Published in final edited form as:
Vasc Med. 2013 October ; 18(5): 263–274. doi:10.1177/1358863X13503695.

n-3 Polyunsaturated fatty acids supplementation in peripheral artery disease: the OMEGA-PAD trial

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Abstract

Despite current consensus guidelines recommending intensive cardiovascular risk factor management for peripheral artery disease (PAD), patients suffering from PAD continue to experience significant morbidity and mortality. This excess morbid burden is at least partially related to impaired vascular function and systemic inflammation. Interventions bridging this gap are critical. Dietary supplementation of n-3 polyunsaturated fatty acids (n-3 PUFA) has been shown to improve endothelial function and reduce inflammation in different cohorts, as well as to decrease cardiovascular events in secondary prevention trials in patients with coronary artery disease, Their effects in the PAD population are, however, less well understood. The OMEGA-PAD trial is a double-blinded, randomized, placebo-controlled trial that examines the impact of a high-dose, short-duration dietary oral supplementation of n-3 PUFA on vascular function and inflammation in patients with established PAD. The purpose of this article is to provide a detailed description of the design and methods of the OMEGA-PAD trial, and a summary of baseline characteristics of the cohort.

Keywords

fatty acids; peripheral artery disease; randomized controlled trials

Introduction and rationale

Peripheral artery disease (PAD) is an epidemic with significant impact on the health of our society. Classically, PAD affects more than 12% of people aged over 65 years, and more

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Declaration of conflicting interest
The authors have no conflicts of interest to declare.

than 20% of those aged above 75.[1] However, a more recent study suggests that in a primary care population, one-third of patients aged 70 years and above suffer from PAD.[2] Once PAD is diagnosed, the annual mortality rate is 4–6%,[3,4] with a 20–60% increased risk of myocardial infarction[5,6] and a 40% increased risk of stroke. With further progression of the disease- to its most advanced stage (critical limb ischemia), the 1-year mortality is increased to 25% and may be as high as 45% in those who have under-gone an amputation.[3,7,8] Overall, the public remains poorly informed about PAD, and there are major knowledge gaps with regards to the definition of PAD, (he risk factors and symptoms of the disease, as well as the association of the disease with the risk of amputation or mortality.9 In 2001, a total of $4.37 billion was spent by Medicare on PAD-related treatment and 88% of these expenditures were for inpatient care.[10] Interestingly, the international Reduction of Atherothrombosis for Continued Health (REACH) Registry reported PAD incurring higher annual mean medication and hospitalization costs annually than coronary artery disease (CAD) and cerebrovascular disease.[11] Interventions to improve the morbidity and mortality of patients with PAD are therefore critical.

n-3 Polyunsaturated fatty acids (PUFA) have long been recognized to reduce cardiovascular disease,[12,13] with a high tissue ratio of n-3/n-6 PUFA leading to a reduced risk of coronary events.[14] Greenland Eskimos who consume a diet high in n-3 PUFA[15] have a low incidence of CAD. Randomized trials evaluating the impact of oily fish or fish oil supplementation on cardiovascular health have demonstrated a decrease in total mortality, a decrease in cardiovascular death, a decrease in sudden cardiac death and a reduction in non-fatal cardiovascular events.[16–19] There is presently more controversy about the benefits of n-3 PUFA in patients with cardiovascular disease. For example, in a recent meta-analysis published last year by Rizos et al., there were no significant benefits of n-3 PUFA on all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke.[20] However, in another meta-analysis published the same year, n-3 PUFA were found to be protective against vascular death.[21] One of the largest trials, the JEL1S study, randomized 18,000 hypercholesterolemic patients to statin and n-3 PUFA supplementation versus statins alone. The group with n-3 PUFA supplementation experienced significantly fewer non-fatal coronary events.[18] Furthermore, n-3 PUFA supplementation was associated with a 55% reduction in major coronary events in the PAD subgroup compared to an 18% relative reduction in the patients without PAD.[22] Since patients with PAD continue to experience a higher risk of cardiovascular events compared to those with CAD,[23,24] they could potentially benefit from improved medical therapy, warranting further investigations on n-3 PUFA in this patient population.

Part of the clinically relevant benefit of fish-oil or n-3 PUFA supplementation is thought to be due to improvement in endothelial function and reduction in inflammation. Supporting evidence of (his proposition includes an improvement in endothelial function in healthy volunteers,[25–27] in obese adolescents[28] and in patients with chronic heart failure,[29] as well as a dramatic reduction in inflammation as measured by C-reactive protein (CRP) in healthy individuals,[30–36] male smokers,[37] obese adolescents,[28] and in patients with rheumatoid arthritis[38] or CAD.[39] Still, evidence for the beneficial effects of n-3 PUFA in patients with PAD is controversial.[40] Although there is some evidence suggesting that consumption of n-3 FAs in the diet may be associated with a decreased prevalence of PAD,[41] the effects of n-3

PUFA on PAD have not been as thoroughly investigated. Several relatively small prospective studies have aimed to assess the effects of fish oil and n-3 PUFA on different parameters related to PAD,[42–49] with different conclusions. One meta-analysis studied the effects of n-3 PUFA in intermittent claudication[50] and concluded that blood viscosity decreases with n-3 PUFA supplementation, but that there is no evidence it significantly affects walking distance, the ankle-brachial index or blood pressure. At the present time, there is also insufficient evidence to know if n-3 PUFA can improve endothelial function and inflammation in patients with PAD.

