant instance of MySQL. The redundant instance is available for restoration of the primary database or for manual failover in the case of primary database failure. Time-stamped backup files are made from the replicated database daily by CHOP Research Information Systems using automated backup routines. Backup files are encrypted and transferred to a secure file server accessible only to designated personnel. A rolling seven-day window

of backup files is maintained in an immediately available online state, with a larger window maintained in a compressed file archive available at a reduced speed of access. Daily destructive database backup files are stored on the database server and are deleted only after successful backup of the entire database to file. In the event of data error, loss or corruption, Research personnel will work with CHOP Research Information Systems to determine the most appropriate recovery strategy.

Data and backups are stored in the CHOP Research Information Systems Storage Area Network (SAN). Access to the SAN directories where data are stored will be limited to Research Information Systems personnel, with authentication performed using CHOP's enterprise Active Directory service.

REDCap's extensive security features will be taken advantage of to limit PHI, and any links between this PHI and all other data, to only the study investigators listed on this IRB protocol. All other users, particularly future recipients of data output from the registry-repository, will only have access to data tagged with a unique research ID number.

9.0 STATISTICAL METHODS:

9.1 Sample size and Power computation

Sample size is based on the primary goal of detecting a 26% reduction in plasma CX3CL1 levels in drug treated participants compared to placebo. We believe that such a difference in CX3CL1 levels represents a clinically important difference with biological implications in setting of obesity, metabolic syndrome and type 2 diabetes. We have chosen TNF α as the primary response variable for the following reasons; (1xxx

The calculations were performed using our data establishing expected CX3CL1 levels in a healthy sample (0.58 +/- 0.17 ng/mL in recent FFAME cohort; n=80). Using a two-sample Wilcoxon test, for 80% power and $\alpha=0.05$, 22 subjects per group will allow detection of an effect size of 0.9σ difference in plasma CX3CL1 levels between Lovaza and placebo (0.14 ng/mL difference—26%, using mean/SD above). Allowing for 20% dropout, 26 subjects per group will be required.

9.2 Statistical Analyses

Analysis will be performed by Dr Shah with support from Penn Statistics faculty with expertise in human translational research (Dr. ProPERT). In the human trial, the primary endpoint will be compared between groups using two-sample Wilcoxon test, with graphical explorations and repeated measured ANOVA to explore the shape of changes over time. Correlations between circulating and adipose CX3CL1/CCL2 and monocyte/macrophage markers, systemic inflammatory markers and metabolic parameters will utilize Spearman's correlation. Differences among conditions in cell studies will be analyzed by ANOVA. All analysis will use STATA 11 (StataCorp).

All secondary/exploratory variables will be assessed as independent responses because they may reflect independent effects of the treatment. The multiple testing of non independent responses may result in type I error and false positive findings. Therefore, any statistically significant differences will be interpreted in terms of the overall significance level, considering multiple comparisons, and ultimately in the context of the clinical significance of the difference.

10.0 STUDY AGENTS

10.1 Preparation and Labeling

The Investigational Drug Service (IDS) pharmacist will ensure that all study drugs are stored in a secured area, under recommended storage conditions (below 86° F and protected from moisture and freezing) in accordance with applicable regulatory requirements and will be dispensed by qualified staff members. The pharmacist will maintain accurate records regarding study drug administration and return.

10.2 Omega-3 fish oil formulation

Prescription grade omega-3 fish oil supplementation will be used in the form of oral Lovaza (omega-3-acid ethyl esters) manufactured by GlaxoSmithKline. Subjects will be randomized to a dosage of either 1 or 4 capsules daily. We will use matching placebo capsules provided by the manufacturer so that all participants are taking 2 capsules twice daily – with food as recommended in clinical practice.

10.3 Study Agent Compliance

Study drug adherence will be monitored by pill count and medication diary and will be assessed by telephone/email contact at 4 weeks after randomization and in person at Visit 4 (8 weeks after randomization). The final analysis of compliance will be performed at this time.

11.0 POTENTIAL RISKS

A complete description of all study related risks is also provided in the Safety Plan (Appendix D). Briefly, risks include (1) general study procedure-related risks (e.g., blood draws, intravenous lines, adipose biopsies), (2) Lovaza related risks [e.g., increase in alanine aminotransferase (ALT) and/or low-density lipoprotein cholesterol (LDL-C) levels], and (3) risks from clinical or laboratory abnormalities that emerge during the study. The process of monitoring the study is outlined in the Safety Plan for proposed Data Safety and Monitoring Board (DSMB) (Appendix D). Additional safety-related details are provided in the Safety Plan

12.0 POTENTIAL BENEFIT OF THE PROPOSED RESEARCH

There is no direct benefit to the participant of this study; however data obtained from this study may be very useful in understanding the role of omega-3 fatty acids on obesity-related inflammatory and metabolic complications. Subjects on study drug may have

temporary improvement in triglyceride levels while in the study. Clinical data relating to general health and risk for metabolic disease (diabetes, cholesterol) will be provided to them and their primary physician if they so wish.

13.0 ADVERSE EVENTS

A complete description of all study related risks, adverse events, adverse event definitions, recording and reporting is provided in the Safety Plan (Appendix D for proposed Data Safety and Monitoring Board (DSMB)). Key aspects are summarized below.

13.1 Non-serious Adverse Events

All other observed or volunteered adverse events regardless of treatment group or suspected causal relationship to study drug will be recorded on the adverse event source document and transcribed onto the case report form. Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of pre-existing illnesses will be recorded. In addition, abnormal objective test findings (e.g., electrocardiogram changes, abnormal laboratory test results) that result in a change in study drug dosage or in discontinuation of the drug, or require intervention or diagnostic evaluation to assess the risk to the patient/participant, will be recorded as adverse events. Clinically significant changes in physical examination findings will also be recorded as adverse events.

Thus, any AE that is not designated as Serious, but is not expected as defined in appendix D, must be recorded on the non-serious AE page of the CRF.

13.2 Serious Adverse Event

All serious adverse events (as defined below) regardless of treatment group or suspected relationship to study drug will be reported to the DSMB, NIH, FDA and University of Pennsylvania IRB as outlined by the Penn Manual for Clinical Research (Office of Human Research).

