Modulation of Esophageal Inflammation in Barrett's Esophagus by Omega-3 Fatty Acids, a Double Blind Placebo Controlled Randomized Pilot Study

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Modulation of Esophageal Inflammation in Barrett's Esophagus by ω3 Fatty Acids, a Double Blind Placebo Controlled Randomized Pilot Study

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BE	Barrett's Esophagus
CFR	Code of Federal Regulations
Cox-2	Cyclooxygenase-2
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EAC	Esophageal adenocarcinoma
FDA	Food and Drug Administration
FFA	Free fatty acid
GCP	Good Clinical Practice
GEJ	Gastroesophageal Junction
HGD	High grade Dysplasia
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IL1β	Interleukin-1 beta
IL6	Interleukin-6
IND	Investigational New Drug Application
IRB	Institutional Review Board
MetS	Metabolic syndrome
NSAIDs	Nonsteroidal anti-inflammatory drugs
ω3 FFA	Omega 3 free fatty acid
PGE2	Prostaglandin E2
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
SQ	Subcutaneous
TNFα	Tumor necrosis factor-alpha

1 Introduction

Increased visceral abdominal fat is associated with increased risk of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC). Novel preliminary data gathered by us has revealed a reflux independent influence of visceral abdominal fat on esophageal pro-inflammatory and pro-neoplastic pathways, mediated via visceral fat produced saturated free fatty acids and activated macrophages. ω3 free fatty acids have been shown to inhibit epithelial PGE2 production by inhibiting COX-2 activity and modulating the macrophage phenotype from proinflammatory to anti-inflammatory. In this pilot randomized double blind placebo controlled study we wish to assess the ability of oral ω3 free fatty acids to downregulate pro-neoplastic and pro-inflammatory pathways in the esophagus. Pilot data from this proposal will enable us to design a larger adequately powered study assessing the role of ω3 free fatty acids as chemoprevention agents in subjects with BE, who are at substantial risk of developing EAC, a lethal cancer with rapidly rising incidence in the United States.

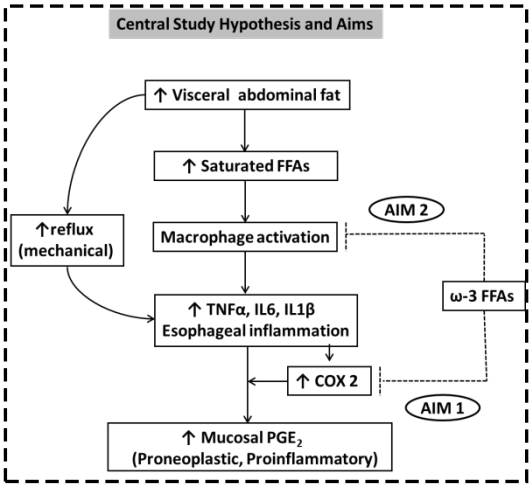
This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 *Background*

BE and visceral abdominal fat: Barrett's esophagus (BE) is characterized by intestinal metaplasia of the esophagus in response to esophageal injury and inflammation [1]. BE is the strongest risk factor and precursor of esophageal adenocarcinoma (EAC) a malignancy with rapidly rising incidence and poor outcomes. In addition to reflux, increased abdominal visceral fat has been identified as an independent risk factor for BE and EAC[2, 3]. Abdominal fat is composed of visceral and subcutaneous fat. Visceral fat may predispose to BE by mechanically increasing reflux and/or by reflux independent mechanisms [4]. We have found that increased visceral abdominal and gastroesophageal junction (GEJ) fat are independently associated with BE, esophageal inflammation and high grade dysplasia (HGD) [5]. Visceral fat is metabolically active, leading to a systemic inflammatory state and the metabolic syndrome. We have also identified Metabolic syndrome as a risk factor for BE, independent of reflux, in a population-based case control study [6], further supporting a reflux independent pathway by which visceral abdominal fat may modulate esophageal inflammation and neoplasia.

Visceral fat, Free Fatty Acids (FFAs) and Macrophage mediated inflammation:

Abdominal visceral fat releases proinflammatory cytokines and saturated FFAs which are known to induce a systemic inflammatory insulin resistant state: which is strongly associated with BE [7, 8]. FFAs function as ligands modulating inflammatory responses, but with different effects. sFFAs exert proinflammatory, while ω3 FFAs exert anti-inflammatory effects [9-12]. sFFAs activate macrophages to a proinflammatory (M1) phenotype [13]. Preliminary data obtained by us show increased levels of serum sFFA in obese BE subjects compared



to controls, as well as higher density of activated macrophages (M1 phenotype) and their products in BE biopsies compared to controls. M1 macrophage produced proinflammatory cytokines (such as TNFα, IL6, IL1β) are known to be overexpressed in BE and are thought to contribute to malignant progression to EAC in BE [14-16].

Anti-inflammatory effects of ω3 FFA: Increased intake of polyunsaturated FFAs and ω3 FFAs in particular, is associated with a 50% lower risk of BE [17]. ω3 FFAs have been shown to have anti-neoplastic activity in the colon. *A major proposed mechanism by which ω3 FFAs exert anti-inflammatory and antineoplastic effect is by modulation of prostaglandin biosynthesis,* by providing an alternate substrate for COX-2 (instead of the ω6 FFA : Arachidonic acid), which leads to decreased PGE2 production, epithelial proliferation and adenoma/aberrant crypt foci number in animal and human trials [9] [18-20]. We have shown in prior studies that increased levels of COX2 are seen with increasing grades of dysplasia in BE and that inhibition of COX-2 activity leads to reduced EAC incidence in an animal model of BE, by reducing PGE2 production [21, 22]. ω3FFAs have also been shown to modulate macrophage phenotype from proinflammatory (M1) to anti-inflammatory (M2). GPR120 has been identified as a likely ω3FFA receptor, mediating the anti-inflammatory and insulin sensitizing effects of ω3FFAs on macrophages and tissue [13]. Our novel central hypothesis is that *oral supplementation with ω3 FFAs in obese BE subjects will lead to downregulation of pro-neoplastic inflammatory pathways in BE by modulating the esophageal macrophage phenotype from proinflammatory to anti-inflammatory.* We propose to test this hypothesis by the following specific aims, using a randomized controlled trial study design:

Specific Aim 1: To estimate the magnitude and variation of changes in esophageal inflammation and injury in BE as measured by tissue PGE2 and the esophageal inflammation score, attributable to ω3 FFA supplementation.

Specific Aim 2: To estimate the magnitude and variation of changes in esophageal macrophage infiltration and modulation of esophageal macrophage phenotype attributable to ω3 FFA supplementation.

