VASCEPA TO ACCELERATE LIPOPROTEIN UPTAKE AND ELIMINATION (THE VALUE STUDY): AN OPEN-LABEL, MECHANISTIC, RANDOMIZED, CONTROLLED, SINGLE-CENTER, ADAPTIVE-DESIGN TRIAL OF ICOSAPENT ETHYL OIL IN DYSLIPIDEMIC PATIENTS

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Study Product: Icosapent Ethyl Oil (AMR101)

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Study Summary

Title	Vascepa to Accelerate Lipoprotein Uptake and Elimination (The VALUE Study): An Open-Label, Mechanistic, Randomized, Controlled, Single-Center, Adaptive-Design Trial of Icosapent Ethyl Oil in Dyslipidemic Patients
Short Title	The VALUE Study
Protocol Number	AMR-01-01-0024
Phase	I *
Methodology	Small-scale, mechanistic randomized controlled clinical pilot experiment comparing the EPA-rich oil Icosapent Ethyl Oil (Vascepa) to usual care among triglyceridemics. Based on the pilot results, the study may be adapted to a definitive hypothesis-testing experiment.
Study Center(s)	Single Center
Objectives	Develop statistical parameters to design a definitive study to test the following mechanistic hypotheses: EPA-rich oil retards VLDL production rate vs usual care EPA-rich oil enhances hepatic clearance of VLDL vs usual care
Number of Subjects	19 subjects for the pilot experiment
Diagnosis and Main Inclusion Criteria	Caucasian triglyceridemics lacking extreme triglyceridemia (i.e. TG < 500 mg/dL)
Study Product, Dose, Route, Regimen	The EPA-rich oil Icosapent Ethyl Oil (Vascepa), two capsules by mouth twice daily, with meals. Subjects not on statins must start a statin to participate.
Duration of administration	At least 16 weeks of Icosapent Ethyl Oil and if starting a statin for the study, at least 20 weeks on the statin. Total duration of participation will be 16 to 28 weeks depending on the length of the screening period.
Reference therapy	Usual care without lipid-altering oils plus background statin
Statistical Methodology	The focus of the pilot phase is to develop estimates of critical statistical parameters, including central tendency and variability. If the study progresses to the confirmatory phase, in general hypothesis testing will be by mixed effects linear regression with group and time as fixed effects and subject as a random effect with the alpha expended below 0.05 to account for the interim look.

*Although the experimental intervention is already approved for marketing, the pilot nature, small sample size, and mechanistic purpose meet the regulatory definition of Phase I (cf. 21 CFR § 312.21 and discussion below).

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Fish oil is used to treat triglyceridemia, with the short-term goal of lowering risk of pancreatitis in those with extreme triglyceridemia, and as an emerging target, the longer-term goal of reducing risk for atherosclerosis.[1, 2] Fish oil that provides a therapeutic dose of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) lowers fasting triglycerides and postprandial triglycerides, and importantly, postprandial triglycerides are an independent risk factor for coronary heart disease (CHD) events. This is probably because the triglyceride-rich lipoproteins (TRLs) and especially their compact remnants also bear cholesterol, and are therefore atherogenic to the extent that their cholesterol contributes to arterial plaque. Accordingly, suppressing fasting and especially postprandial triglyceridemia is expected to benefit atherosclerosis and in turn reduce the incidence of CHD events. This concept is currently being tested in a landmark randomized clinical trial using Vascepa, a highly purified EPA-only omega-3 oil. Triglyceride-rich lipoproteins are largely a postprandial phenomenon, as alimentary fat is packaged as chylomicrons (CMs). Since we spend most of the day in the postprandial state, this is actually the dominant physiology. As such, it is not surprising the postprandial triglyceridemia is superior to fasting triglycerides as an indicator of CHD risk.[3, 4] In the post-absorptive state, VLDL is the major TRL, whose TG-depleted remnants are cholesterol-rich and therefore atherogenic. Because CM's and VLDL compete for lipoprotein lipase (LPL), postprandial chylomicronemia retards VLDL catabolism. Several common medical conditions involve postprandial triglyceridemia, including the atherogenic dyslipidemic triad and conditions associated with insulin resistance, including obesity, metabolic syndrome, pre-diabetes, and diabetes.

1.2 Investigational Agent

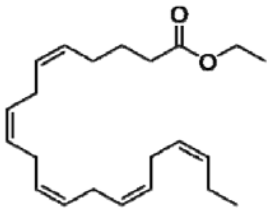
Most fish oils combine two types of oil: EPA and DHA. The EPA+DHA preparations typically include roughly equal concentrations of each, or at least neither has a decisive majority. Vascepa is unique in that it is highly enriched with EPA with very minor amounts of DHA, effectively constituting a pure EPA preparation. Though the EPA in Vascepa is derived from fish, the EPA is highly-refined and may not share the adverse event profile typical of other approved drugs in this class. Between EPA and DHA, EPA is thought to be the more active of the two in terms of remediating dyslipidemia. Remarkably, the EPA-rich fish oil differs from EPA+DHA combinations in that it does not raise LDL cholesterol (LDL-c) as EPA+DHA does, thus implicating DHA in the rise in LDL-c.[5] Increased LDL-c is a disconcerting side effect for a strategy that seeks to reduce CHD, since elevated LDL-c is a potent risk for atherosclerosis. Thus, apart from preventing pancreatitis, it seems counterintuitive to accept an increase in LDL-c from combination EPA+DHA fish oil products. On the other hand, the EPA-rich preparation may be optimal because it does not increase LDL-c.