A serious adverse event (SAE) is any AE that:

- ◆ Results in death
- ◆ Is life-threatening (at immediate risk of death from the event as it occurred)
- ◆ Requires inpatient hospitalization (overnight stay) or prolongs a current hospitalization
- ◆ Causes a persistent or significant disability/incapacity
- ◆ Is a congenital anomaly/birth defect in the offspring of a patient who received test article

And may be any event that:

- ◆ Requires intervention to prevent one of the outcomes listed above

The investigator should exercise medical and scientific judgment when deciding whether expedited reporting is appropriate in other situations not strictly meeting the listed criteria above.

13.3 Intensity

The investigator is responsible for ensuring that AEs are documented on the appropriate page of the CRF according to the following descriptors:

Mild: associated with no limitation of usual activities or only slight discomfort

Moderate: associated with limitation of usual activities or significant discomfort

Severe: associated with inability to carry out usual activities or very marked discomfort

13.4 Relationship to Test Article

The relationship of AEs to study drug will be assigned by the investigator according to the following definitions:

Definitely: a reaction that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the suspected study drug; and that cannot be reasonably explained by the known characteristics of that subject/patient's clinical state.

Probable: a reaction that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the suspected study drug; and that is unlikely explained by the known characteristics of that subject/patient's clinical state.

Possible: a reaction that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the suspected study drug; but that could readily have been produced by a number of other factors.

Unlikely: a reaction that does not follow a reasonable temporal sequence from administration of the test article. However, causality from the test article cannot be ruled out.

None: a reaction for which sufficient data exist to indicate that the etiology is unrelated to the study drug.

13.5 Pretreatment-Emergent Events

Events that occur between the time the informed consent document is signed for the study and the time when the patient is first exposed to study drug as “pretreatment-emergent” events. These pretreatment-emergent events will not be collected as adverse events, unless the event increases in intensity or frequency after study article administration.

13.6 Recording and Documenting AEs and SAEs

The investigator or study team member must completely and promptly record each AE in the database on the AE record, even if the relationship of an AE to the study drug is assessed by the investigator to be “unlikely” or “none”. The investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms.

14.0 INVESTIGATOR OBLIGATIONS

This section reviews each investigator’s obligations according to federal regulations.

14.1 Adverse Event Reporting Obligations

The investigator is responsible for recording AE’s reported by the subject or discovered by any other means during the study. In addition, AE’s classified as “serious” must be reported to the DSMB, NIH, and University of Pennsylvania IRB as outlined by the Penn Manual for Clinical Research (Office of Human Research).

14.2 Study Records and Source Documents

Federal regulations and The University of Pennsylvania policy require that, following completion of a clinical study, a copy of all records of that study be maintained by the Principal Investigator. Completed original CRFs and clarification queries, dated, and signed by the investigator, will be kept at the study site beyond locked door and key. Hard copies of data forms will also be kept on file at The University of Pennsylvania or its designee.

In order to assure the accuracy of data collected in the CRFs, it is mandatory that The University of Pennsylvania or its designees, as well as representatives of the FDA or other pertinent regulatory agencies, have access to original source documents (e.g., patient records, patient charts, laboratory reports). During the review of these documents, the anonymity of the patient will be respected with strict adherence to professional standards of confidentiality.

The University of Pennsylvania reserves the right to terminate the study for refusal of the investigator to supply source documentation of work performed in this clinical study.

15.0 REFERENCES

1. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007;117:175-84.
2. Fujisaka S, Usui I, Bukhari A, et al. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes* 2009;58:2574-82.
3. Aron-Wisnewsky J, Tordjman J, Poitou C, et al. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. *J Clin Endocrinol Metab* 2009;94:4619-23.
4. Weisberg SP, Hunter D, Huber R, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 2006;116:115-24.
5. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab* 2008;8:301-9.
6. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 2008;57:3239-46.
7. Duffaut C, Zakaroff-Girard A, Bourlier V, et al. Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. *Arterioscler Thromb Vasc Biol* 2009;29:1608-14.
8. Westcott DJ, Delproposto JB, Geletka LM, et al. MGL1 promotes adipose tissue inflammation and insulin resistance by regulating 7/4hi monocytes in obesity. *J Exp Med* 2009;206:3143-56.
9. Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nature medicine* 2009;15:930-9.
10. Lumeng CN, Maillard I, Saltiel AR. T-ing up inflammation in fat. *Nature medicine* 2009;15:846-7.
11. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature medicine* 2009;15:914-20.
12. Winer S, Chan Y, Paltser G, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nature medicine* 2009;15:921-9.

13. Ito A, Suganami T, Yamauchi A, et al. Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. *J Biol Chem* 2008;283:35715-23.
14. Chen A, Mumick S, Zhang C, et al. Diet induction of monocyte chemoattractant protein-1 and its impact on obesity. *Obes Res* 2005;13:1311-20.
15. Shah R, Hinkle CC, Ferguson JF, et al. Fractalkine is a novel human adipochemokine associated with type 2 diabetes. *Diabetes* 2011;60:1512-8.
16. Tacke F, Randolph GJ. Migratory fate and differentiation of blood monocyte subsets. *Immunobiology* 2006;211:609-18.
17. Puglisi MJ, Hasty AH, Saraswathi V. The role of adipose tissue in mediating the beneficial effects of dietary fish oil. *J Nutr Biochem* 2011;22:101-8.
18. Mohammadi E, Rafrat M, Farzadi L, Asghari-Jafarabadi M, Sabour S. Effects of omega-3 fatty acids supplementation on serum adiponectin levels and some metabolic risk factors in women with polycystic ovary syndrome. *Asia Pac J Clin Nutr*;21:511-8.
19. Zhou Y, Tian C, Jia C. Association of fish and n-3 fatty acid intake with the risk of type 2 diabetes: a meta-analysis of prospective studies. *Br J Nutr*;108:408-17.
20. Siegel G, Ermilov E. Omega-3 fatty acids: Benefits for cardio-cerebro-vascular diseases. *Atherosclerosis*.
21. Sasaki J, Yokoyama M, Matsuzaki M, et al. Relationship between coronary artery disease and non-HDL-C, and effect of highly purified EPA on the risk of coronary artery disease in hypercholesterolemic patients treated with statins: sub-analysis of the Japan EPA Lipid Intervention Study (JELIS). *J Atheroscler Thromb* 2012;19:194-204.
22. Saito Y, Yokoyama M, Origasa H, et al. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis* 2008;200:135-40.
23. Oikawa S, Yokoyama M, Origasa H, et al. Suppressive effect of EPA on the incidence of coronary events in hypercholesterolemia with impaired glucose metabolism: Sub-analysis of the Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis* 2009;206:535-9.
24. Marchioli R, Schweiger C, Tavazzi L, Valagussa F. Efficacy of n-3 polyunsaturated fatty acids after myocardial infarction: results of GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto

Miocardico. Lipids 2001;36 Suppl:S119-26.

25. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999;354:447-55.

26. Bosch J, Gerstein HC, Dagenais GR, et al. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. N Engl J Med;367:309-18.

27. Calder PC, Yaqoob P. Marine omega-3 fatty acids and coronary heart disease. Curr Opin Cardiol;27:412-9.

28. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology? Br J Clin Pharmacol.

29. Mayser P, Mrowietz U, Arenberger P, et al. Omega-3 fatty acid-based lipid infusion in patients with chronic plaque psoriasis: results of a double-blind, randomized, placebo-controlled, multicenter trial. J Am Acad Dermatol 1998;38:539-47.

30. Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. A 12-month, double-blind, controlled study. Arthritis Rheum 1994;37:824-9.

31. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr 1997;65:1011-7.

32. Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med 1996;334:1557-60.

33. Sijben JW, Calder PC. Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. Proc Nutr Soc 2007;66:237-59.

34. Dangardt F, Osika W, Chen Y, et al. Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents. Atherosclerosis 2010;212:580-5.

35. Vedin I, Cederholm T, Freund Levi Y, et al. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. The American journal of clinical nutrition 2008;87:1616-22.

36. Luu NT, Madden J, Calder PC, et al. Dietary supplementation with fish oil modifies the ability of human monocytes to induce an inflammatory response. J

Nutr 2007;137:2769-74.

37. Bouwens M, van de Rest O, Dellschaft N, et al. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. *The American journal of clinical nutrition* 2009;90:415-24.
38. Madden J, Shearman CP, Dunn RL, et al. Altered monocyte CD44 expression in peripheral arterial disease is corrected by fish oil supplementation. *Nutr Metab Cardiovasc Dis* 2009;19:247-52.
39. Mullen A, Loscher CE, Roche HM. Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages. *J Nutr Biochem* 2010;21:444-50.
40. Brown AL, Zhu X, Rong S, et al. Omega-3 fatty acids ameliorate atherosclerosis by favorably altering monocyte subsets and limiting monocyte recruitment to aortic lesions. *Arterioscler Thromb Vasc Biol*;32:2122-30.
41. Todoric J, Loffler M, Huber J, et al. Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. *Diabetologia* 2006;49:2109-19.
42. Baranowski M, Enns J, Blewett H, Yakandawala U, Zahradka P, Taylor CG. Dietary flaxseed oil reduces adipocyte size, adipose monocyte chemoattractant protein-1 levels and T-cell infiltration in obese, insulin-resistant rats. *Cytokine*;59:382-91.
43. Titos E, Rius B, Gonzalez-Periz A, et al. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. *J Immunol*;187:5408-18.
44. Hensler M, Bardova K, Jilkova ZM, et al. The inhibition of fat cell proliferation by n-3 fatty acids in dietary obese mice. *Lipids Health Dis*;10:128.
45. White PJ, Arita M, Taguchi R, Kang JX, Marette A. Transgenic restoration of long-chain n-3 fatty acids in insulin target tissues improves resolution capacity and alleviates obesity-linked inflammation and insulin resistance in high-fat-fed mice. *Diabetes* 2010;59:3066-73.
46. Kanarek N, Ben-Neriah Y. Regulation of NF-kappaB by ubiquitination and degradation of the I kappa Bs. *Immunol Rev*;246:77-94.
47. Novak TE, Babcock TA, Jho DH, Helton WS, Espat NJ. NF-kappa B inhibition by omega -3 fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L84-9.

48. Draper E, Reynolds CM, Canavan M, Mills KH, Loscher CE, Roche HM. Omega-3 fatty acids attenuate dendritic cell function via NF-kappaB independent of PPARgamma. *J Nutr Biochem*;22:784-90.
49. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*;142:687-98.
50. Oh DY, Olefsky JM. Omega 3 fatty acids and GPR120. *Cell Metab*;15:564-5.
51. Ichimura A, Hirasawa A, Poulain-Godefroy O, et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature*;483:350-4.
52. Liu D, Wang L, Meng Q, Kuang H, Liu X. G-protein coupled receptor 120 is involved in glucose metabolism in fat cells. *Cell Mol Biol (Noisy-le-grand)*;Suppl.58:OL1757-62.
53. McMurray DN, Bonilla DL, Chapkin RS. n-3 Fatty acids uniquely affect anti-microbial resistance and immune cell plasma membrane organization. *Chem Phys Lipids*;164:626-35.
54. Mattila KJ, Nieminen MS, Valtonen VV, et al. Association between dental health and acute myocardial infarction. *BMJ (Clinical research ed)* 1989;298:779-81.
55. Zeigler CC, Persson GR, Wondimu B, Marcus C, Sobko T, Modeer T. Microbiota in the oral subgingival biofilm is associated with obesity in adolescence. *Obesity* 2012;20:157-64.
56. Myles IA, Pincus NB, Fontecilla NM, Datta SK. Effects of parental omega-3 Fatty Acid intake on offspring microbiome and immunity. *PloS one* 2014;9:e87181.
57. Tabbaa M, Golubic M, Roizen MF, Bernstein AM. Docosahexaenoic acid, inflammation, and bacterial dysbiosis in relation to periodontal disease, inflammatory bowel disease, and the metabolic syndrome. *Nutrients* 2013;5:3299-310.
58. Shah R, Lu Y, Hinkle CC, et al. Gene profiling of human adipose tissue during evoked inflammation in vivo. *Diabetes* 2009;58:2211-9.