The OMEGA-PAD trial aims to assess the impact of high-dose, short-term, n-3 PUFA supplementation on endothelial function and inflammation in patients with established symptomatic PAD (Figure 1). The results obtained will be correlated with the omcga-3 index, a validated marker of tissue n-3 PUFA content and an independent graded risk factor for death from CAD.[51,52] The scientific knowledge obtained from this study could have a significant clinical impact resulting in improved treatment and outcomes for patients suffering from PAD. The results of this study will also provide valuable mechanistic insight into PUFA as well as inform on this class of agents for cardiovascular outcome trials. At the present time, despite guidelines from the American Heart Association (AHA) of n-3 PUFA and diet in general,[53,54] the evidence for PAD remains elusive. If successful, the present study will provide direct evidence of a beneficial impact of dietary n-3 PUFA supplementation on the vascular health of the PAD population, and help define a treatment goal (omcga-3 index threshold) to guide such recommendations.

Methods

Study design

The OMEGA-PAD trial is a randomized, double-blind, placebo-controlled trial. A total of 80 patients aged 50 years or above with lower extremity PAD in the form of intermittent claudication and an ankle–brachial index (ABI) of <0.9 presenting to the vascular surgery clinic at the Veterans Affairs Medical Center in San Francisco (SFVAMC) will be randomized to 2.2 g oral n-3 PUFA twice daily (total of 4.4 g/day) or a matched placebo for 1 month. Measurements will be done at baseline and after 1 month (Figure 2 and Table 1). The primary endpoint is a change in endothelial function measured by brachial artery flow-mediated, endothelium-dependent vasodilation (FMD). Secondary endpoints include a change in inflammatory and anti-inflammatory markers such as high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), soluble intracellular adhesion molecule-] (sICAM-1), tumor necrosis factor-α (TNF-α) and 15-epimeric lipoxin A4 (15-epi-LXA4), and improvement in lipid profile (low-density lipoprotein (LDL), triglycerides, high-density lipoprotein (HDL)), blood pressure and patient-perceived walking impairment as determined by the Walking Impairment Questionnaire.[55] The omega-3 index will be measured to correlate observed effects with changes in the red blood cell (RBC) content of n-3 PUFA.

Ethics

Institutional review board approval was granted for this study by the Committee on Human Research at the University of California, San Francisco (UCSF), The study was registered with ClinicalTrials.org (NCT01310270).

Study population, site and recruitment

The subjects recruited for this study will include males and females with PAD, age 50 years and above. The patients targeted are those with intermittent claudication (Rutherford grade I–3), Inclusion criteria include; (1) intermittent claudication (mild-severe); (2) resting or exercise ABI<0.9; and (3) age ≥ 50. Lxolusion criteria include: (1) critical limb ischemia (rest pain or tissue loss); (2) hypersensitivity/ allergies to fish or seafood; (3) significant renal, hepatic or inflammatory disorder; (4) concurrent severe infections; (5) acute illness or major surgery within 30 days; and (6) receiving immunosuppressive medications. Eighty subjects will be recruited from the vascular surgery clinic at the SFVAMC. All patients will undergo a complete history and physical examination. Demographic information, cardiovascular history (e.g. coronary artery disease, cerebrovascular disease), risk factors (hypertension, diabetes, hypercholesterolemia, cigarette smoking, renal insufficiency), medications, and pertinent cardiovascular examination findings will be documented.

Randomization procedures and nutritional intervention

After an individual is recruited and determined to be eligible, the participant will be randomized to one of two groups: n-3 PUFA or placebo, n-3 PUFA supplementation is achieved with four capsules of ProOmega® twice daily (Nordic Naturals, Watsonville, CA, USA), corresponding to a total of 4.4 g/day. Each ProOmega capsule contains 325 mg of eicosapentaenoic acid (EPA) and 225 mg of doco-sahexaenoic acid (DHA). We chose the dose of 4 g/day, which corresponds with the AHA's recommendations for the treatment of hypertriglyceridemia.[40,41] In order to achieve this target dose, a total of eight tabs of ProOmega (325 mg EPA/225 mg DHA) are used, corresponding to a total dose of 4.4 g/day of n-3 PUFA. The placebo group will take the same number of capsules containing an inactive substance (soybean; Nordic Naturals). The placebo capsules are designed to be the same color and shape as the treatment capsules. Prior to the randomization visit, project staff will contact the assigned research pharmacist who will generate the block randomization process. Four subjects will be randomized per block (total of 20 blocks) with a ratio of 1:1 for each block. A 1-month period was chosen in order to assess the impact of acute oral supplementation of fish oil, as other investigators have demonstrated that 1 month of treatment was sufficient to see changes in the lipid profile.[44]

Study protocol

Individuals who are interested in the study will undergo an initial screening questionnaire with the study staff in clinic or over the phone. Those meeting the inclusion criteria will be invited to enter the research study, at which time informed consent will be obtained by study staff in accordance with requirements of the Committee for Human Research.