AMR101 is a highly purified EPA-only oil and is the non-branded (i.e. not possessing any ink/markings on the capsule) version of Vascepa; the latter is commercially available for the treatment of very high TG levels. The following information is paraphrased or excerpted from the Vascepa Prescribing Information:

1.2.1 Description

VASCEPA, a lipid-regulating agent, is supplied as a 1-gram amber-colored, liquid-filled soft gelatin capsule for oral administration.

Each VASCEPA capsule contains 1 gram of icosapent ethyl. Icosapent ethyl is an ethyl ester of the omega-3 fatty acid eicosapentaenoic acid (EPA). The empirical formula of icosapent ethyl is $C_{22}H_{34}O_2$ and the molecular weight is 330.51. The chemical name for icosapent ethyl is ethyl all-cis-5,8,11,14,17-icosapentaenoate with the following chemical structure:



1.2.2 Clinical Pharmacology

1.2.2.1 Mechanism of Action

Studies suggest that EPA reduces hepatic very low-density lipoprotein triglycerides (VLDL-TG) synthesis and/or secretion and enhances TG clearance from circulating VLDL particles. Potential mechanisms of action include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase (DGAT); decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity.

1.2.2.2 Pharmacokinetics

Absorption: After oral administration, VASCEPA is de-esterified during the absorption process and the active metabolite EPA is absorbed in the small intestine and enters the systemic circulation mainly via the thoracic duct lymphatic system. Peak plasma concentrations of EPA were reached approximately 5 hours following oral doses of VASCEPA. VASCEPA was administered with or following a meal in all clinical studies; no food effect studies were performed. Take VASCEPA with or following a meal.

Distribution: The mean volume of distribution at steady-state of EPA is approximately 88 liters. The majority of EPA circulating in plasma is incorporated in phospholipids, triglycerides and cholesteryl esters, and <1% is present as the unesterified fatty acid. Greater than 99% of unesterified EPA is bound to plasma proteins.

Metabolism and Excretion: EPA is mainly metabolized by the liver via beta-oxidation similar to dietary fatty acids. Beta oxidation splits the long carbon chain of EPA into acetyl Coenzyme A, which is converted into energy via the Krebs cycle. Cytochrome P450-mediated metabolism is a minor pathway of elimination of EPA. The total plasma clearance of EPA at steady state is 684 mL/hr. The plasma elimination half-life ($t_{1/2}$) of EPA is approximately 89 hours. VASCEPA does not undergo renal excretion.

1.2.2.3 Drug-Drug Interactions

VASCEPA was studied at the 4 g/day dose level with the following medications which are typical substrates of cytochrome P450 enzymes, and no drug-drug interactions were observed:

Omeprazole: In a drug-drug interaction study with 28 healthy adult subjects, VASCEPA 4 g/day at steady-state did not significantly change the steady-state AUC_T or C_{max} of omeprazole when co-administered at 40 mg/day to steady-state.

Rosiglitazone: In a drug-drug interaction study with 28 healthy adult subjects, VASCEPA 4 g/day at steady-state did not significantly change the single dose AUC or C_{max} of rosiglitazone at 8 mg.

Warfarin: In a drug-drug interaction study with 25 healthy adult subjects, VASCEPA 4 g/day at steady-state did not significantly change the single dose AUC or C_{max} of *R*- and *S*- warfarin or the anti-coagulation pharmacodynamics of warfarin when co-administered as racemic warfarin at 25 mg.

Atorvastatin: In a drug-drug interaction study of 26 healthy adult subjects, VASCEPA 4 g/day at steady-state did not significantly change the steady-state AUC_T or C_{max} of atorvastatin, 2-hydroxyatorvastatin, or 4-hydroxyatorvastatin when co-administered with atorvastatin 80 mg/day to steady-state.

1.2.2.4 Specific Populations

Gender: When administered VASCEPA in clinical trials, plasma total EPA concentrations did not differ significantly between men and women.

Pediatric: The pharmacokinetics of VASCEPA has not been studied in pediatric patients.

Hepatic or Renal Impairment: VASCEPA has not been studied in patients with renal or hepatic impairment.

SOURCE: Vascepa Prescribing Information, 1/2015

1.3 Preclinical Data

This section is not pertinent, as EPA-rich oil is already approved for human use and related fish oils enjoy a long history of use for the population under investigation. Accordingly, the observations that motivated the present mechanistic study are from clinical studies. Likewise, there are sufficient safety and tolerability data from man at this point.