APPENDIX A

Study Visits and Procedures

Procedure	Visit 1	Visit 2	Visit 3	Visit 4
	Screen	Randomization	Contact Follow-up	Completion
	Week -2 to -4	Week 0	Week 4	Week 7-9
Participant Questionnaire completion ¹	X			
Informed consent	X			
History & physical	X			
3 day diet record, Food History questionnaire		X	X	
Electrocardiogram	X			X
Blood pressure, heart rate, weight, height & waist measures	X	X		X
Glucose and insulin measurement	X			X
Urine pregnancy test ²	X	X		X
Review medications	X	X	X	X
Dispense study medication/placebo pills		X		
Comprehensive Metabolic Lab Panel ³	X			X
Complete blood count (CBC)	X			X
Lipid profile ⁴	X			X
Blood and urine collected for efficacy measures		X		X
Dietary assessment of high omega-3 fish consumption (questionnaire)	X	X	X	X
Saliva Collection		X		X
Adipose biopsy		X		X

- ¹ The questionnaire along with a copy of the consent form will be mailed to subjects for completion prior to the study visit in order to save time on the day of their screening visit. After consent is obtained, a coordinator will review the questionnaire with the participant for completeness.
- ² Standard urine pregnancy test performed on females of child-bearing potential only
- ³ Comprehensive Metabolic panel includes: sodium, potassium, chloride, carbon dioxide, glucose, blood urea nitrogen, creatinine, calcium, total protein, albumin, AST, ALT, alkaline phosphatase, and total bilirubin
- ⁴ Includes TC, LDL-C, VLDL, HDL-C, TG

APPENDIX B

Pre-screening E-mail Questionnaire

Hello,

Thank you for your interest in the FAIR study. We are looking for healthy volunteers to participate in a study to better understand the anti-inflammatory benefits of prescription fish oil supplements that are currently used to help people with cholesterol problems. If you qualify, your participation would last about 3 months during which time there are 3 visits to the Clinical and Translational Research Center (CTRC) at the Hospital of the University of Pennsylvania.

Visit 1 is an outpatient screening visit which is about 2 hours long.

Visit 2 is a baseline visit which lasts about 2 hours

Visit 3 is a follow-up visit conducted via e-mail and/or telephone (you do not need to come to the CTRC)

Visit 4 is a completion visit which lasts about 2 hours

Visits may include blood sampling, urine collection, blood pressure measurements, fat-tissue sampling, fasting and brief physical exams.

Volunteers who complete all study visits will be compensated \$180 for time and travel.

If you would like more information and to see if you may qualify, please answer the questions below and reply back to me. A member of our study team will be in touch with you as soon as possible.

- 1) Name:
- 2) Gender:
- 3) Phone Number:
- 4) Email:
- 5) Age:
- 6) How did you hear about the study?
- 7) Do you have a history of diabetes (type 1 or type 2) or are you on any medications to lower your blood sugar?

- 8) Do you have any history of abnormal heart rhythm or other heart problems?
 1. If yes, what are they?
- 9) Do you have any other medical conditions for which you are under a doctor's care?
 1. If yes, what are they?
- 10) What is your height?
- 11) What is your weight?
- 12) How often do you eat-- as a main course-- tuna or other non-fried fish? (One serving size is 3 oz = in size to a deck of cards. Note: One serving of non-fried fish could include: 1, 3 oz. filet of salmon OR 6 pieces of sushi OR 8 sushi rolls OR ~4 tuna fish (salad) sandwiches, etc.)
 - A) Once or less per month
 - B) 2-3 times per month
 - C) Once per week
 - D) Twice per week
 - E) More than twice per week
- 13) Have you taken any fish oil supplements in the past 3 months?
- 14) What medicines, including supplements (like Omega-3 or fish oil) and vitamins, do you currently take and how often do you take them?
 - 1.
 - 2.
 - 3.
 - 4.
 - 5.
- 15) Do you have any history of gall bladder disease?
- 16) Do you have any allergies to fish?
- 17) Do you currently smoke or use any other tobacco product (snuff, chew, dip, etc.)?
 1. If no, have you used tobacco in the last 30 days?
- 18) On average, how many days per week do you drink alcohol?
- 19) On a day when you drink alcohol, how many alcoholic drinks do you have?
- 20) When you drink alcohol, is it beer, wine or liquor?
- 21) How many ounces are in each drink?
- 22) Do you have high blood pressure?
- 23) Do you have a history in your immediate family of pre-mature heart disease or stroke (i.e., mother/sister before age 65 or father/brother before age 55)?
 1. If yes, who and at what age?
- 24) Have you been treated for cancer?
 1. If yes, are you still being treated?
 - i. If you are not still being treated, when was your last treatment?
- 25) Are you HIV + or do you have AIDS?
- 26) *Females only:* Are you currently pregnant or breast-feeding?

- 27) Are you currently enrolled in another research study?
1. If yes, when will your participation end?
- 28) Have you participated in a clinical research study within the last 6 weeks?
- 29) In terms of race, do you consider yourself a Caucasian, African American, Asian, Pacific Islander, Hawaiian Native, American Indian or Alaskan Native?

I look forward to your reply and thank you for your time.

Sincerely,

APPENDIX C

Health Screening Questionnaire

Screening Health Questionnaire

Personal Information:

Name: _____ *Home*
phone: _____

Address: _____ *Work* *phone:*
(optional): _____

City, State, Zip: _____ *Cell phone (optional):* _____

Occupation: _____

Email: _____

Fax *#:*

Contact *Person:* _____

Phone: _____

Physicians:

1) Primary MD : _____

Address: _____

Phone: _____

Fax #: _____

2) Other MD: _____

Address: _____

Specialty: _____

Phone: _____

Fax #: _____

Are you currently enrolled or have you participated in any other research studies within the past 6 weeks?