Specific Aim 3: To estimate the magnitude and variation in changes in esophageal mucosal impedance attributable to ω3 FFA supplementation.

Specific Aim 4: To define the baseline esophageal mucosal microbiome and the potential change in the esophageal mucosal microbiome attributable to ω3 FFA supplementation.

Significance and Innovation:

The prevalence of BE has been estimated to be 1.6-2.0% in the United States. The incidence of EA has increased exponentially in the Western world in parallel with the prevalence of obesity, now estimated to affect > 30% of the US population. *Radiofrequency ablation has emerged as an alternative modality to reduce the risk of progression to EAC in dysplastic BE, but is expensive, with potential endoscopic complications and a fairly high risk of recurrent IM (30% at 2 years) following successful ablation.* This study uses a safe compound (ω3 FFA) based on a novel biologically plausible, mechanistic hypothesis and strong supportive preliminary data to

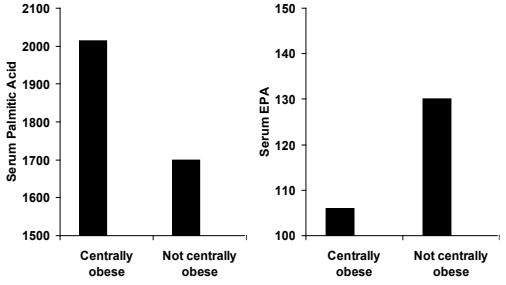
attenuate and inhibit proinflammatory and pro-neoplastic pathways in subjects with BE who are at high risk of developing EAC, a lethal malignancy with rapidly increasing incidence. While used extensively in preclinical and clinical studies in the colon with encouraging results, there is no data on their ability to inhibit proinflammatory and neoplastic pathways in subjects with BE. If successful, this novel trial will lay the ground work for a larger adequately powered trial to demonstrate the use of ω3 FFA as a *novel chemoprevention compound* in subjects with BE to decrease EAC incidence. While inhibition of proinflammatory pathways in BE is possible with NSAIDs (and Aspirin), their use is associated with adverse effects including cerebrovascular and gastrointestinal bleeds even at low doses. ω3 FFA may provide a safer alternative to NSAIDs for chemoprevention in BE. Additionally, given the close association of obesity and esophageal injury, ω3 FFAs could also be used as an adjunct or alternative to proton pump inhibitors (PPIs) in modulating esophageal inflammation and injury, particularly in those patients with an incomplete response to PPIs. This can provide a novel alternative to PPIs in the management of patients with obesity and reflux induced esophageal injury, which is a very prevalent problem in the United States.

Preliminary data:

*1. Increased_visceral and GE junction fat area are independently associated with BE, esophageal inflammation and HGD [5]***.** GE junction, visceral and subcutaneous (SQ) fat area were assessed in age and gender matched 50 BE cases and 50 controls, using CT scans. BE was strongly associated with visceral and GE junction fat area independent of BMI. There was no association with SQ fat area, suggesting a non-mechanical influence of visceral fat on BE pathogenesis. Furthermore, increased visceral fat area (but not SQ fat area) was also independently associated with severe esophageal inflammation and HGD, further suggesting a non- mechanical influence of visceral fat on esophageal inflammation and carcinogenesis.

*2. Metabolic Syndrome is a risk factor for BE independent of reflux[6]***.** We studied the association between BE and metabolic syndrome in a population based case control study: 309 subjects from Olmsted County, MN were studied (103 BE cases, 103 reflux + controls and 103 reflux - controls). 64% of cases, 49% of reflux + controls and 51% of reflux - controls had MetS. MetS was associated with a 2 fold increased risk of BE with reflux (+) controls (OR 1.9, 95% CI 1.1, 3.6, p=0.04) and reflux (–) controls (OR 1.9, 95% CI 1.1, 3.4 p=0.04) on multivariable regression analysis.

Fig 1: Serum saturated and ω3 FFA (EPA) levels in BE subjects with and without central obesity



*3. Centrally obese BE patients have higher serum sFFA levels and lower ω3 FFA levels than age, gender and BMI matched controls***.** Fasting serum from 10 controls without BE and 20 age and gender matched BE cases was analyzed for serum FFA profiles, using mass spectroscopy. Patients with BE had higher saturated FFA and lower ω3 FFA levels than those without BE (figure 1).

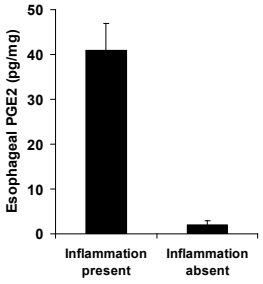
4. Central obesity increases risk of esophagitis and metaplasia. In a population based screening study [23], 60 age, gender and reflux symptom stratified subjects were randomized to evaluation by sedated endoscopy, unsedated transnasal endoscopy or capsule endoscopy. 11 subjects with esophagitis or BE had higher waist circumference and waist hip ratios (measures of central obesity) than those without (table 1).

Table 1: Comparison of anthropometric parameters in subjects with and without esophageal inflammation and metaplasia.

Variable, Mean (SEM)	BE or esophagitis (N=11)	No BE or esophagitis (N=47)
Waist circumference (cm)	100.3 (4.3)	95.8 (2.1)
Waist hip ratio	0.94 (0.02)	0.90 (0.01)
BMI	30.9 (1.7)	29.0 (0.8)

5. Esophageal tissue PGE2 accurately reflects endoscopic and histologic esophageal injury. Tissue esophageal PGE2 levels were measured in biopsies of 10 subjects with no symptoms of, endoscopic or histologic evidence of reflux and 10 subjects with esophagitis or BE. Levels in subjects with esophageal injury (mean 42 pg/mg) were significantly higher than those with no symptoms or signs of esophageal injury (mean 2 pg/mg), p=0.03. (Figure 2).

Fig 2: Esophageal PGE2 in subjects with and without esophageal inflammation



6. BE patients have denser macrophage infiltration than controls. IHC for CD68 and HAM56 (macrophage markers) was performed on esophageal biopsies from 5 BE cases (without endoscopic evidence of esophagitis) and 5 controls.[24] Mean counts of CD68 positive cells in BE were higher compared to controls (p=0.06) (Figure 3).

7. Sub-characterization of macrophages in BE: Increased M1 like macrophages in BE. IHC using double labeling for CD68 and CD206 was performed on esophageal biopsies from subjects with BE [24]. Cells double labeling for CD68 and CD206 are M2 like cells and those labeling for only CD68 are M1 like cells. M2 like macrophages constituted a *minority (30%)* of all CD68 positive cells, indicating a preponderance of M1 like cells in BE tissue (Figure 4)

Fig 3: Columnar metaplasia is associated with increased macrophage infiltration A-C: IF for CD68 on distal esophageal biopsies c (panel A), with BE (panel B) and for HAM56 in BE tissue (panel C) show increased CD68+/HAM56 cell count and stain area in BE cases than controls (panel D is graphical representation of immunostaining).