1.4 Clinical Data to Date

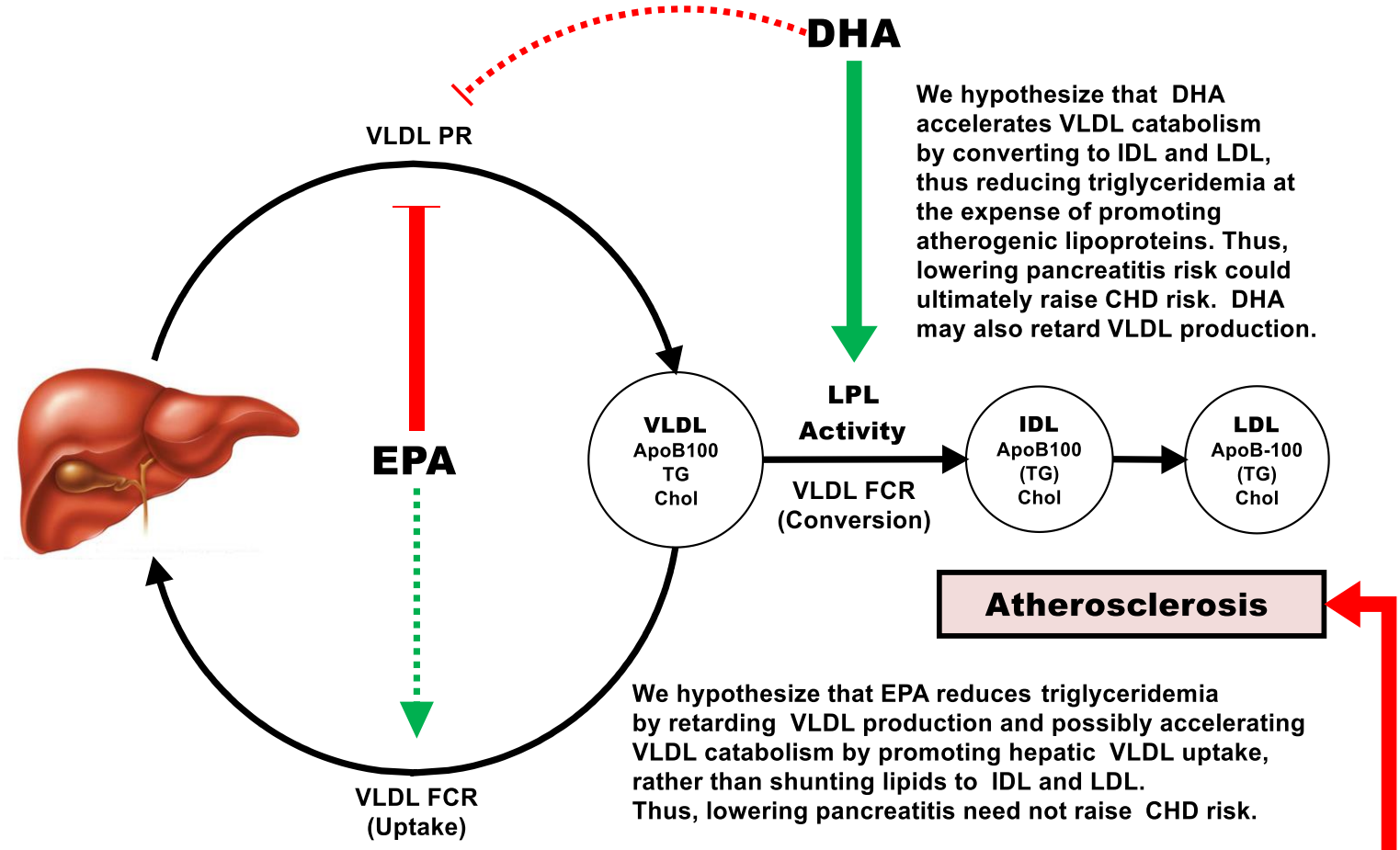
The mechanism of action EPA+DHA fish oil in general is not well established, and there are very little mechanistic data for EPA or DHA separately. We therefore propose a clinical trial to confirm the mechanism of EPA using tracer kinetics to determine its effect on production and catabolism of the relevant lipids. Though this would be interesting in its own right (for example to identify new targets of therapy), we will link the kinetics to EPA's improvement of postprandial triglycerides, thus affirming that the mechanism mediates the putative atheroprotective property. Specifically, we expect EPA-rich oil to suppress postprandial triglyceridemia by retarding CM and VLDL production, while enhancing particle clearance. Hence, our approach is not to merely elucidate the mechanism of EPA, but to learn whether EPA mediates fish oil's most likely clinical benefit with respect to atherosclerosis, namely, suppressing postprandial triglyceridemia.

Given preliminary evidence that EPA is distinct from DHA, we think it is also very important to formally test differences. The study is designed as a two-stage adaptive design, with the first step a Pilot Stage, followed by a Confirmation Stage. In the pilot stage we will compare EPA-rich oil to usual care. If the study progresses to the Confirmation Stage, we will then add an active control, EPA-poor/DHA-rich oil, thus providing a head-to-head comparison of EPA-rich and EPA-poor oil. If the study progresses to the Confirmation Stage, we would be able to make EPA-poor oil that appears identical to EPA-rich oil, thus allowing us to randomize to double-blinded oil vs open-label usual care. We expect EPA-poor oil to worsen LDL-c kinetics compared to EPA-rich oil, whereas we think EPA-rich oil will have no ill effect on LDL, similar to usual care.

At this stage of the study protocol, we will only test EPA-rich oil against usual care in the Pilot Stage. Though we describe the Confirmation Stage in general terms throughout this protocol as part of our overall approach, we would not proceed to the Confirmation Stage without a major revision to the protocol, so that we can appropriately update information from the Pilot Stage that informs the Confirmation Stage, and will provide other essential details about the Confirmation Stage during that revision.