Yes / No

If yes, what date did the most recent study end? ____/____/____

Demographics:

Gender: M / F

In terms of ethnicity, do you consider yourself Hispanic or Latino? Yes/No

In terms of race, do you consider yourself:

Date of Birth: _____

1-American Indian/Alaska Native Yes / No

Age: _____

2-Asian Yes / No

Height: _____

3-Black or African American Yes / No

Weight: _____

4-Native Hawaiian/Pacific Islander Yes / No

5-White Yes / No

6-other _____

Dietary Assessment of Omega-3 Intake

1) How often do you eat-- as a main course-- tuna or other non-fried fish? (One serving size is 3 oz = in size to a deck of cards. Note: One serving of non-fried fish could include: 1, 3 oz. filet of salmon OR 6 pieces of sushi OR 8 sushi rolls OR ~4 tuna fish (salad) sandwiches, etc.)

A) Once or less per month

B) 2-3 times per month

C) Once per week

D) Twice per week

E) More than twice per week

2) Do you currently take fish oil (Omega-3) supplements? Yes / No

3) Do you currently eat foods and/or drink beverages that are fortified with Omega-3 fatty acids? Yes / No

Heart Risk Assessment:

1) Do you currently smoke cigarettes/cigars/pipe or use other tobacco products (snuff, chew, etc.)? Yes / No

If yes, how many/much per day or week? Number/amount: _____ Per: day / week (circle)

If no, have you ever smoked or used tobacco products (snuff, chew, etc.)? Yes / No

If yes, when did you quit? Month: _____ Day: _____ Year: _____

For how many years did you/have you smoked or used tobacco products (snuff, chew, etc.)? _____

2) Do you drink alcohol? Yes / No If yes, How much do you drink?

- a) >1 drink per day
- b) 1 drink per day
- c) <1 drink per day but > 1 drink per week
- d) < 1 drink per week

3) How often do you exercise?

(for example: walking, running,

biking, swimming or gym work-out)

- a) every day
- b) three times a week
- c) once a week
- d) once a month or less

e) not at all

4) Do you have or did you ever have High Blood Pressure? Yes / No

5) Do you have or did you ever have High Cholesterol? Yes / No

6) Do you have diabetes? Yes / No

7) Do you have heart rhythm abnormalities? Yes/ No

8) If Female:

Are you pregnant? Yes / No

Are you breast-feeding? Yes / No

When was your last menstrual period? _____

Postmenopausal? Yes / No / don't know

If no, Contraception being used:

Abstinence

Barrier: condoms, cervical cap, diaphragm

Surgical (tubes tied, partner vasectomy)

Hormonal contraception (pill/patch/shot)

Intrauterine device (IUD)

Have you had both ovaries surgically removed? Yes/No

If post-menopausal, are you taking hormone replacement therapy? Yes / No

Family History:

Do you have a first degree (*mother, father, sister, or brother*) family history of heart attack or stroke? Yes / No

Please answer below for each member of your family.

		<u>Age at time of heart</u>		
<u>History of heart attack or stroke?</u>		<u>attack or stroke?</u>	<u>Current Age?</u>	<u>Alive?</u>
<i>Biological mother</i>	Yes / No	_____	_____	_____
	Yes / No / don't know			
Biological father	Yes / No	_____	_____	Yes / No / don't know
Biological brothers	1) Yes / No / not applicable	_____	_____	Yes / No / don't know
or half-brothers	2) Yes / No / not applicable	_____	_____	Yes / No / don't know
	3) Yes / No / not applicable	_____	_____	Yes / No / don't know
	4) Yes / No / not applicable	_____	_____	Yes / No / don't know
Biological sisters	1) Yes / No / not applicable	_____	_____	Yes / No / don't know
or half-sisters	2) Yes / No / not applicable	_____	_____	Yes / No / don't know
	3) Yes / No / not applicable	_____	_____	Yes / No / don't know
	4) Yes / No / not applicable	_____	_____	Yes / No / don't know

*if >8 siblings, list on back of this sheet

General Health / Medical History:

- 1) Do you have any allergies to fish? Yes / No
- 2) Have you ever had cancer? Yes / No
 - a. If yes, what type? _____
 - b. If yes, do you still have cancer? Yes / No
 - i. If no, when did you go into remission (*no more cancer symptoms*)? Month: _____
Year: _____
- 3) Are you HIV+ or do you have AIDS? Yes / No
- 4) Do you have problems with your kidneys? Yes / No
- 5) Have your doctors ever diagnosed with any of the following:
 - a) Asthma (including childhood or exercise induced asthma) Yes / No
 - b) Rheumatoid Arthritis Yes / No
 - c) Inflammatory Bowel Disease (e.g., Ulcerative Colitis or Crohn's disease)? Yes / No
- 6) List any other medical conditions you have been diagnosed with.

Cardiovascular History:

1. Have your doctors ever diagnosed you with:

Heart disease Yes / No

What? _____

Heart attack Yes / No

Angioplasty or stent of a heart (coronary) artery Yes / No

Heart bypass surgery Yes / No

Stroke Yes / No

Ministroke or TIA Yes / No

Neck (carotid) artery blockage/narrowing/bypass surgery Yes / No

Blocked/narrowed arteries in your legs Yes / No

Bypass surgery of your leg arteries Yes / No

Angioplasty/stent in arteries in your legs Yes / No

2. a) Has your doctor ever told you that you have angina? Yes / No

b) Do you have a history of chest pain/discomfort brought on
by exertion and relieved by rest? Yes / No

c) Do you have a history of chest pain relieved by medication? Yes / No

_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Other Past Medical History Not Covered:

Condition

Year of diagnosis

APPENDIX D

Safety Plan

Title of Study Protocol: Fish oils and Adipose Inflammation Reduction (FAIR Study)

Principal Investigator: Rachana Shah, MD, MsTR

Sponsors of Protocol: National Institutes of Diabetes, Digestive, and Kidney Disease

Grant Number: 1K23DK095913-01A1 (NIDDK)

Title of Grant: Omega-3 PUFA Suppress Adipochemokines (PI:Shah)

IRB Protocol Number: 818359

Date of Document: *June 14, 2013*

SAFETY PLAN OVERVIEW

It is recognized that there is some inherent risk in this study because of 1) the outpatient treatment with prescription omega-3 fatty acids and 2) multiple blood draws and two adipose biopsies. An important perspective, however, is that the study population, while obese, will be relatively young and free of cardiovascular and other significant disease. Moreover, the study drug is used for a short duration in an FDA-approved formulation, dosage, and population.