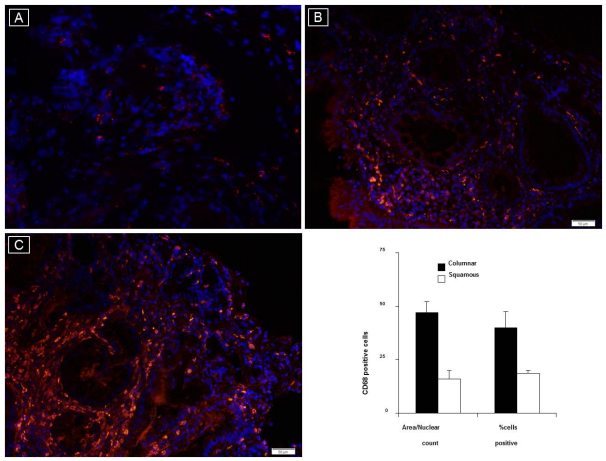
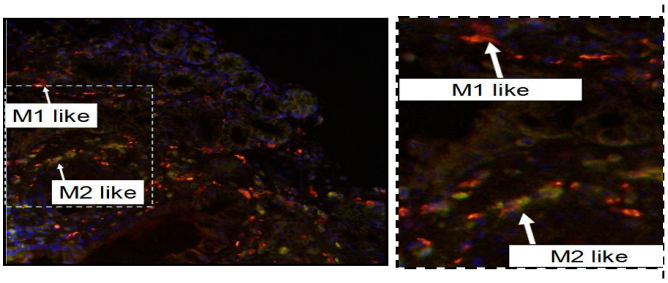
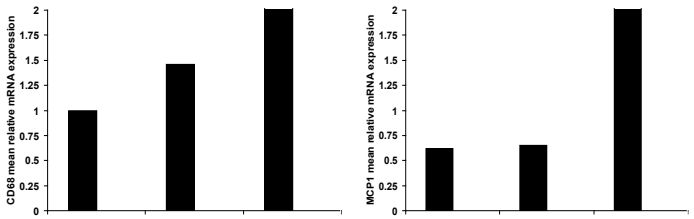


Fig 4: Columnar metaplasia is associated with increased M1 like macrophage infiltration. Immunofluorescence with double labeling for CD68 and CD206 on distal esophageal biopsies from subjects with BE.



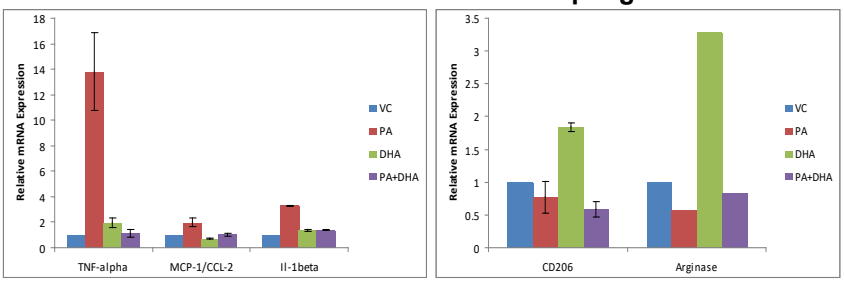
8. *M1 Macrophage markers are more prevalent in BE compared to squamous tissue.* Esophageal biopsies were obtained from patients with and without BE on endoscopy and snap frozen at -80^0 C. RNA was extracted from these biopsies using the RNAase easy kit. qPCR was performed for macrophage (CD68), M1 (MCP-1) and M2 (IL10, arginase) markers. Results show increased expression of CD68 RNA in BE tissue compared to that of normal squamous tissue (p=0.02). MCP1, a M1 macrophage marker was also greater in BE tissue than in squamous tissue (p=0.03) (figure 5).

Fig 5: Higher CD68 and MCP1 (M1 macrophage marker) mRNA in BE compared to squamous esophageal biopsies.
qPCR for CD68 and MCP in squamous and BE biopsies.



9. Influence of saturated and ω3 FFA on macrophages in vitro. THP-1 cell line, a human monocyte cell line was differentiated into macrophages using previously described methods [25]. Macrophages were treated with palmitic acid (PA), a saturated FA or docosahexanoic acid (DHA), a ω3 FFA, or both in combination. RNA was isolated using the RNAase easy kit (Qiagen). qPCR was then performed for macrophage (CD68), M1 (IL1β, MCP-1) and M2 (CD206, arginase) markers. Gene expression analysis was performed by calculating the ΔC_t for each target gene using β actin as the reference gene. As seen in the results from figure 6a and 6b, exposure of macrophages to PA led to a proinflammatory response while administering DHA with PA lead to a substantial blunting of the proinflammatory response. Exposure to DHA led to the induction of M2 markers (CD206 and Arginase) in macrophages.

Fig 6A and 6B: Exposure of macrophages to satFFA leads to a proinflammatory response which is blunted by DHA (an ω3FFA) and induction of M2 macrophage markers.



Hence in these preliminary data we have discovered increased visceral fat, serum saturated FFAs and M1 proinflammatory macrophage infiltration in BE and have found that exposure of macrophages to ω3FAs is able to blunt the proinflammatory and neoplastic cascade induced by saturated FFAs and induce a M2 phenotype. These data help support and justify our hypothesis of Specific Aims 1 & 2 and their feasibility.

2 Study Objectives

Specific Aim 1: To estimate the magnitude and variation of changes in esophageal inflammation and injury in BE as measured by tissue PGE2 and the esophageal inflammation score, attributable to ω3 FFA supplementation.

Specific Aim 2: To estimate the magnitude and variation of changes in esophageal macrophage infiltration and modulation of esophageal macrophage phenotype attributable to ω3 FFA supplementation.

Specific Aim 3: To estimate the magnitude and variation in changes in esophageal mucosal impedance attributable to ω3 FFA supplementation.

Specific Aim 4: To define the baseline esophageal mucosal microbiome and the potential change in the esophageal mucosal microbiome attributable to ω3 FFA supplementation.