Subjects will come into the hospital for an initial visit for baseline measurements and then commence the study drug or placebo for I month (Figure 2). At the end of the month, they

will return for a second visit when study measurements will be repeated (Table 1). In addition to the primary and secondary endpoints described above, other measurements will include bilateral ABIs, bioelectric impedance analysis (BIA), and arterial tonometry to correlate with FMD results. Subjects will also be instructed to complete a nutrition questionnaire (Block 2005 Food Frequency Questionnaire; NutritionQuest, Berkeley, CA, USA) based on their diet,[56] two surveys for post-traumatic stress disorders,[57,58] and one 8-item patient health questionnaire for depression.[59]

Measurements

Demographics, anthropometries, medical history, and hemodynamic measurements—Demographic data (including age, race, sex, hip and waist circumference, body mass index (BM1), prior supplement use, exercise frequency), cardiovascular history (e.g. CAD, cerebrovascular disease, previous procedures), risk factors (hypertension, diabetes, hypercholesterolemia, cigarette smoking, renal insufficiency), medications, and pertinent cardiovascular examination findings will be recorded. Blood pressure will be recorded by an indirect sphygmomanometer.

Vascular reactivity of brachial arteries—Flow-mediated vasodilation will be performed according to current guidelines and standards[60,61] and as already described by our group[62] and other investigators.[63–66] Subjects will be asked to be fasting (≥8 hours) and free of nicotine (≥ 4 hours). A history of recent medications will be recorded. Subjects will be allowed to rest for 10 minutes in a supine position in a darkened room at 23°C. The subject's arm will be extended onto a movement-constraining pillow with the palmar aspect oriented anteriorly. A 5 Cm tourniquet blood pressure cuff will be placed on the upper arm distal to the insertion of the deltoid. The length of the brachial artery will be surveyed by B-mode ultrasound (Philips HD11) using a broadband linear array transducer with a 3–12 MHz range {Philips LI2-3) until a straight segment with a visible registration structure can be located. The probe will be oriented so that the artery is at least 3 cm deep to the surface of the skin, the focus aligned with the deep boundary of the vessel, and clearly demarcated intima/lumen boundaries are visible.

Prior to cuff inflation, the baseline diameter of the vessel and blood-flow velocity will be recorded for 60 seconds using electrocardiogram (EKG)-gated image capture software (Brachial Imager; Medical Imaging Applications LLC, Coralville, 1A, USA). Baseline blood-flow velocity will be recorded for 60 seconds using an insonation angle of 60°. The Doppler sample gate will be positioned to cover the center, but not the edges, of the lumen. The probe will not be moved between measurements.

The blood pressure cud will then be inflated to the greater of 250 mmHg or 50 mmHg above the subject's systolic blood pressure for a period of 5 minutes. Recording of the B-mode images will begin 10 seconds prior to cuff release. Blood-flow velocity will be assessed for a period of 30 seconds post-cuff release using the methods described above. 13-mode images will be recorded until 3 minutes post-cuff release.

Analysis of the images will be performed using continuous edge-detection software (Brachial Analyzer; Medical Imaging Applications LLC). Baseline diameter will be

recorded as the mean of 60 seconds of data. From hyperemia recordings, the exact moment of cuff release will be noted, Hyperemia diameter will be calculated using a pre-determined time window (55–65 seconds post-cuff release). FMD% will be calculated as [(60 s hyperemia diameter – avg baseline diameter/avg baseline diameter)* 100]. The vasoreactivity index was calculated by normalizing FMD to brachial stimulus ratio, with brachial stimulus ratio corresponding to hyperemia flow divided by baseline flow. FMD in healthy subjects is expected to be above 7%[60] and has been reported to range between 0.20 and 19.2%.[67] Our preliminary data are comparable to other investigators in the PAD population (5–9%).[62,63,65]

Time-averaged velocity measurements will be obtained using the peak-velocity method. Average velocity at baseline will be obtained from 60 seconds of data. Velocity of the hyperemia stimulus will be calculated as the mean velocity of the first four heart beats following cuff-release. Both mean velocity and the velocity time integral will be recorded.

Quality control will be assessed at each point of the measurements. Image quality will be evaluated by a second observer and graded on a 6-point scale that includes; registration structure (landmark), horizontally directed artery, correct longitudinal alignment, clearly visualized near wall intimal medial thickness (1MT) and far wall IMT, and at least 5 mm of clearly visualized artery.

Tonometry—Measurements of arterial stiffness will be performed with an applanation tonometer (SphygmoCor CP (Tonometry System; Itasca, 1L, USA) and the provided analysis software. After measuring the subject's blood pressure and applying EKG leads, the applanation tonometer probe will be placed over the subject's radial, carotid, and femoral arteries for about 3 minutes to capture pulse wave tracings of the artery of interest. The tracings will be used in the Fourier transform algorithm to calculate several parameters including ascending aortic blood pressure, aortic pulse pressure and augmentation index, ejection duration, and pulse wave velocity between any two sites in the vasculature.