It will be the responsibility of the PI (Dr. Shah) and these collaborative investigators to plan, implement, and continuously monitor patient safety for the FAIR study. Furthermore, a Data Safety Monitoring Board (DSMB) has been established by the PI and approved by the NIH-NIDDK, which will include expertise in Endocrine, and Cardiovascular disease, clinical research and clinical trials as well as biostatistics. The DSMB will meet at least bi-annually in order to review the overall conduct of the study, patient enrollment, and non-serious and serious adverse events (AEs and SAEs). The study team will prepare pre-specified reports and an analysis of the statistical significance of these events. In order to further ensure the safety of study patients, the study team will implement a safety plan, divided into five components: 1) staff safety training, 2) patient baseline health and safety assessment, 3) patient monitoring, 4) patient safety education and 5) adverse event and serious adverse event collection and reporting, 6) participant discontinuation and stopping criteria.

1.0 Staff Safety Training

All study staff will be required to complete Protection of Human Research Subjects Training (e.g., Collaborative IRB Training Initiative (CITI) Training) and Health Insurance Portability and Accountability Act (HIPAA) Training, as mandated by the University of Pennsylvania. In addition to these courses, as part of the initial training for the FAIR Study, all staff members (e.g. research coordinators and assistants) will be trained to identify specific and extensive exclusion criteria for the protocol and to identify situations or circumstances that may be associated with a heightened safety concern (e.g., specific monitoring criteria during the outpatient treatment protocol). This training will take place before contact with study patients, and will be documented in the regulatory binder. This will entail a formal PI-directed in-service training sessions first for all sub-investigators, project managers, research coordinators and assistants.

2.0 Participant Baseline Health and Safety Assessment

Baseline Health Assessment: Prior to participation in the FAIR Study, each participant will undergo a baseline health assessment at the screening visit composed of the following: a history and physical examination, an electrocardiogram, safety laboratory testing (fasting glucose, electrolytes, BUN, creatinine, hematocrit, hemoglobin, and platelet count, AST, ALT), and a urine pregnancy test for women of child-bearing potential. The purpose of these baseline measures are: (1) to evaluate baseline health; (2) to obtain relevant data that may include or exclude a participant from the study; and (3) to provide a baseline to identify and interpret potential changes in patient health during the patient's participation in the study. According to enrollment criteria (Section 5.4 of Protocol), participants with any inter-current illness, significant current or past health history, regular medication use (except contraception), any abnormal physical exam findings or laboratory findings outside of the pre-specified range will be excluded from the study.

3.0 Participant Monitoring

The regular monitoring of the status of each participant is an essential component of the FAIR Study safety plan. Each study staff member will be trained to identify exclusion criteria and situations during the study protocol that may be associated with increased risk of adverse events.

3.1 General Risk Considerations

The risks associated with blood draws include mild discomfort, occasional bruising and a small risk (< 1%) of infection. The total amount of blood that will be drawn at the screening visit is approximately 20ml. The total amount of blood that will be drawn at the randomization visit (Visit 2) is 50ml and again 50 mL at the completion visit (Visit 4). Overall, this is significantly less than 1 pint of blood which is well within the recommended allowance over a span of 2 months. The risks associated with subcutaneous fat aspirations include bleeding, bruising, infection and pain. These will be minimized by using a trained physician to perform the procedures, administration of anesthesia to reduce pain, and careful monitoring of the aspirated sites by nursing.

3.2 Risks for Adverse Events During Treatment with LOVAZA (omega-3-acid ethyl esters) Capsules

Please refer to Appendix E for complete prescribing information.

Description: LOVAZA, a lipid-regulating agent, is supplied as a liquid-filled gel capsule for oral administration. Each 1-gram capsule of LOVAZA (omega-3-acid ethyl esters) contains at least 900 mg of the ethyl esters of omega-3 fatty acids. These are predominantly a combination of ethyl esters of eicosapentaenoic acid (EPA - approximately 465 mg) and docosahexaenoic acid (DHA - approximately 375 mg). LOVAZA capsules also contain the following inactive ingredients: 4 mg α -tocopherol (in a carrier of partially hydrogenated vegetable oils including soybean oil), and gelatin, glycerol, and purified water (components of the capsule shell).

Mechanism of Action: The mechanism of action of LOVAZA is not completely understood. Potential mechanisms of action include inhibition of acyl CoA:1,2-diacylglycerol acyltransferase, increased mitochondrial and peroxisomal β -oxidation in the liver, decreased lipogenesis in the liver, and increased plasma lipoprotein lipase activity. LOVAZA may reduce the synthesis of triglycerides (TGs) in the liver because EPA and DHA are poor

substrates for the enzymes responsible for TG synthesis, and EPA and DHA inhibit esterification of other fatty acids.

Pharmacokinetic and Bioavailability Studies: In healthy volunteers and in patients with hypertriglyceridemia (HTG), EPA and DHA were absorbed when administered as ethyl esters orally. Omega-3-acids administered as ethyl esters (LOVAZA) induced significant, dose-dependent increases in serum phospholipid EPA content, though increases in DHA content were less marked and not dose-dependent when administered as ethyl esters. Uptake of EPA and DHA into serum phospholipids in subjects treated with LOVAZA was independent of age (<49 years versus ≥49 years). Females tended to have more uptake of EPA into serum phospholipids than males.

Adverse Reactions: Treatment-emergent adverse events reported in at least 1% of patients treated with LOVAZA 4 g per day or placebo during 8 randomized, placebo-controlled, double-blind, parallel-group studies for HTG are listed in Table 3 of Appendix E. Adverse events led to discontinuation of treatment in 3.5% of patients (n=226) treated with LOVAZA and 2.6% of patients (n=228) treated with placebo.

In some patients, increases in alanine aminotransferase (ALT) levels without a concurrent increase in aspartate aminotransferase (AST) levels were observed. ALT levels will be monitored before and after LOVAZA treatment. If a subject's ALT level is greater than 1.5 x ULN, then the subject will be excluded from the study.