3 Study Design

3.1 *General Design*

In this pilot randomized double blind placebo controlled study we wish to assess the ability of oral ω3 free fatty acids to downregulate pro-neoplastic and pro-inflammatory pathways in the esophagus. Pilot data from this proposal will enable us to design a larger adequately powered study assessing the role of ω3 free fatty acids as chemoprevention agents in subjects with BE, who are at substantial risk of developing EAC, a lethal cancer with rapidly rising incidence in the United States. Our plan is to complete the study on 80 participants, 40 in each group randomized to omega-3 or placebo. Subjects between 18-85 years of age, with known BE (no dysplasia and low-grade dysplasia) will be identified using an existing GI database for participation in this study. Invitation letters will be mailed to eligible subjects. Subjects meeting inclusion and exclusion criteria will be screened and consented. Once consent has been obtained baseline assessment and endoscopy with biopsy/brushings, and endoscopic mucosal impedance measurement will be completed. A single slice CT scan of the abdomen will also be obtained at baseline for each subject to quantify visceral and subcutaneous fat area in the abdomen. Subjects will then begin treatment and follow-up for the next 6 months. A final visit for evaluation and collection of tissue samples will be conducted at the end of the study. No risks of any particular seriousness or severity are anticipated. Blood and tissue samples will be obtained and used in this study as outlined in section 3.2. Any remaining samples will be stored in Dr. Iyer -80C freezer located at Joseph M97 and used for future research in Barrett's esophagus.


Eligibility assessment
Baseline assessment
Randomization
Drug X 6m
Placebo X 6m
Final assessment
Final assessment

3.2 *Study Endpoints*

The primary analysis objective in this pilot study is to estimate response magnitude and variation in esophageal inflammation and injury in BE as measured by tissue PGE2 , the esophageal inflammation score, esophageal macrophage infiltration and modulation of esophageal macrophage phenotype attributable to ω3 FFA supplementation. This will be the information needed to design a larger adequately powered study to study the efficacy of w3FFA as a chemoprevention agent in BE.

Aim 1: The **primary response** outcome is tissue PGE2 levels and the main secondary response outcome is the histologic score of 3 or 4 (severe grades of inflammation). Other **secondary outcomes** include pre and post supplementation serum and tissue ω3 FFA levels.

Aim 2: The **primary response** outcome is the cytokine expression profile in esophageal biopsies as measured by qPCR [IL1β, IL6 (M1 markers) versus IL1, IL10 (M2 markers)]. **Secondary response outcomes** include change in esophageal macrophage counts and phenotype proportions (total, %M1, %M2).

Aim 3: The primary response outcome is change in esophageal mucosal impedance (baseline-post treatment) which is a physiologic correlate of the epithelial barrier function, with ω3 FFA supplementation. Secondary outcomes include the association of esophageal mucosal impedance with measures of central obesity (waist circumference, waist hip ratio and abdominal visceral fat area on CT) at baseline and following treatment.

Aim 4: The primary response outcome is the change in the esophageal microbiome in BE subjects following ω3 FFA supplementation. Secondary outcomes will be the association of the microbiome pattern with measures of central obesity ((waist circumference, waist hip ratio and abdominal visceral fat area on CT).

Assays performed at baseline and at 6 months will include:
Aim 1: Primary outcome: Tissue PGE2 in esophageal biopsies. This will be performed by previously described methods [21] on esophageal biopsies snap frozen at -80^0 C in Dr.Buttar's laboratory.

Secondary outcomes: 1. Tissue inflammation score (grade 0-3). This will be performed on esophageal H&E stained sections as previously described [5], by Dr.Lewis, an experienced gastrointestinal pathologist. 2. Esophageal tissue EPA levels: this will be performed on esophageal biopsies obtained at baseline and at 6 months, in the Metabolomics Core laboratory. 3. Serum ω3 FFA levels: will be performed using mass spectroscopy/liquid chromatography in the Immunochemistry Core laboratory at baseline and 6 months.

Aim 2: Primary outcome: 1. Quantification of esophageal macrophage infiltration (and subtypes) by qPCR for CD68 (macrophage marker) TNFά and MCP1 (M1 markers) and CD206,IL1, IL10, as M2 markers). This will be performed in collaboration with Dr. Gores (see preliminary data and letter of support) using previously described standard methodology [29].

Secondary outcomes : Esophageal macrophage counts (using CD68 and HAM 56 as markers) and proportion of M1 and M2 (CD206) using immunofluorescence will be performed using previously described methods [24]. Cells double labeled for CD206 and CD68 will be categorized as M2 cells and those staining only with CD68 will be categorized as M1 cells. Cell counts will be performed as previously described.

Aim 3: Esophageal mucosal impedance measurements will be obtained using a esophageal mucosal impedance catheter at 2 cm, 5 cm and 7 cm above the GEJ. At least one measurement will be obtained in the squamous mucosa at 3 cm above the BE segment [30].

Aim 4: The esophageal microbiome will be characterized from biopsies and brushings from the squamous and columnar epithelium using standard methods.

Sequencing : Biopsy samples and brushings will be collected in cryo-vials and flash frozen in liquid nitrogen. All samples with be stored at -80 till they are ready for processing.
Next Generation sequencing:
Bacterial DNA will be enriched in biopsy and brushings using NEBNext microbiome DNA enrichment kit if concentrations are low. Amplicons spanning the variable region 4 (V4) of bacterial 16SrRNA will be generated using a barcoded reverse primer (515F, 806R). Samples will sequenced using the MiSeq platform at Mayo Genome Facility to obtain 300bp paired end reads. Based on our previous experience we will sequence at most 100 samples in one sequencing run and we expect ~30,000 high quality reads per sample.

3.3 *Primary Safety Endpoints*

ω3 FFAs have an excellent safety and tolerability profile. Most common side effects associated with their use in human trials include diarrhea, indigestion, nausea, fishy taste and belching. Total incidence of GI disturbances in phase 2 and 3 clinical trials has varied between 1.4 – 4.9%, with a very low rate of study termination [27]. The rate of study withdrawal was 3.4% in a phase 3 study using EPA [20].
We will specifically assess the prevalence of adverse effects in subjects randomized to drug or placebo. Adverse effects which have been reported in the literature, may include:

1. Gastrointestinal adverse effects such as: abdominal pain, nausea, vomiting, diarrhea.
2. Altered (fishy) taste in the mouth. The preparation used in this trial using orange oil to mask this effect.
3. Allergic reactions.
4. ω3 FFAs have antiplatelet activity, however increased rates of bleeding have not been seen in large clinical cardiovascular trials [28]. We will specifically assess rates of any mucosal or skin bleeds in monthly telephone calls (1,2,3,4,5 months) and at the 6 month visit.

4 Subject Selection Enrollment and Withdrawal

Male and female patients between 18-85 years of age, with known BE (no dysplasia and low-grade dysplasia) will be identified using an existing GI database for participation in this study.

4.1 *Inclusion Criteria*

- Presence of BE defined as ≥ 1 cm of visible columnar mucosa in the distal esophagus with intestinal metaplasia on histology.
- Absence of high grade dysplasia or EAC on baseline histology.
- BMI > 30 kg/m^2 (this has been shown to correlate strongly with increased abdominal visceral fat)[26] or Waist circumference > 102 cm in men, > 88 cm in women.
- Ability to give informed consent.