Ankle-brachial index—Ankle–brachial indices will be measured using current guidelines and standards.[68] Systolic blood pressures of the brachial, posterior tibial and dorsalis pedis arteries will be measured bilaterally. For each lower extremity, the highest systolic pressure of the two pedal pulses will be divided by the highest systolic pressure of the two brachial arteries.

Body fat composition—Body fat composition will be measured using bioelectric impedance analysis (RJL Quantum J01Q; RJL Sciences, Clinton Township, Ml, USA). Resistance and reactance will be used to calculate impedance [(resistance^2+ reactance^2)$^{0.5}$], which is used to calculate fat free mass (FFM) [(0.8787*ht^2)/(impedance$^{0.485l}$*25.22)+(0 . 0813*wt)+0.071]. FFM is used to calculate body fat mass [wt FFMJ and fat percentage [(body fat mass/wt)*100].

Inflammatory markers—Inflammation will be measured through inflammatory biomarkers including hsCRP, IL-6, sICAM-1, TNF-α and 15-epi-LXA4.[69,70] Ten milliliters of whole venous blood will be collected in a fasting stale in a tiger-top tube, clotted for a

minimum of 30 minutes at room temperature, and centrifuged at 2800 rpm for 10 minutes at 4°C. Serum will be stored at −80°C until assayed for IL-6, sICAM-1, and TNF-α per standard kit protocol (R&D Systems Inc., Minneapolis, MN, USA). The typical coefficients of variation for IL-6, slCAM-1, and TNF-α are 7.4%, 4.6%, and 5.4%, respectively. The lower limits of detection are 0.04 pg/ml, 0.1 ng/ml, and 0.11 pg/ml, respectively. Plasma will be assayed for hsCRP the same day as collection by the SFVAMC lab per standard methodology (Analyzer; Beckman Coulter, Miami, FL, USA), The coefficient of variation for hsCRP using this procedure is 5.1%, In healthy individuals, CRT is <1.0 mg/dl.

n-Fatty acid measurements—The omega-3 index represents the red blood cell (RBC) content of the two major long-chain n-3 FAs, LPA and D11A.[71] It equates to LPA I D1IA as a percent of total RBC FAs and has been identified as a biomarker for increased risk for CAD, especially sudden cardiac death, it is read as high, intermediate, and low-risk levels of the omega-3 index (<4%, 4–8%, >8%, respectively). Ten milliliters of whole venous blood will be collected in a fasting slate in an HDTA tube, and centrifuged at 2800 rpm for 10 minutes at 4°C within 30 minutes of collection. Packed RBCs will be stored al −80°C until assayed for n-3 FAs, n-6 FAs, arachidonic acid (AA), HPA, D11A, omega-3 index., saturated fat, trans fat, and monounsaturated fat. The RBCs will be analyzed according to the HS-Omega-3 Index® methodology.[72] Briefly, fatly acid methyl esters are generated from RBCs by acid transesterification with boron trifluoride and analyzed by capillary gas chromatography using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD, USA) equipped with a SP2560, 100-m column (Supelco, Inc., Bellefonte, PA, USA). Fatty acids arc identified by comparison with a standard mixture of fatty acids characteristic of erythrocytes and reported as a percentage of total identified fatty acids after response factor correction. Trans fats are defined as the sum of the trans isomers of oleic acid (C18: 1) and linoleic acid (C18:2n-6; i.e. the C18 trans). The typical coefficient of variation for the HS-Omega-3 Index (EPA+DHA) using this procedure is 3% and 7% for trans fats. The average omcga-3 index in the US population is 4.5%, with values ranging from 2.7% in the lowest 5th percentile to 8.8% in the highest 95th percentile.[73]

Renal, lipid and metabolic measurements—Blood samples will be collected in a fasting state for measurement of creatinine (Cr), estimated glomerular filtration rate (eGFR), albumin, hemoglobin Alc (HbAlc) as well as total cholesterol, triglycerides, LDL and HDL. Plasma will be assayed for these analytes the same day as collection by the SFVAMC lab per standard methodology (Beckman Coulter Analyzer). Serum will be isolated at the same time points for homocysteine and assayed the same day as collection by the SFVAMC lab per standard methodology (Architect i1000 Analyzer; Abbott Laboratories, Lake Forest, IL, USA).

Walking Impairment Questionnaire (WIQ)—The Walking Impairment Questionnaire (WIQ) is a validated instrument that assesses the patient-perceived walking impairment.[55] This questionnaire will be administered by research staff and measures patient-perceived walking capacity and limitation across three domains; distance, speed, and stair-climbing. The WIQ Distance score is calculated after the patient ranks, on an ordinal Likert scale, his or her degree of difficulty walking specific distances (indoors at home to five blocks). The

WIQ Speed score uses a similar method; patients are asked to rank their degree of difficulty walking a fixed distance at difference speeds (walking slowly to jogging). The WIQ Stair score asks patients to rank their difficulty climbing one to three flights of stairs. To score the WIQ, the individual items are multiplied by a weighted factor, the products are then summed and divided by the maximum possible score yielding a percent. For example, the WIQ Distance multiplies different distances (20 to 1500 feet; 6.1 to 457.2 meters). Each distance is multiplied by the respondent's difficulty ranking (0 to 4). The sum of these is divided by the total possible score. The WIQ Speed score assigns weights to different speeds (1 to 5 mph; 1.6 to 8 km/h). The WIQ Stair score assigns a proportional weighting to the number of flights of stairs (one to three).