1.4.1 Mechanistic Hypothesis

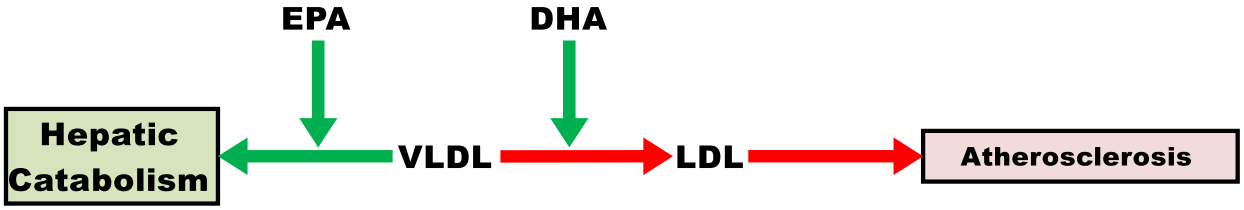
We hypothesize that EPA and DHA reduce the VLDL pool by different mechanisms, with EPA primarily limiting VLDL production and DHA primarily converting VLDL to IDL and LDL. Reducing the VLDL pool by either mechanism is expected to benefit postprandial triglyceridemia because less VLDL will compete with CM for LPL, thus aiding CM clearance.



Expected Changes in Lipid Kinetics from EPA vs DHA

		VLDL FCR						
	VLDL PR	LPL Activity	VLDL Conversion	VLDL Uptake	VLDL Pool	IDL PR	LDL PR	LDL Pool
DHA	⇔⇓	⇑	⇑	⇔	⇓	⇑	⇑	⇑
EPA	⇓	⇔	⇔	⇔⇑	⇓	⇔	⇔	⇔

Specifically, we hypothesize that EPA-rich oil will significantly retard the VLDL-apo B-100 production rate (PR) and the VLDL-TG PR,[6] and expect these for the most part mediate the reduction of postprandial triglyceridemia. Namely, we expect VLDL-apo B-100 PR and perhaps VLDL-TG PR to predict the clinical benefit of reduced postprandial TG as the incremental area under the curve (iAUC) of TG on an oral fat tolerance test.



At the same time, we expect EPA-poor to increase the LDL pool by decreasing hepatic clearance of VLDL and accelerating conversion of VLDL to LDL, effectively shunting cholesterol to LDL rather than back to the liver.[7] If correct, preferentially delivering cholesterol to LDL that might otherwise return to the liver is expected to have a relatively adverse effect on atherosclerosis. Alternatively, if EPA-rich oil preferentially shunts cholesterol from VLDL to the liver by accelerating VLDL catabolism, this is expected to be atheroprotective. Thus, distinguishing these effects would be of great interest to the field.

There is reason to believe that EPA+DHA fish oil stimulates the overall VLDL-apo B-100 fractional catabolism rate (FCR), specifically, by stimulating VLDL conversion to LDL. However, prior studies have thus far failed to prove a statistically-significant increase in VLDL-TG FCR from EPA+DHA. Failing that, it is hard to discern whether the latter is from a true null effect versus a Type II error related to small sample sizes or use of active controls. In any case, prior literature leads us to think that EPA+DHA accelerates conversion of VLDL to LDL, resulting in a shunting of lipids to IDL and LDL rather than returning to the liver directly for catabolism. Given its penchant for raising fasting LDL-c, it follows that DHA is the likely culprit which shunts lipids to LDL. If correct, we would expect EPA-poor oil to reduce the VLDL-apo B-100 and TG PR and pool size, but with the adverse effect of enriching LDL particles with TG and/or cholesterol. Conversely, we expect EPA-rich oil to reduce the VLDL-apo B-100 and TG PR and pool size without increasing LDL PR or enriching LDL particles with TG and/or cholesterol. Given the importance of the LDL-c effect clinically, we propose formally testing cholesterol kinetics by labeling cholesterol in addition to apo B-100 and TG. We believe this would decisively settle the question as to whether and to what extent DHA raises LDL-c by comparing EPA-poor oil to usual care. At the same time, we would have comparable data for EPA-rich oil, so that comparing EPA-rich oil to usual care proves the concept that EPA averts the anticipated adverse effects of DHA on LDL-c.

Demonstrating this has important clinical implications. Since statins accelerate apo B-100 FCR by promoting hepatic uptake, a complementary strategy for reducing fasting and postprandial TG would be to retard production via fish oil and accelerate hepatic catabolism without shunting lipids to LDL. If our hypothesis is correct, EPA would fit this strategy, but DHA would not, to the extent that it shunts lipids to LDL. This would support the use of a pure EPA product rather than EPA+DHA. Though this information would be motivating in and of itself, again, the ability to link it directly to postprandial benefits during an oral fat load would provide a powerful affirmation of clinical relevance, since postprandial TG is such a decisive risk factor for CHD events compared to fasting TG.[3, 4]