In some patients, LOVAZA increased low-density lipoprotein cholesterol (LDL-C) levels. This tends to be most notable in patients with more severe hypertriglyceridemia in whom Lovaza causes the greatest redistribution of cholesterol from VLDL to LDL particles coincident with reduction in triglyceride rich VLDL particles. In such patients, Lovaza increases the size and composition of LDL from small, dense particles (which are thought to be atherogenic due to their susceptibility to oxidation), to large buoyant particles. These larger particles have a greater affinity for cholesterol receptors and are catabolized rapidly. Indeed, total cholesterol in atherogenic apoB lipoproteins (e.g., estimated by calculation of non-HDL cholesterol) tends to fall on Lovaza consistent with redistribution of cholesterol from VLDL to LDL with increased size of LDL particles rather than any overall increase in atherogenic apoB-cholesterol containing particles [10-13]. Thus we will exclude from participation any subject with LDL-C at screening of greater than 190 mg/dl and LDL-C will be repeated at completion of the study (visit 4).

The most common adverse effects reported with LOVAZA are burping, infection, flu symptoms, upset stomach, a change in sense of taste, back pain and skin rash. Monitoring for these adverse events will be conducted at the follow-up Visit 3 (telephone / email visit at 4 weeks post randomization). If these events worsen and/or become extremely bothersome over time, the subject will be discontinued from the study as outlined below in Section 6.1.

There is a possible association between LOVAZA and more frequent recurrences of symptomatic atrial fibrillation or flutter in patients with paroxysmal or persistent atrial fibrillation, particularly within the first months of initiating therapy. We will exclude from the study any subject with diagnosis of cardiac arrhythmias or abnormal ECG on screening. ECG will be repeated at completion of study (visit 4) and any new arrhythmia noted as an adverse event.

3.5 Risk for Development of Clinical or Laboratory Abnormalities during the Study

Subjects will have screening laboratories done at Visit 1 to assess for any safety concerns that would prompt exclusions from the study and as a baseline for drug effect. These clinical and laboratory parameters are measured to assure the participant meets eligibility criteria and that he/she is generally in good health prior to enrollment in the study. The development of significant clinical or laboratory abnormalities (based on repeat laboratory evaluation at the completion visit, Visit 4) would prompt an Adverse Event Report and a notification of the participant and his or her personal physician.

At the end of the inpatient endotoxemia visit (Visit 5), at the Day 2, 6am time point, blood will be drawn to determine if any safety issues have arisen during the inpatient visit. For example, the presence of persistent anemia, biochemical abnormalities (e.g., elevated LFTs (>1.5 x ULN) or hemoglobin < 10g/dL), or clinical abnormalities (e.g., IV line or adipose biopsy site inflammation, infection or hematoma) would prompt a Adverse Event Report, a formal review of the safety for other participants as well as notification of the participant and his or her personal physician for appropriate follow up care.

3.6 DSMB Participant Monitoring

The PI has assembled an NIDDK-NIH approved DSMB comprised of investigators independent of the study. The primary responsibilities of the DSMB will be to approve the final safety plan (i.e. this document), to decide whether the study is being conducted in accordance with the agreed upon safety plan, to review AEs, and SAEs, to decide whether modifications to the protocol should be recommended, and ultimately to recommend whether participation can continue. The DSMB will likely perform its first formal review of the conduct, progress, and participant safety aspects of the study within three-six months after the first participant is enrolled. Thereafter, the DSMB will meet to perform a similar review periodically while there is active participant participation in the study.

4.0 Patient Safety Education

During the initial enrollment phase, all prospective participants will have the entire protocol described to them in detail and will be informed of all risks and potential benefits of participation. This explanation is a part of the informed consent process. Once enrolled, subjects will be educated about the risks of LOVAZA responses, , adipose biopsies and other study procedures. All patients will be given practical advice on identifying and reporting any adverse events including infection, inflammation or bleeding at biopsy sites. Patients will be asked to notify the research study investigator, coordinator or CTSC nursing staff in if they are experience symptoms related to the above-mentioned risks or any other adverse events.

5.0 Adverse Event and Serious Adverse event data collection and reporting

Participants will be closely monitored for all non-serious and serious adverse events. Proper recording and reporting of all adverse events is essential to maintaining and evaluating participant safety. All research staff will be trained on the proper method of recording adverse events. All non-serious and serious adverse events will be reviewed by the PI and included in the Open Reports submitted to the DSMB prior to scheduled meetings.

5.1 Adverse Event (AE)

An adverse event is described as any untoward medical event that occurs during a clinical study which results in the participant experiencing a new and significantly abnormal symptom, physical sign, or laboratory value, or the clinically significant worsening of an already existing symptom, physical sign, abnormal laboratory value, whether or not the event is considered to be related to the therapy. Significant laboratory abnormalities are defined according to screening criteria. Importantly, all screened participants with any significant medical history, physical exam abnormality or safety laboratory abnormality that could potentially be associated with increased risk of adverse events are excluded in the protocol. An Adverse Event Report must be completed for all AEs.

5.2 Serious Adverse Event (SAE)

A serious adverse event is described as any event that is 1) life threatening or fatal, 2) results in significant or persistent disability, 3) requires or prolongs hospitalization, 4) results in a congenital anomaly or birth defect, or 5) represents other significant hazards or potentially serious harm to research participants or others, in the opinion of the investigators.

5.3. Serious Adverse Event Categories

Serious Unexpected Adverse Event: Development or identification of a sudden catastrophic or life-threatening illness (e.g., myocardial infarction, stroke, pulmonary embolism, sustained hypotension or significant bradycardia) or an injury resulting in intensive care unit hospitalization, persistent disability, and/or death. Given the absence of baseline medical conditions at enrollment, all serious adverse events will be categorized as unexpected.

5.4 Serious Adverse Event Reporting

In the event of a serious adverse event, notification within 24 hours of learning of the event must be provided to the Institutional Review Board, CTCRC Research Subject Advocate and Directorship, DSMB, and the NIDDK Program Scientist. As a precaution, at baseline all participants will be instructed to notify the study staff as soon as possible after a serious adverse event. The study staff will develop a report detailing the event. It should include details such as a thorough description of the event, an estimated severity of the event, the frequency or the event, any prior indications that a problem existed, as well as time patterns. In an effort to fully document the SAE, a copy of all corresponding progress notes, physical exams, laboratory test results, and diagnostic tests will be requested, as supporting documentation.