Adverse events

Adverse events will be regularly assessed by telephone every week as well as at the end of the study. All events will be recorded, whether elicited by the study staff or reported by the subject at clinic visits or via telephone contact. Additionally, all participants will be asked to contact study staff in the event of hospitalization, emergency room visits or any other unexpected medical event.

Statistical analysis

The sample size for this study was calculated using a paired t-test. Our study evaluates the hypothesis that n-3 PUFA supplementation is associated with an improvement in brachial artery FMD. Based on the available data from the literature, the closest estimate of an effect size that can be expected is a 40% increase in FMD. For example, Schiano et al. demonstrated a 3.3% (6.7–10.0%) absolute increase in FMD with n-3 PUFA supplementation,[48] which corresponds to a 49% relative increase in FMD. In the hypercholesterolemic patients, Goodfellow et al. demonstrated a doubling of FMD with n-3 PUFA supplementation.[74] Hence, a 40% increase in FMD would be considered conservative. Based on data from a similar patient population at Brigham and Women's Hospital,[75] a baseline value of 9.21±4.6% is expected in the PAD population. With a power of 0.9 and a 40% increase in brachial artery diameter, a sample size of 33 patients per group (66 total) should lead to significance. Considering a possible 10% loss of patients during the study due to loss of follow-up, discontinuing participation in the study, we plan to randomize 40 patients per group for a total of 80 patients participating in the study.

For subsequent analysis, all variables will be summarized by appropriate descriptive statistics at each time point. The intervention group will be compared to the placebo group for homogeneity on demographic and baseline clinical variables via the chi-squared test or Student's t-test (depending on the type of variable). Through stratified randomization, these variables are anticipated to be comparable in both groups. All analyses will be based on intention to treat (all participants randomized will be analyzed based upon their assigned group). The significance level will be preset at 0.05 and two-sided tests will be performed. Results will be correlated with the change in the omega-3 index after intervention. Statistical analyses will be performed using Stata/SE 12 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics of the first 50 randomized patients in the OMEGA-PAD trial

Recruitment for the OMEGA-PAD trial started in April 2011. Fifty male veterans have been enrolled and randomized (Table 2). The mean age is 67±9 years. The mean index limb ABI is 0.80±0.19. CAD is present in 36%, hypertension in 92%, and diabetes mellitus in 32%. A total of 47% are current smokers and 45% are former smokers. The mean HbAlc is 8±1, LDL 93±37 mg/dl, triglycerides 160±98 mg/dl and HDL 43±12 mg/dl. The baseline brachial FMD is 7±4%, indicating an overall impairment of endothelial function in this cohort. The inflammatory burden of this cohort is substantial – as evidenced by hsCRP 4.5±4.8 mg/l. The baseline ornega-3 index is 4.9±0.2%.

Discussion

PAD is an epidemic with significant impact on the health of the aging population. In addition to better understanding the effects of n-3 PUFA supplementation on vascular health in patients with symptomatic PAD, we expect that this study will serve as a pilot study for a larger randomized trial assessing the effects of n-3 PUFA supplementation on disease progression and clinical events in patients with PAD. Successful prevention of critical limb ischemia and treatment of the disease at an early stage could have a tremendous impact in terms of the economic benefits to the individual as well as the healthcare system, and more importantly a significant improvement to the quality of life of thousands of patients.

Significance of the study and contribution to knowledge development

This study is innovative in several areas of PAD research. First, it is presently unknown whether a short-term dietary intervention (high-dose n-3 PUFA oral supplementation) has the potential to reverse key features (endothelial dysfunction and inflammation) of vasculopathy associated with chronic PAD. Second, this study captures clinically relevant physiological end points of dietary supplement (fish oil) which could potentially become an alternative to drug therapies for the treatment of PAD. Current medical therapies for PAD are limited to antiplatelet and lipid-lowering medications, and there is a great need for additional pharmacotherapy to reduce the burden of this disease. It is known that elderly patients have a lower compliance to statins compared to younger patients.[76,77] n-3 PUFA supplementation may therefore represent a cheaper and better tolerated alternative for that population, with the overall goal of slowing and potentially reversing disease progression.

The definition of a relationship between dietary omega-3 supplementation and inflammatory markers in a PAD cohort is another novel aspect of this trial. Epidemiologic evidence linking inflammation to the future development and severity of PAD has been established.[78–81] CRP is a classical acute-phase marker and a member of the pentraxin family of innate immune response proteins.[82] It is synthesized by the liver and plasma concentrations can increase 1000-fold in response to an inflammatory stimulus.[83] Inflammation in general, and CRP in particular, has been correlated with impaired endothelial function in vivo and in vitro,[84,85] and hsCRP levels have been inversely correlated with acetylcholine-induced, endothelial-dependent forearm blood flow.[86] hsCRP

as well as IL-6 and ICAM have been found to be increased in patients with PAD.[75,87,88] Emerging evidence suggests that omega-3 FAs are biologically linked to the endogenous resolution pathway of PAD[89–91] and 15-epi-LXA4 levels have been found to be reduced in PAD patients.[88] This study will allow us to correlate levels of pro- and anti-inflammatory markers in response to omega-3 supplementation in a cohort with PAD. Furthermore, the inflammatory markers chosen for this trial have already been found to be correlated with clinical events in PAD patients in our group and in others.[75,88,92]