As before, prior studies suggest a greater role for fish oils in retarding the apoB-100 and TG PR than in accelerating conversion to LDL or hepatic catabolism. That said, the literature is notable for relatively small sample sizes, in which catabolism appeared to accelerate, but not within the range of statistical confidence. In other words, the failure to prove decisive effects on overall FCR may in fact represent a Type II error. We propose examining the effect of EPA-rich oil on the overall apoB-100 and TG FCR, as our design affords a more realistic opportunity to rule in or rule out accelerated FCR for several reasons. First, by simultaneously measuring the kinetics of doubly-labeled apoB-100 with or without TG and cholesterol, the resulting compartmental model would have more power to discriminate changes due to greater sensitivity of the multiple substrate model. Second, if needed we would enhance the sample size in the Confirmation Stage. Third, we may add an EPA-poor oil arm, further increasing the overall sample contributing to the statistical models. Thus, we believe our approach is uniquely positioned to answer once and for all whether fish oil, especially EPA-rich oil, accelerates VLDL hepatic catabolism. If we showed that EPA-rich oil not only retarded VLDL PR but at the same time accelerated hepatic VLDL FCR, and that without shunting lipids to LDL, this would affirm multiple benefits that would probably never be replicable with EPA+DHA.

1.5 Summary

Despite widespread use in clinical practice, the mechanism by which fish oil alters lipids is not well established, and this may be because the different components have distinct effects on lipid kinetics. Strikingly, there is evidence that one component, DHA, may actually suppress postprandial and fasting triglyceridemia by shunting lipids into atherogenic lipoprotein species. If true, DHA may protect from pancreatitis at the expense of promoting atherosclerosis. On the other hand, there is emerging evidence to suggest EPA accelerates VLDL hepatic uptake with minimal or no shunting to the atherogenic lipoproteins. We propose what we believe to be the first human experiment to rigorously test these concepts, and decisively distinguish the two, while linking these mechanistic outcomes to a robust assessment of triglycerides in the postprandial state.

1.6 Dose Rationale and Risk/Benefits

1.6.1 Experimental Medication

In this Pilot Stage, the experimental medication is AMR101, an EPA-rich oil marketed as Vascepa (icosapent ethyl). Detailed information can be found in the accompanying Prescribing Information.

1.6.1.1 Dose Rationale for Experimental Medication

The dose of Vascepa in this study is the same dose used in clinical practice: 2 caps twice daily, with meals. The rationale is that this dose has well-described safety, tolerability, and efficacy data for the population of reference: dyslipidemic patients with suboptimal triglycerides. This dose is also FDA-approved as a pharmaceutical to lower TGs in extreme hypertriglyceridemia (≥500 mg/dL). Because the question is mechanistic, we decided not to require extreme triglyceridemia in this study. Avoiding this rarer condition limits confounding by other triglyceride-lowering medications and the excessive variability of triglycerides at the high end, and also facilitates recruitment.

1.6.1.2 Risk/Benefits of Experimental Medication

The following excerpt from the Prescribing Information describes adverse events seen with Vascepa, namely, arthralgia and oropharyngeal pain:

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Adverse reactions reported in at least 2% and at a greater rate than placebo for patients treated with VASCEPA based on pooled data across two clinical studies are listed in Table 1.

Table 1. Adverse Reactions Occurring at Incidence >2% and Greater than Placebo in Double-Blind, Placebo-Controlled Trials*

Adverse Reaction	Placebo (N=309)		VASCEPA (N=622)	
	n	%	n	%
Arthralgia	3	1.0	14	2.3

*Studies included patients with triglycerides values of 200 to 2000 mg/dL.

An additional adverse reaction from clinical studies was oropharyngeal pain.

SOURCE: Vascepa Prescribing Information 1/2015

The Investigator Brochure for AMR101 lists additional events and the incidence for placebo:

Adverse Event Preferred Term	Placebo (N=309)		AMR101 2 g/day (N=312)		AMR101 4 g/day (N=310)	
	n	%	n	%	n	%
Abdominal Distension	4	1.3	2	0.6	3	1.0
Abdominal Pain	4	1.3	3	1.0	1	0.3
Arthralgia	3	1.0	9	2.9	5	1.6
Bronchitis	8	2.6	5	1.6	3	1.0
Constipation	4	1.3	2	0.6	2	0.6
Diabetes Mellitus	5	1.6	5	1.6	1	0.3
Diarrhoea	15	4.9	13	4.2	9	2.9
Dizziness	2	0.6	3	1.0	4	1.3
Eructation	7	2.3	2	0.6	2	0.6
Fatigue	4	1.3	2	0.6	4	1.3
Flatulence	4	1.3	4	1.3	4	1.3
Gastroesophageal Reflux Disease	2	0.6	1	0.3	4	1.3
Headache	4	1.3	1	0.3	2	0.6
Muscle Spasms	5	1.6	2	0.6	0	0.0
Nasopharyngitis	8	2.6	6	1.9	1	0.3
Nausea	11	3.6	10	3.2	6	1.9
Non-Cardiac Chest Pain	1	0.3	4	1.3	3	1.0
Oedema Peripheral	2	0.6	7	2.2	3	1.0
Oropharyngeal Pain	1	0.3	2	0.6	5	1.6
Pain	0	0.0	4	1.3	1	0.3
Pain in Extremity	3	1.0	2	0.6	4	1.3
Prurtius	5	1.6	1	0.3	2	0.6
Upper Respiratory Tract Infection	6	1.9	4	1.3	8	2.6
Urinary Tract Infection	9	2.9	5	1.6	7	2.3