4.2 *Exclusion Criteria*

- Allergy to ω3 FFAs, fish or shellfish.
- Presence of high grade dysplasia or cancer on histology.
- Pregnant and or breastfeeding women
- Presence of esophagitis on initial endoscopy or symptoms of refractory GERD (heartburn or regurgitation ≥ 2 times a week) indicative of uncontrolled gastroesophageal reflux.
- Inability to give informed consent.
- Currently taking ω3 FFA as prescription.
- Anti-coagulant therapy (Plavix, Warfarin, Coumadin)
- AST or ALT level > three times upper limit of normal at baseline
- LDL > 200 mg/dl at baseline.
- INR > 2

4.3 *Subject Recruitment, Enrollment and Screening*

Subjects between 18-85 years of age, with known BE (no dysplasia and low-grade dysplasia) will be identified using an existing GI database for participation in this study. Invitation letters will be mailed to eligible subjects. Subjects will be called to discuss study and scheduled for a screening visit. Subjects taking ω3 FFA as a supplement will discontinue use at least four weeks prior to visit 1. Subjects will report fasting 12 hours for this visit. Subjects meeting inclusion and exclusion criteria will be screened and consented. Baseline assessment will include the measurement of height, weight, waist circumference, hip circumference and waist/hip ratio using standard methods of anthropometric measurements. A comprehensive medication list will be obtained. Patients will also fill out the Reflux Symptom Questionnaire (RSQ)/GER Questionnaire a validated instrument to assess reflux symptoms All patients will continue their baseline PPI regimen to control gastroesophageal reflux. 20cc of blood will be drawn after 12 hours of fasting to obtain a baseline serum FFA profile . Clinical baseline blood will be drawn for inclusion/exclusion: INR, LDL, AST and ALT. Endoscopy will then be performed in a standard manner. Six biopsies will be obtained from the GE junction, and seven biopsies from the BE mucosa 1 cm above the GE junction. Two biopsies from each site will be formalin fixed and paraffin embedded (for inflammatory score assessment) and the remainder frozen at -80 C for subsequent analysis. An additional seven biopsies will be obtained from the squamous mucosa at least 3 cm above the proximal end of the BE segment. Two will be placed in formalin (or other fixative) and paraffin embedded (IHC for macrophage cell counts) and the remainder will be snap frozen at -80^0 C (PGE2 levels). Endoscopic brushings of the Barrett's segment as well as squamous tissue will be obtained and frozen for each subject. Endoscopic mucosal impedance measurement will be completed during endoscopy. A single slice CT scan of the

abdomen will also be obtained at baseline for each subject to quantify visceral and subcutaneous fat area in the abdomen.

Patients will be randomized to either a ω3 FFA preparation or a matching placebo for *six months*. A randomization schedule will be generated in the Division of Biomedical Statistics and Informatics and sent to the research pharmacy. Investigators and patients will be blinded to drug versus placebo assignment and only research pharmacy personnel and statisticians will have access to the assignments until the study is completed. Medications (drug and matching placebo) will be dispensed through the Mayo research pharmacy. Phone calls will be made by the study coordinator every month to the patients to assess for any adverse effects (presence and grade) and reinforce compliance. Compliance will also be assessed by pill counts during monthly call.

Patients will undergo repeat assessment at six months following randomization. This will include anthropometry (similar to baseline), a fasting blood draw for serum FFA profile, LDL, AST, ALT, INR and endoscopy with research biopsies as outlined at baseline. Subjects will also fill out the Reflux Symptom Questionnaire (RSQ)/GER Questionnaire. Investigators and patients will be blinded to drug versus placebo assignment. Both endoscopic procedures will be conducted in the outpatient CTSA unit on Charlton 7.

5 Study Drug

5.1 *Description*

Drug and placebo:
The DHA/EPA and placebo softgels will be supplied by Sancilio and Company, Inc. (Riviera Beach, FL) and stored in the Mayo Clinic Research Pharmacy at room temperature. Active drug will consist of 1200 mg of a ω3 FFA preparation containing 675 mg EPA and 300 mg DHA. Patients randomized to active drug will be placed on 3 capsules a day of this preparation. A matching placebo (1200 mg of ethyl oleate 3 capsules a day) will be provided to subjects randomized to placebo. Both preparations will contain orange oil to mask the gustatory effect of fish oils. Both drug and placebo will be provided by Sancilio & Company

5.2 *Treatment Regimen*

Investigators, patients and statisticians will be blinded to drug versus placebo assignment. Patients randomized to active drug will be placed on 3 capsules a day of this preparation taken orally for six months. A matching placebo (1200 mg of ethyl oleate 3 capsules a day) will be provided to subjects randomized to placebo taken orally for six months. Both groups will take 2 capsules with breakfast and 1 capsule with their evening meal.

5.3 *Method for Assigning Subjects to Treatment Groups*

A randomization schedule will be generated in the Division of Biomedical Statistics and Informatics and sent to the research pharmacy. Investigators and patients will be blinded to drug versus placebo assignment and only research pharmacy personnel and statisticians will have access to the assignments until the study is completed. Medications (drug and matching placebo) will be dispensed through the Mayo research pharmacy.

5.4 *Subject Compliance Monitoring*

Phone calls will be made by the study coordinator every month to the patients to assess for any adverse effects (presence and grade) and reinforce compliance. Compliance will also be assessed by pill counts during this telephone call. Adverse effects mentioned in section 3.3 will be specifically assessed in the phone calls (see phone call script).

5.5 *Prior and Concomitant Therapy*

A complete list of medications which the patient is currently consuming (both prescription and over the counter) will be obtained at the baseline visit.
Patients currently taking ω3 FFA s as prescription medications will be excluded from the study.

5.6 *Receiving, Storage, Dispensing and Return*

The research pharmacy will maintain the double-blind status of the study until all of the main outcomes have been measured. The DHA/EPA and placebo softgels will be supplied by Sancilio and Company, Inc. (Riviera Beach, FL) and stored in the Mayo Clinic Research Pharmacy at room temperature. The research pharmacy will assign participants to treatment or placebo based on a randomization table prepared by a statistician. Each participant will be instructed to swallow 2 softgels in the morning and 1 in the evening with meals (morning and evening) for a total of 3 softgels per day. The DHA/EPA softgels will each contain 1200mg of DHA/EPA for a total daily dosage of 3.6g/day. The placebo softgel will contain 1200mg of oleate. The pharmacy will maintain records of receipt, dispensation, and return pill counts for compliance.