Another unique aspect of our study is the correlation of endothelial function and inflammatory changes with the actual incorporation of n-3 PUFA in the R.BC membrane using the omega-3 index, a validated marker of tissue n-3 content. Impaired FMD of the brachial artery independently predicts cardiovascular events in patients undergoing vascular surgery,[93,94] emphasizing the relationship between markers of inflammation, thrombosis, and endothelial function.[95] Our study will provide direct evidence linking n-3 PUFA supplementation to vascular phenotype via changes in cellular lipid metabolism.

Lastly, the preliminary data accumulated during the proposed study may lead to the development of a laboratory treatment target for the use of n-3 PUFA supplements in PAD. The omega-3 index has been correlated with CAD. A subsequent trial based on the data acquired in this study could validate a suggested threshold level of the omega-3 index in a larger population with PAD. Overall, we feel that this trial will potentially bring another paradigm to clinical practice with the potential addition of n-3 PUFA supplementation to the medical management of patients, particularly elderly patients with PAD. Future trials could evaluate the effects of n-3 PUFA on the functional status of patients with PAD.

Brachial artery FMD in this cohort is significantly depressed compared with other cohorts and therefore we feel this represents a vulnerable cohort for incident and recurrent cardiovascular events. It is not currently known whether or not improvement of endothelial function in this cohort will translate into improvement in clinical outcomes and this represents one of the first attempts to mitigate risk in this population. A recent meta-analysis suggested that the association between FMD and the estimated 10-year risk of CAD assessed by the Framingham risk score was strongest in low-risk populations compared to medium or high-risk populations.[96] In the first 50 patients randomized to this study, the Framinghum risk score (10-year cardiovascular risk) is 19%. Therefore, we feel studies in high-risk populations such as ours will provide clarity on this issue and guide future investigations.

Additional limitations of study/design/methods

It is possible that patients may experience an aftertaste after taking the fish oil capsules, possibly affecting their compliance with treatment. However, we will perform a pill count when patients return after the treatment period. Furthermore, the omega-3 index should help us confirm that the n-3 PUFA supplementation was taken by quantifying the incorporation into the RBC membrane. Additionally, hsCRP and other biomarkers may be affected by concurrent infectious or inflammatory illness unrelated to vascular disease per sc. However, with proper selection and exclusion criteria as outlined, the baseline levels should be minimally affected by such factors. The magnitude of an important association may be less

than hypothesized, in which case an inadequate sample size may fail to detect potentially significant relationships between markers and endothelial function.

Another major limitation is related to unknown aspects of the dosing scheme. A high-dose and short-duration intervention was chosen but a longer treatment and/or higher dose may be required to have a beneficial effect. Furthermore, the very short-term follow-up period will limit the ability to discern effects that may require more time to be observed. These unknown aspects could, however, be studied in further trials.

The study population, composed of mostly male veterans who are predominantly Caucasian, may not be fully representative of the wider PAD population. Furthermore, the inflammatory phenotype of the population studied at the SFVAMC may be skewed compared to the typical PAD patient with regards to levels of psychological and psychosocial factors affecting overall stress and inflammation.[97]

Conclusion

The OMEGA-PAD trial will test the novel hypothesis that short-term, intensive n-3 PUFA supplementation improves functional and inflammatory parameters in a cohort at high vascular risk. The results of this study will provide valuable mechanistic insight into PU FA as well as inform on this class of agents for cardiovascular outcome trials to ultimately decrease morbidity burden in this patient population.