More conservatively, low occurrences of other adverse events might be foreseeable based on experience from other lipid-altering oils. For example, in a long-term clinical trial Yokoyama et al. found that 1.8 g/day of ethyl-EPA was well tolerated:[8]

	Control (n=9319)	EPA (n=9326)	p value
Total number of adverse experiences (%)	2004 (21·7%)	2334 (25·3%)	<0·0001
Newly diagnosed cancer			
Total	218 (2·4%)	242 (2·6%)	0·26
Stomach	37 (0·4%)	53 (0·6%)	0·09
Lung	37 (0·4%)	32 (0·3%)	0·54
Colorectal	29 (0·3%)	26 (0·3%)	0·68
Breast	21 (0·2%)	16 (0·2%)	0·41
Common adverse experiences			
Pain (joint pain, lumbar pain, muscle pain)	180 (2·0%)	144 (1·6%)	0·04
Gastrointestinal disturbance (nausea, diarrhoea, epigastric discomfort)	155 (1·7%)	352 (3·8%)	<0·0001
Skin abnormality (eruption, itching, exanthema, eczema)	65 (0·7%)	160 (1·7%)	<0·0001
Haemorrhage (cerebral, fundal, epistaxis, subcutaneous)	60 (0·6%)	105 (1·1%)	0·0006
Abnormal laboratory data			
Total	322 (3·5%)	378 (4·1%)	0·03
CPK increased	116 (1·2%)	126 (1·4%)	0·52
GOT increased	38 (0·4%)	59 (0·6%)	0·03
Sugar blood level increased	27 (0·3%)	38 (0·4%)	0·17

CPK=creatine phosphokinase. GOT=glutamic oxaloacetic transaminase.

A mixture of EPA and DHA oils marketed as Lovaza has reported the following adverse events:

Table 1. Adverse Reactions Occurring at Incidence ≥3% and Greater than Placebo in Clinical Trials of LOVAZA

Adverse Reaction[a]	LOVAZA (N = 655)		Placebo (N = 370)	
	n	%	n	%
Eructation	29	4	5	1
Dyspepsia	22	3	6	2
Taste perversion	27	4	1	<1

[a] Trials included subjects with HTG and severe HTG.

Other adverse events included the following:

Body System	Symptom
Digestive System	Constipation Gastrointestinal disorder Vomiting
Metabolic and Nutritional Disorders	Increased ALT Increased AST
Skin	Pruritis and rash

SOURCE: Lovaza Prescribing Information

Another EPA and DHA mixture marketed as Epanova has reported the following adverse events:

Table 1. Adverse Reactions Occurring at Incidence >3% and Greater than Placebo in Placebo-Controlled Trials*

Adverse Reaction	Placebo N=314	EPANOVA 2g N=315	EPANOVA 4g N=315
Diarrhea	2%	7%	15%
Nausea	1%	4%	6%
Abdominal pain or discomfort	2%	3%	5%
Eructation	<1%	3%	3%

* Trials included subjects with hypertriglyceridemia of varying severity.

Other adverse events included abdominal distension, constipation, vomiting, fatigue, nasopharyngitis, arthralgia, and dysguesia. Epanova contains EPA and DHA in the free-fatty acid form, as opposed to the ester forms that are present in Vascepa, Epadel, and Lovaza, which may explain the higher prevalence of gastrointestinal adverse events.

SOURCE: Epanova Prescribing Information

1.6.2 Risks Associated with Other Aspects of the Study

In studies involving prolonged treatment with medications, the most important risks are those associated with the therapy itself. However, there is also a possibility of risk associated with 1) background therapy given to all participants and 2) procedures conducted at the study visits, especially the prolonged visits. Background therapy includes statins for subjects who join the study statin-naïve, and hematinics given in accordance with Red Cross guidance to facilitate hematopoiesis. In general statins can cause muscle aches, can raise glucose levels slightly, and can elevate transaminases. Unlike chronic therapies, procedural risks tend to be short-lived and mild, and given their brief nature, tend to be less disruptive to subject's daily lives. For example, iron can cause constipation and can turn stools dark or even black. Nevertheless, we have consolidated information on possible procedural risks to better inform prospective subjects of what to expect from participation and help investigators evaluate adverse events. Procedural risks including chronic medications are also discussed further in the Procedural Standard Operating Procedures (SOP).

To summarize, risks beyond those of the experimental medication include potential risks from obligatory chronic statin therapy for subjects who initiate statins under the study, hematinics, venipuncture and intravenous catheterization, large-volume phlebotomy, heparin-stimulated lipase activity determination, the oral fat tolerance test, and even risks from the prolonged study visits themselves. Again, these risks are presented in detail in the SOP's, most of which have been through review by the Penn IRB for prior studies.

1.6.3 Overall Risk/Benefit

In general, lipid-altering oils including EPA-rich oil are very well tolerated. Given the manageable side effects and modest risk, we do not expect significant risk from the experimental medication. Likewise, background therapy given to all groups is generally well-tolerated. For example, among subjects who start a statin, the statins are generally well tolerated, especially at the submaximal doses we would prefer.