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 *Visit 1*

Subjects taking ω3 FFA as a supplement will discontinue use at least four weeks prior to visit 1. Subjects will report fasting 12 hours to the Charlton CRU and have a brief consultation with a gastroenterologist specializing in Barrett's esophagus. Baseline assessment will include the measurement of height, weight, waist circumference, hip circumference and waist/hip ratio using standard methods of anthropometric measurements. A comprehensive medication list will be obtained. Subjects will also fill out the Reflux Symptom Questionnaire (RSQ)/GER Questionnaire a validated instrument to assess reflux symptoms. All subjects will continue their baseline PPI regimen to control gastroesophageal reflux. 20cc of blood will be drawn after 12 hours of fasting to obtain a baseline serum FFA profile. Clinical baseline blood will be drawn for inclusion/exclusion: INR, LDL, AST and ALT. Endoscopy will then be performed in a standard manner. Brushings will be obtained from the Barrett's segment as well as squamous tissue. Biopsies will be obtained from the GE junction, from the BE mucosa 1 cm above the GE junction and from the squamous mucosa 3cm above the proximal Barrett's segment. Endoscopic mucosal impedance measurement will be completed as described in the methods section above. A single slice CT scan of the abdomen will also be obtained at baseline for each subject.

Subjects will be randomized to either a ω3 FFA preparation or a matching placebo for *six months*. A 3 month supply of study medication will be given to subjects at randomization. Telephone calls will be made by the study coordinator every month to the subjects to assess for any adverse effects (presence and grade) and reinforce compliance. Compliance will also be assessed by pill counts during this call. An additional 3 month supply of study medication will be mailed to subject by research pharmacy after the 2 month telephone call.

6.2 *Visit 2*

Subjects will undergo repeat assessment at six months following randomization. This will include anthropometry (similar to baseline), a fasting blood draw and endoscopy with research biopsies and brushings as outlined at baseline. Subjects will also fill out the Reflux Symptom Questionnaire (RSQ)/GER Questionnaire. Remaining medication will be counted and recorded.

If the participant is to be scheduled for a clinically indicated upper endoscopy and procedures that are ordered cannot be done on the Clinical Research Unit, (i.e. radiofrequency ablation, endoscopic mucosal resection, esophageal dilation, endoscopic ultrasound) the research portion of the procedure will be done during the clinically indicated upper endoscopy. These research procedures will include biopsies and brushings of the esophagus as well as mucosal impedance.

Schedule of Events

Study Activity	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 6 + 7 days
Informed consent	X							
History	X							
Concurrent meds	X							
Physical exam (ht, wt, hip/waist, VS)	X						X	
Blood draw (FFA, LDL, AST, ALT) INR)	X						X	
Endoscopy with bx/brushing and mucosal impedance measurement	X						X	
Randomization	X							
Adverse event evaluation		X	X	X	X	X	X	X
Pill count		X	X	X	X	X	X	
Single slice CT scan of abdomen	X							
Questionnaire	X						X	

7 Statistical Plan

7.1 *Sample Size Determination*

Statistical Analysis

Up to 90 patients with Barrett's will be identified and randomized to either drug or placebo. The primary analysis objective in this pilot study is to estimate response magnitude and variation which will be the information needed to design a larger adequately powered study to study the efficacy of w3FFA as a chemoprevention agent in BE. The data will be summarized as mean, standard deviation, as well as median, and interquartile range, overall (baseline) and by treatment group (post-supplementation at 6 months). In addition, 95% confidence intervals for response mean (or median) treatment group values and 95% confidence intervals for treatment group differences in means (or medians) will also be calculated. Exploratory descriptive summaries of the response data will also be generated after stratifying on ASA intake.

Aim 1: The primary response outcome is tissue PGE2 levels and the main secondary response outcome is the histologic score of 3 or 4 (severe grades of inflammation). Other secondary outcomes include pre and post supplementation serum and tissue ω3 FFA levels.

Aim 2: The primary response outcome is the Cytokine expression profile in esophageal biopsies as measured by qPCR [IL1β, IL6 (M1 markers) versus IL1, IL10 (M2 markers)]. Secondary response outcomes include change in esophageal macrophage counts and phenotype proportions (total, %M1, %M2).

Aim 3: The esophageal mucosal impedance of the squamous epithelium and columnar epithelium will be summarized as mean (SD) or median (IQR) depending on data normality. The data will be summarized (baseline and post-supplementation) by treatment group. Change in impedance (Baseline – post supplementation) will be compared using appropriate statistical tests. Secondary outcome analysis will include testing the association between esophageal mucosal impedance and measures of central obesity (including waist circumference, waist hip ratio and abdominal visceral fat area on CT scan).

Aim 4:
We will align the paired end reads using PANDASeq given our small amplicon size (~392bp) which allows for significant overlap in the two reads. Data will be processed using QIIME1.8.0 (Quantitative Insights Into Microbial Ecology) analysis pipeline. OTUs (Operational Taxonomic Units) will be determined at 97% sequence similarity using uclust, taxonomy will be assigned using RDP classifier against the GreenGenes database, and a phylogenetic tree will be built using FastTree. The OTU table will be rarified at a single sequencing depth. We will compare alpha diversity using both phylogenetic (PD whole tree) and non-phylogenetic measures (Shannon, observed species and Chao1) to assess evenness and richness of distribution. We will compare beta diversity using both phylogenetic (UniFrac) and count based metrics (Bray-Curtis).

Statistical analysis of differences between groups and changes within the microbiome will be performed such as using PERMANOVA to compare beta diversity. Differences in individual taxa between groups will be assessed using ANOVA with correction for multiple hypotheses testing using FDR. A p-value of <0.05 will be considered statistically significant. Additional

analysis to impute potential changes in gene content (PICRUSt) and pathways (HUMAnN) will be done depending on the results of initial analysis.

Pitfalls and Alternative approaches: It is possible that recruitment to this trial may be challenging. However, more than 400 patients with BE are seen annually in Rochester and the PI has successfully led and participated in many prospective studies which have recruited BE patients for clinical trials. It also possible, though unlikely, that the in-vitro effects of ω3 FFAs may not translate into clinical effects in terms of alterations in tissue PGE2 and macrophage counts. We will also consider assessing the influence of ω3 FFA on BE cell survival, a known biological correlate to neoplastic progression in BE, using Ki-67-Caspase dual staining to assess other biologic effects of ω3 FFA. Concomitant ASA use may confound the effects on PGE2 levels. Given that this is a preliminary study, we will collect data on ASA and NSAID use and perform analysis stratified on ASA and NSAID use. The randomized study design will however mitigate this confounding effect to a large extent. It is possible that the dose of ω3 FFA used in this proposal may be inadequate for a clinical effect in vivo. We have chosen the 3.6 grams/day dose as described in prior human clinical trials: this dose has been shown to be well tolerated and able to produce meaningful clinical response in human trials. We also have proposed to target subjects with a BMI>30 given that these subjects with increased visceral fat are most likely to benefit from ω3 FFAs.