Acknowledgments

Funding

This work was supported by start-up funds from the University of California, San Francisco and the Northern California Institute for Research and Education. The project described was supported by a Society for Vascular Surgery Seed Grant and by Award Number KL2RR024130 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. The funding organizations were not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. This publication was also supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI Grant Number KL2 TR000143. Its contents arc solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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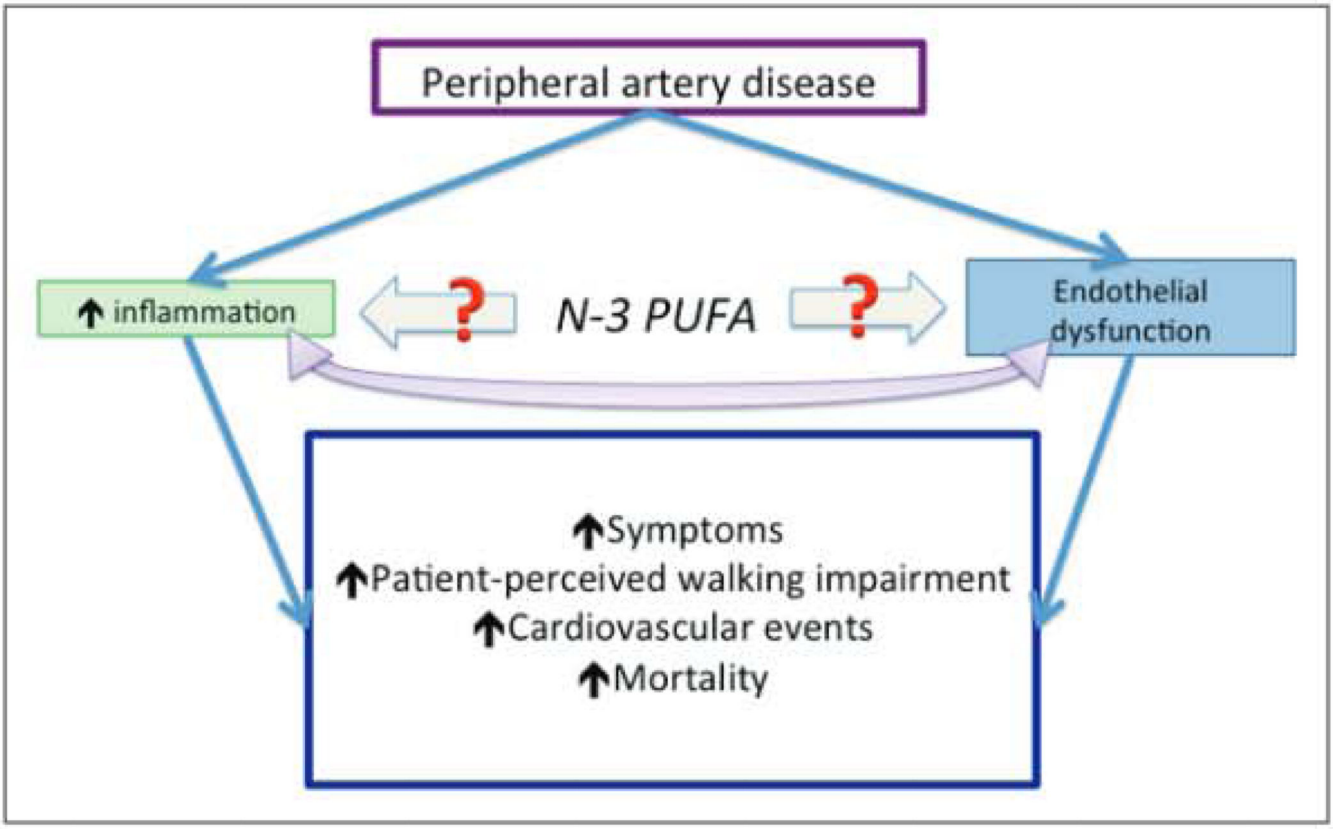

Figure I.
OMEGA-PAD trial.