A theoretical benefit of participation is that treatment with lipid-altering oil may improve triglycerides in subjects with dyslipidemia. Though short-term treatment is of little consequence over the long term, information about a given subject's response to a marketed therapy could guide their clinician in making long-term therapeutic decisions. Thus, a potential benefit of the study is that we will determine the efficacy and tolerability among subjects randomized to EPA-rich oil. Importantly, we will share the clinically-relevant laboratory results with the subjects and their physicians if requested (n.b. we encourage subjects to share these results with their physician). As for risk, the protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

On balance, we believe there is reasonable individual and generalized benefit when compared to the risk of participation.

2 Study Objectives

2.1 Mechanistic Hypotheses

2.1.1 Hypothesis M1 (Primary Hypothesis, Pilot and Confirmation Stages)

EPA-rich oil retards the VLDL production rate compared with usual care and in the confirmation stage, compared to EPA-poor oil, as determined by apoB-100 before and after therapy by compartmental modeling.

2.1.2 Hypothesis M2 (Pilot and Confirmation Stages)

EPA-rich oil hastens hepatic VLDL catabolism compared with usual care, and in the confirmation stage, EPA-poor oil, as determined by apoB-100 before and after therapy by compartmental modeling.

2.1.3 Hypothesis M3 (Contingent, Confirmation Stage Only)

EPA-poor oil A) hastens production of atherogenic cholesterol-rich lipoproteins IDL and LDL compared with usual care and B) retards hepatic VLDL catabolism, consistent with the molecular hypothesis that EPA-poor oil shunts the remnant lipids and apolipoproteins from VLDL to IDL, and in turn, to LDL, for example, by increasing lipoprotein lipase activity. This will be determined by the LDL apoB-100 before and after therapy by compartmental modeling. To confirm the proposed molecular mechanism, we will also determine LPL activity after heparin loading.

2.2 Clinical Hypotheses

2.2.1 Hypothesis C1 (Pilot and Confirmation Stages)

EPA-rich oil suppresses postprandial triglyceridemia compared to usual care, and does so in proportion to retarded VLDL production and hastened hepatic VLDL catabolism.

2.2.2 Hypothesis C2 (Contingent, Confirmation Stage Only)

EPA-poor oil suppresses postprandial triglyceridemia compared to usual care, and does so in proportion to hastened VLDL conversion to IDL and LDL rather than retarded VLDL production or hastened hepatic uptake.

3 Study Design

3.1 General Design

3.1.1 Overview of Study Design

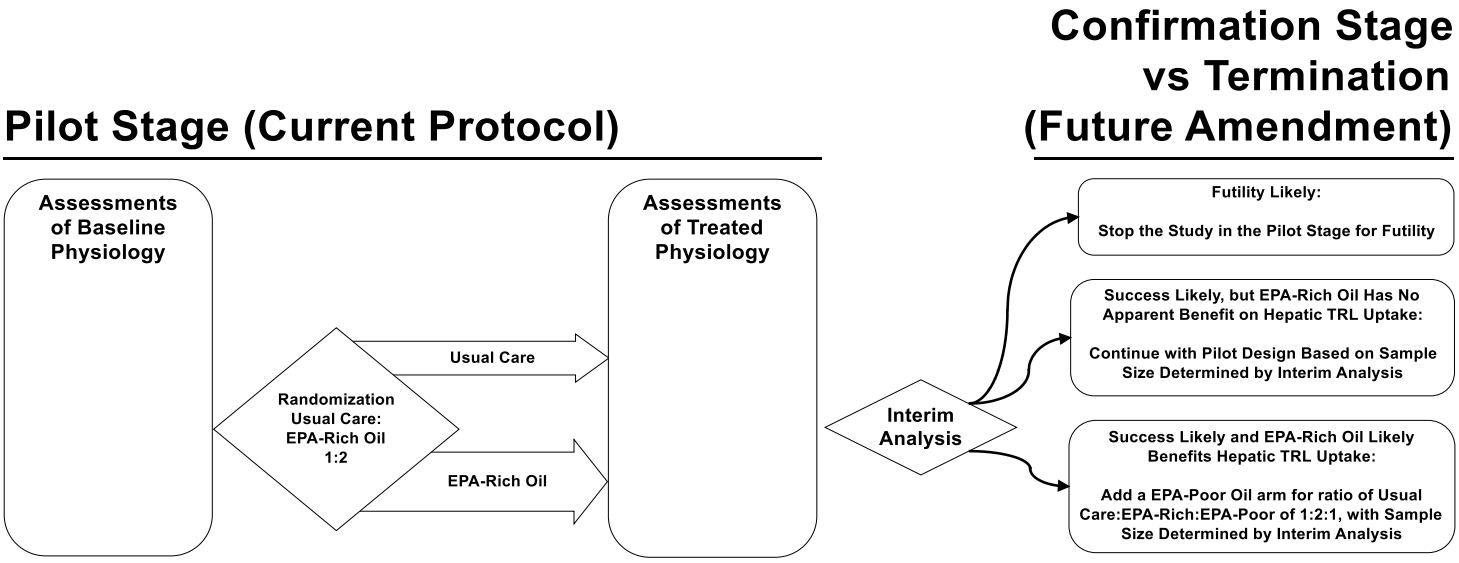
This is an adaptive-design, parallel group, randomized, prospective, inactive- and potentially active-controlled clinical lipid kinetics experiment to determine the mechanism by which EPA-rich oil suppresses postprandial triglyceridemia.