5.5 Adverse Event and Serious Adverse Event Attribution and Grading

The PI will evaluate all AEs and SAEs for attribution based on the following reporting guidelines:

Reporting Guidelines for Adverse Event Attribution Description

1. Unrelated: The AE is clearly not related to the intervention
2. Unlikely: The AE is doubtfully related to the intervention
3. Possible: The AE may be related to the intervention
4. Probable: The AE is likely related to the intervention
5. Definite: The AE is clearly related to the intervention

In addition to attribution, the following FDA reporting guideline for AE grading will be used:

FDA Reporting Guidelines for Adverse Event Grade Description

1. Grade 1: Mild AE
2. Grade 2: Moderate AE
3. Grade 3: Severe AE
4. Grade 4: Potentially Life-threatening AE

Action taken in response to the adverse events will be classified as:

- 1) No action taken
- 2) Study Intervention suspended (specify length of suspension)
- 3) Study Intervention terminated and the patient has been withdrawn

Finally, the study team will work with the IRB, DSMB and other regulatory organization in applying the most up-to-date and appropriate Reporting Guidelines for participants in this study protocol.

6.0 Participant Discontinuation and Stopping Criteria

6.1 Individual Participant Study Discontinuation/Completion

A subject may be withdrawn from the study at any time at either the investigator's discretion or the participant's request. An effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study. The primary reason for discontinuing participation in the study must be stated in the CRF and may include, but is not limited to, one of the following:

- ◆ Development of any abnormality in blood counts, blood chemistries, or urinalysis because of which the investigator deems continued participation in the study to be ill-advised
- ◆ Use of unapproved concomitant medications
- ◆ Occurrence of intolerable AEs
- ◆ Withdrawal of consent by patient
- ◆ Development of an intercurrent illness, injury, medical condition or medication use likely to interfere with patient safety, the overall assessment, or the required administration of study medication
- ◆ Failure to follow the protocol including non-compliance with the study medications (less than 80% by pill count at visit 4)
- ◆ Development of any condition for which the investigator feels withdrawal is justified

The procedure to discontinue a subject from the study is as follows:

- ◆ record any AEs since previous visit
- ◆ record any medications
- ◆ record vital signs
- ◆ perform a physical examination (if discontinuation is due to an AE)
- ◆ collect blood for clinical laboratory analysis

Patients who discontinue from the study because of an AE(s) will be treated and followed according to established medical practice.

6.2 Stopping Criteria

Participant visits and participant enrollment may be discontinued if any clinically significant unexpected adverse events occur in participants that are deemed possibly, probably or definitely related to the study procedures.

RISK BENEFIT RATIO

We would rate the risks associated with this study to be moderate.

There is no direct benefit to the participant of this study, however data obtained from this study may be very useful in expanding scientific knowledge. The primary risks have been highlighted in this document, and reflect in minor or rare study drug-related symptoms, multiple blood draws and several adipose biopsies. Overall, we consider the risk-benefit to be acceptable for the scientific knowledge gained.

REFERENCES

1. Michie, H.R., et al., *Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings*. *Surgery*, 1988. **104**(2): p. 280-6.
2. Revhaug, A., et al., *Inhibition of cyclo-oxygenase attenuates the metabolic response to endotoxin in humans*. *Arch Surg*, 1988. **123**(2): p. 162-70.
3. Martich, G.D., A.J. Boujoukos, and A.F. Suffredini, *Response of man to endotoxin*. *Immunobiology*, 1993. **187**(3-5): p. 403-16.

4. Suffredini, A.F., et al., *The cardiovascular response of normal humans to the administration of endotoxin*. N Engl J Med, 1989. **321**(5): p. 280-7.
5. Barber, A.E., et al., *Glucocorticoid therapy alters hormonal and cytokine responses to endotoxin in man*. J Immunol, 1993. **150**(5): p. 1999-2006.
6. Lehrke, M.R., M.P.; Millington, S.C.; Iqbal, N.; Rader, D.J.; Lazar, M.A., *An Inflammatory Cascade Leading to Hyperresistinemia in Humans*. PLOS Medicine, 2004. **[in press]**.
7. Shah, R., et al., *Gene profiling of human adipose tissue during evoked inflammation in vivo*. Diabetes, 2009.
8. McGillicuddy, F.C., et al., *Inflammation impairs reverse cholesterol transport in vivo*. Circulation, 2009. **119**(8): p. 1135-45.
9. Anderson, P.D., et al., *Innate immunity modulates adipokines in humans*. J Clin Endocrinol Metab, 2007. **92**(6): p. 2272-9.
10. Davidson, M.H., et al., *Efficacy and tolerability of adding prescription omega-3 fatty acids 4 g/d to simvastatin 40 mg/d in hypertriglyceridemic patients: an 8-week, randomized, double-blind, placebo-controlled study*. Clin Ther, 2007. **29**(7): p. 1354-67.
11. Sadovsky, R., et al., *Dispelling the myths about omega-3 fatty acids*. Postgrad Med, 2008. **120**(2): p. 92-100.
12. Bays, H.E., et al., *Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications*. Expert Rev Cardiovasc Ther, 2008. **6**(3): p. 391-409.
13. Bays, H., *Clinical overview of Omacor: a concentrated formulation of omega-3 polyunsaturated fatty acids*. Am J Cardiol, 2006. **98**(4A): p. 71i-76i.
14. Mullington, J., et al., *Dose-dependent effects of endotoxin on human sleep*. Am J Physiol Regul Integr Comp Physiol, 2000. **278**(4): p. R947-55.
15. Jilma, B., *Safety of endotoxin challenge in healthy volunteers: bradycardia*. Intensive Care Med, 2005. **31**(3): p. 496.
16. van Eijk, L.T., et al., *Severe vagal response after endotoxin administration in humans*. Intensive Care Med, 2004. **30**(12): p. 2279-81.

APPENDIX E

LOVAZA PRESCRIBING INFORMATION