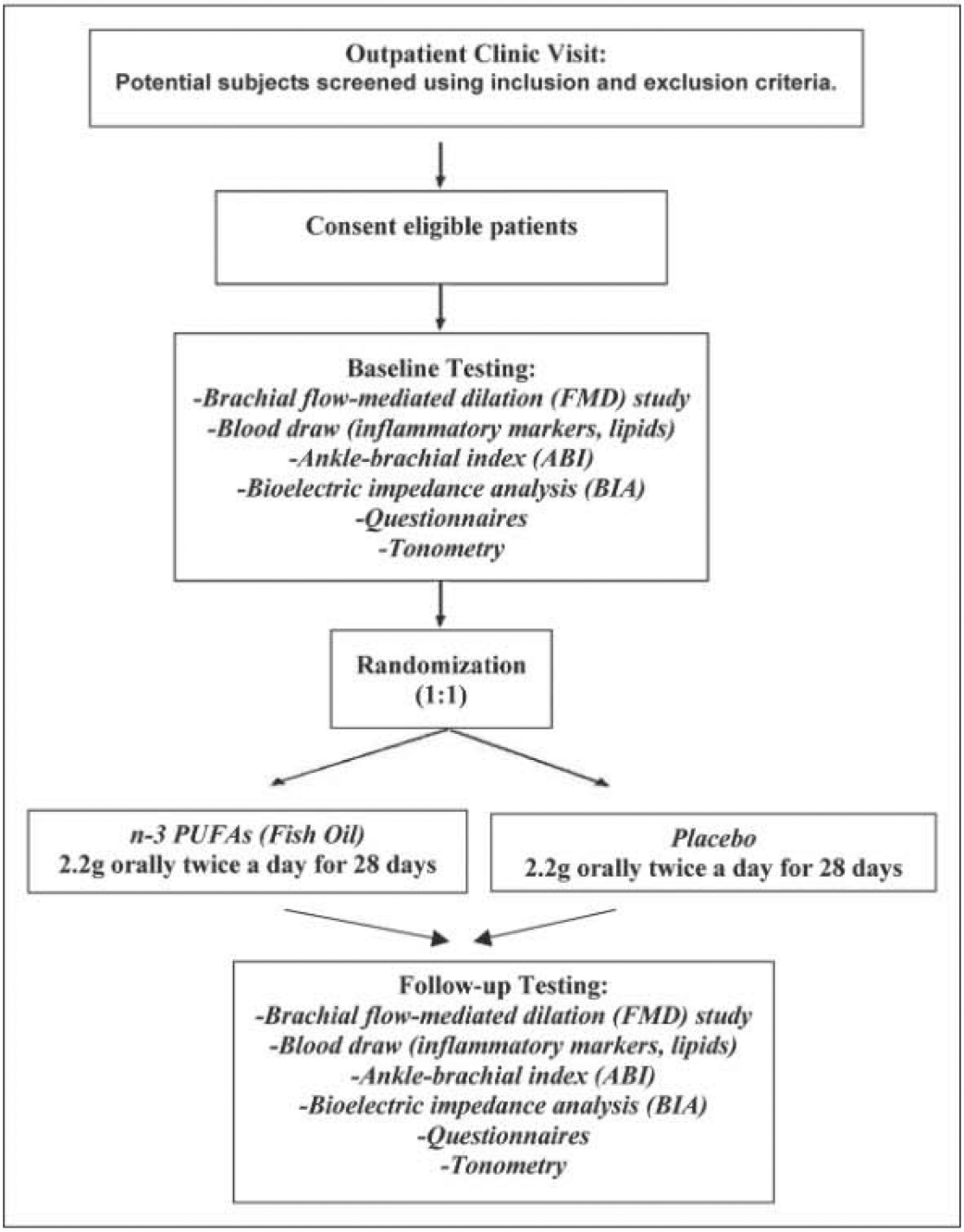

Figure 2.
Study protocol.

Table I

Testing measurements

Measures	Baseline	One-month follow-up
1. Demographics, anthropometrics, medical history	X	
2. Hemodynamic measurements	X	X
3. Brachial flow-mediated vasodilation (FMD) study	X	X
4. Arterial Tonometry	X	X
5. Ankle-brachial index	X	X
6. Body fat composition	X	X
7. Inflammatory markers	X	X
8. Omega-3 index	X	X
9. Lipid, renal, and metabolic markers	X	X
10. Walking Impairment Questionnaire	X	X
11. Nutritional Questionnaire (Block 2005 Food Frequency Questionnaire)	X	
12. Three PTSD/Depression questionnaires (PHQ-9, CIV-C, PTSD Scale)	X	

Table 2

Baseline characteristics of the first 50 individuals recruited for the OMEGA-PAD trial.

General characteristics	Frequency count (%)	Mean	SD
Age, years		67	9
Male	50 (00%)		
Caucasian	36 (72%)		
Veteran	47 (00%)		
Left ABI		0.80	0. 9
Right ABI		0,82	0.20
Rutherford			
Mild claudication	2 (24%)		
Moderate claudication	6 (32%)		
Severe claudication	22 (44%)		
BMI		28	4
Waist:hip ratio		.0	0.06
Omega index {%)		4.9	0.2
Comorbidities			
Coronary artery disease	8 (36%)		
Hypertension	46 (92%)		
Hyperlipidemia	45(90%)		
Diabetes mellitus	6 (32%)		
Surgical history			
Index bypass	7(4%)		
Index percutaneous	3 (27%)		
Systolic blood pressure (mmHg)		36	8
Diastolic blood pressure (mmHg)		76	9
Medications			
Aspirin	28 (58%)		
Ace inhibitor	8(38%)		
B-blocker	26 (54%)		
Statin	43(90%)		
PAD risk factors			
Smoking, current	23 (47%)		
Smoking, former	22 (45%)		
Pack years, years*packs/day		5 .36	37.58
Cholesterol, mg/dl		66.24	4 .36
Triglycerides, mg/dl		60	98
HDL, mg/dl		43	2
LDL. mg/dl		93	37
Serum creatinine (CR). mg/dl		.05	0.34
eGFR, ml/min		79	23
Albumin, g/dl		4.02	0.35

General characteristics	Frequency count (%)	Mean	SD
Homocysteine, μmol/l		3.62	4.4
HbA c. %		8	
Inflammation			
hsCRP, mg/l		4.5	4.8
IL-6. pg/ml		.6	0.9
ICAM-1, ng/ml		2.6	5.0
TNF-α. pg/ml		2.3	.6
Brachial FMD and tonometry			
Brachial FMD, %		7. 9	4. 9
Brachial artery baseline diameter, cm		0.38	0.06
Brachial artery mean velocity, m/s		0. 5	0.07
Brachial artery baseline flow, ml/min		03.46	56.06
Brachial artery baseline shear stress, dynes/cm^2		.49	5.58
Brachial artery reactive hyperemia diameter, cm		0.40	0.06
Brachial artery reactive hyperemia velocity, m/s		0.67	0.28
Brachial reactive hyperemia flow, ml/min		5 3.48	248.29
Brachial reactive hyperemia shear stress. dynes/cm^2		47.37	20.34
Stimulus ratio		4.88	2.3
Patient-perceived walking performance			
Walking distance {score: 0– 00)		26	23
Walking speed (score: 0– 00)		26	24
Stairs (score: 0– 00)		32	25

ABI, ankle-brachlal Index; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukim-6; ICAM-1, intracellular adhesion molecule-; TNF-α, tumor necrosis factor-α FMD, flow-mediated, endothelium-dependent vasodilation