Feasibility and Timeline: We anticipate recruitment to be complete in 6-9 months following IRB approval (which has been initiated). Assays and analysis should be completed in 3 months following completion of the trial and publication thereafter. Assays have been previously performed and validated in multiple published manuscripts by collaborators and Core laboratories. Preliminary data obtained from this trial will be crucial in supporting an aim of a R01 application focused on assessing the mechanistic relationship between BE and central obesity.

8 Safety and Adverse Events

Adverse event grading
Attribution scale. An adverse event is defined as both an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. All events will be graded as to their attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any event that is reported to either the principal investigator or his designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such.

Adverse Events
Safety of ω3 FFA: ω3 FFAs have an excellent safety and tolerability profile. Most common side effects associated with their use in human trials include diarrhea, indigestion, nausea, fishy taste and belching. Total incidence of GI disturbances in phase 2 and 3 clinical trials has varied between 1.4 – 4.9%, with a very low rate of study termination [27]. The rate of study withdrawal

was 3.4% in a phase 3 study using EPA [20]. ω3 FFAs have antiplatelet activity, however increased rates of bleeding have not been seen in large clinical cardiovascular trials [28].

Risk Monitoring/Risk Reduction:
The risk of anaphylactic reactions will be reduced by excluding any potential participants with a history of allergies to fish or shellfish. Participants will be instructed to immediately discontinue the study medication and contact the study team if they experience any signs of allergic reaction (i.e., rash, swelling, itching, cramps, wheezing, upset stomach, loose stool, nasal congestion, fainting, dizziness). If the investigators suspect that such symptoms are due to the EPA/DHA, the participant will be excluded from the study and referred to the appropriate health care provider.

8.1 *Definitions*

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

In case of a serious adverse event, the PI or a member of the study team will contact the research pharmacy for unblinding of the randomization code.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as *7 days* following the last administration of study treatment.

8.2 *Recording of Adverse Events*

The study team will assess subjects for adverse events monthly via telephone. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form. The clinical course of each event will be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period will be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 *Reporting of Serious Adverse Events and Unanticipated Problems*

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

All adverse events will be reported to the Mayo Clinic IRB.
Serious adverse events are defined as:

- Death
- Life Threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

The PI will report serious adverse events to the chair of the IRB using the Serious Adverse Event (SAE) Reporting Form. The PI will sign each Serious Adverse Event form. A copy of the current consent form will be submitted with the actual risk highlighted in the current consent form. Or, if the PI recommends any changes to the consent form document, a printed and electronic revised consent form will be attached.

The IRB office will review the incoming reports and triage according to the nature of the event:

- Reports of serious adverse events that resulted in taking immediate action will be given first priority.
- Reports of serious adverse events for which the PI recommends changes to the consent form document will be given 2nd priority.
- All other reports will then be prioritized.

The members will review SAE form and supporting materials. If additional information is necessary, the IRB will contact the PI (or study coordinator if the PI is unavailable) by conference call. After review of the information, the IRB will make an initial determination of the seriousness of the event and determine what actions, if any will be required.

FDA regulations do not require non-serious adverse events (those that do not fall into the categories outlined in step 3 above) to be reported to the IRB. If non-serious adverse events are reported to the IRB, these reports will be signed by a member of the Subcommittee and returned to the investigator. The IRB will not retain a copy of these materials in the IRB office or files.

For serious adverse events that the IRB determines to be unrelated to the study drug/device/intervention, the original report and supporting materials will be kept in the IRB file. The IRB will review such reports, and if the IRB agrees with the determination that the event was unrelated to study drug/device/intervention, then a copy of the report will be returned to the PI. The IRB office will retain a list of the studies for which unrelated serious adverse events are reported. Serious but unrelated adverse event reports will not be included in the minutes of the meeting.

For all serious adverse event reports that are determined by the IRB to be definitely, probably, or possibly related to the study drug/device/intervention, or if it is unknown what the relationship is at the present time, the IRB will review the reports and related materials and include in the minutes of the meeting. The IRB meeting minutes will be referred to the Full Board for final action. For these adverse events which are unexpected and require a change in the consent form, the Chair or a Vice-Chair will be the primary reviewer upon referral to the Full Board.

The convened IRB shall take whatever action(s) it deems appropriate. These actions may include but are not limited to:

- modification of the protocol
- modification of the consent form document
- modification to the timetable for continuing review requirements,
- suspension of new enrollment into the study
- suspension of the study, or
- termination of the study.

Any studies that are suspended or terminated will be promptly reported to NIH that has provided funding for the study and/or to the FDA if the study involves an IND or an IDE.

All other events not requiring suspension or termination shall be reported to the FDA through the normal reporting channel (notification from the investigator to the sponsor to the FDA).

The convened IRB will generate a subsequent minute excerpt only if additional action is taken (i.e. approval of revised consent form, revisions to the protocol, etc.).

For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 *Stopping Rules*

Definite termination criteria:

- Request by subject to leave study.
- Evidence of deliberate non-compliance.
- Pregnancy
- Alcohol abuse; illicit drug abuse.

Potential termination criteria include:

- Development of acute or chronic condition that may impact on metabolic variables or requiring medications likely to impact on metabolic variables or likely to result in subject being unable to participate. Subjects will be reviewed on a subject-by-subject basis and all subjects reported to the Safety-Monitoring Panel.

Specific Action plans are pre-assigned for:

- Depression or low mood. Here, all patients will be asked to be evaluated by their primary

care physician and if appropriate referred to a psychiatrist. The opinion of the psychiatrist will be used to determine continuance or termination in the protocol.

This does not represent a comprehensive listing of criteria or causes. All subjects who withdraw or whom are withdrawn from the study or whom are considering/being consider for withdrawal will be referred to the Safety-Monitoring Panel. These subjects data will be scrutinized (whether terminated or not), separately to assess for association of intervention with a specific adverse outcome(s).

8.5 *Medical Monitoring*

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Internal Data and Safety Monitoring Board

This will consist of Dr. Elizabeth Rajan and Dr. Michael Levy (both Staff Gastroenterologists at Mayo Clinic, Rochester). They will meet with the PI every 6 months to review recruitment and any adverse effects in subjects recruited into the study.

9 Data Handling and Record Keeping

9.1 *Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

The following study specific measures will be taken:

- Protection of subject privacy: Medical history and physical examination are performed, and a brief questionnaire is administered. Blood is drawn for screening purposes. All of these materials are obtained for research purposes only, and data are kept in strict confidence. No information will be given to anyone without permission from the subject. Our consent form includes the Informed Consent statement required by Mayo for these types of studies. This statement guarantees the confidentiality.

- Database protection: The database is secured with password protection behind the Mayo Firewall and accessible only to study team. Electronic communication with our outside collaborators involves only coded, unidentifiable information. Subject folders are kept in institutionally secured office rooms and laboratory.
- Confidentiality during adverse event reporting: Adverse event reports and annual summaries will not include subject-identifiable material.

9.2 *Source Documents*

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 *Records Retention*

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

All subject-specific data and Case Report Forms will be coded using a unique study number for each individual subject. Confidentiality of all medical records is strictly maintained by established procedures. The original study data are kept in the principal investigators' laboratory/office and are entered into a secure computer database password protected under a secure server space behind the Mayo firewall allocated for use by only the study team. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" Whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 *Study Monitoring Plan*

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide clinical monitoring for the trial as a service for the sponsor-investigator. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the

protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration regulations.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 *Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-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. This 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 Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 *Funding Source*

This study is financed through the Mayo Foundation Division of Gastroenterology.

12.2 *Subject Stipends or Payments*

Subjects completing the study will be given $250 remuneration.

13 References

1. **Sharma, P., *Clinical practice. Barrett's esophagus.* N Engl J Med, 2009. 361(26): p. 2548-56.**
2. **Corley, D.A., et al., *Abdominal obesity and body mass index as risk factors for Barrett's esophagus.* Gastroenterology, 2007. 133(1): p. 34-41; quiz 311.**
3. **Edelstein, Z.R., et al., *Central adiposity and risk of Barrett's esophagus.* Gastroenterology, 2007. 133(2): p. 403-11.**
4. **El-Serag, H., *Role of obesity in GORD-related disorders.* Gut, 2008. 57(3): p. 281-4.**
5. **Nelsen, E.M., et al., *Distribution of Body Fat and Its Influence on Esophageal Inflammation and Dysplasia in Patients With Barrett's Esophagus.* Clin Gastroenterol Hepatol, 2012.**
6. **Cadman, L., et al., *Metabolic Syndrome is a Risk Factor for Barrett's Esophagus: A Population Based Case Control Study.* Gastroenterology, 2011. 140(5): p. S178-S178.**
7. **Ryan, A.M., et al., *Barrett esophagus: prevalence of central adiposity, metabolic syndrome, and a proinflammatory state.* Ann Surg, 2008. 247(6): p. 909-15.**
8. **Greer, K.B., et al., *Association of insulin and insulin-like growth factors with Barrett's oesophagus.* Gut, 2011.**
9. **Cockbain, A.J., G.J. Toogood, and M.A. Hull, *Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer.* Gut, 2012. 61(1): p. 135-49.**
10. **Odegaard, J.I. and A. Chawla, *Mechanisms of macrophage activation in obesity-induced insulin resistance.* Nat Clin Pract Endocrinol Metab, 2008. 4(11): p. 619-26.**
11. **Odegaard, J.I., et al., *Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance.* Nature, 2007. 447(7148): p. 1116-20.**
12. **Odegaard, J.I., et al., *Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance.* Cell Metab, 2008. 7(6): p. 496-507.**
13. **Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-98.**
14. **Tselepis, C., et al., *Tumour necrosis factor-alpha in Barrett's oesophagus: a potential novel mechanism of action.* Oncogene, 2002. 21(39): p. 6071-81.**
15. **Zhang, H.Y., et al., *Cancer-related inflammation and Barrett's carcinogenesis: interleukin-6 and STAT3 mediate apoptotic resistance in transformed Barrett's cells.* Am J Physiol Gastrointest Liver Physiol, 2011. 300(3): p. G454-60.**
16. **Dvorakova, K., et al., *Increased expression and secretion of interleukin-6 in patients with Barrett's esophagus.* Clin Cancer Res, 2004. 10(6): p. 2020-8.**
17. **Kubo, A., et al., *Effects of dietary fiber, fats, and meat intakes on the risk of Barrett's esophagus.* Nutr Cancer, 2009. 61(5): p. 607-16.**
18. **Anti, M., et al., *Effects of different doses of fish oil on rectal cell proliferation in patients with sporadic colonic adenomas.* Gastroenterology, 1994. 107(6): p. 1709-18.**
19. **Cheng, J., et al., *Increased intake of n-3 polyunsaturated fatty acids elevates the level of apoptosis in the normal sigmoid colon of patients polypectomized for adenomas/tumors.* Cancer Lett, 2003. 193(1): p. 17-24.**
20. **West, N.J., et al., *Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis.* Gut, 2010. 59(7): p. 918-25.**
21. **Buttar, N.S., et al., *The effect of selective cyclooxygenase-2 inhibition in Barrett's esophagus epithelium: an in vitro study.* J Natl Cancer Inst, 2002. 94(6): p. 422-9.**

22. **Buttar, N.S., et al., *Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus.* Gastroenterology, 2002. 122(4): p. 1101-12.**
23. **Chang, J.Y., et al., *Population screening for barrett esophagus: a prospective randomized pilot study.* Mayo Clin Proc, 2011. 86(12): p. 1174-80.**
24. **Choi, K.M., et al., *CD206-positive M2 macrophages that express heme oxygenase-1 protect against diabetic gastroparesis in mice.* Gastroenterology, 2010. 138(7): p. 2399-409, 2409 e1.**
25. **Park, E.K., et al., *Optimized THP-1 differentiation is required for the detection of responses to weak stimuli.* Inflammation research : official journal of the European Histamine Research Society ... [et al.], 2007. 56(1): p. 45-50.**
26. **Alberti, K.G., P. Zimmet, and J. Shaw, *Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation.* Diabet Med, 2006. 23(5): p. 469-80.**
27. ***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(9177): p. 447-55.**
28. **Harris, W.S., *Expert opinion: omega-3 fatty acids and bleeding-cause for concern?* Am J Cardiol, 2007. 99(6A): p. 44C-46C.**
29. **Malhi, H., et al., *Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis.* J Biol Chem, 2006. 281(17): p. 12093-101.**30. **Katzka DA, Ravi K, Geno DM, Smyrk TC, Iyer PG, Alexander JA, Mabary JE,Camilleri M, Vaezi MF. *Endoscopic Mucosal Impedance Measurements Correlate With Eosinophilia and Dilation of Intercellular Spaces in Patients With Eosinophilic Esophagitis. Clin* Gastroenterol Hepatol. 2015 Jan 13. pii: S1542-3565(15)00009-9. doi:10.1016/j.cgh.2014.12.032. [Epub ahead of print] PubMed PMID: 25592662**