3.1.2 Selection of Controls

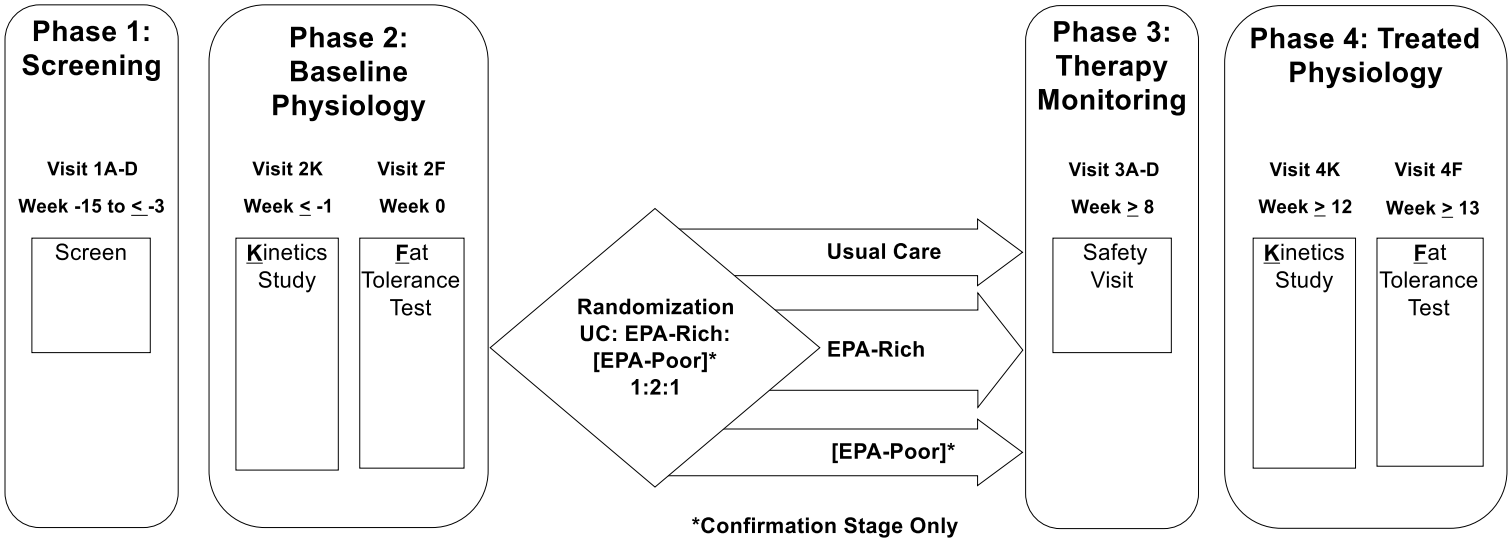
Since oils used for "placebos" are actually bioactive lipids, there is no practical placebo for fish oil. Accordingly, the inactive control is open-label usual care. On the other hand, the active control for EPA-rich oil would be EPA-poor oil, which can be masked to look like EPA-rich oil. Thus, we decided on an **open-label negative control** in the Pilot Stage (i.e. active EPA-rich oil vs usual care sans oil) and potentially a **double-blinded active control** in the Confirmation Stage (i.e. masked and blinded EPA-rich and EPA-poor oils). The study takes advantage of an adaptive approach consisting of two stages: an initial Pilot Stage, and a Confirmation Stage contingent on results from the Pilot Stage following an interim analysis.

3.1.3 Adaptive Design Elements

The adaptive approach allows us to terminate the study for futility if the interim analysis suggests a benefit from EPA-rich oil is unlikely. On the other hand, information from the pilot stage will help us definitively power the remainder of the study to answer our mechanistic questions, allowing us to enroll enough subjects to limit the potential for Type II error. This is particularly import because prior literature is hard to interpret because reported null effects are vulnerable to Type II error due to small samples, and especially because the studies used a metabolically-active oil as the control rather than an inactive control. Accordingly, the sample size for the Pilot Stage is determined by the number of observations needed to develop high-quality statistical parameters to support the interim analysis, whereas the sample size for the Confirmation Stage is to be determined by the interim analysis. The interim analysis will also help us decide whether to ultimately include EPA-poor oil as an active control. If we see evidence that EPA-rich oil retards the VLDL production rate and/or accelerates the hepatic VLDL catabolic rate as expected, this would justify an active control against EPA-poor oil to determine whether this benefit is indeed related to EPA; otherwise, an EPA-poor arm would probably not be worth the effort, and would not be added. The Figure below provides an overview of the adaptive elements of the study design.



3.1.4 Overview of Study Phases and Duration



The study is implemented in four phases, starting with at least one pre-enrollment screening visit in Phase 1. During Phase 1, prospective subjects are consented and screened for elibility, and may start statins or may otherwise have repeat visits to determine suitability for participation. There are two phases encompassing the visits to assess the outcomes comprising the major aims, featuring identical procedures: Phase 2 to assess baseline physiology and 4 to assess treated physiology. Phase 3 involves at least one safety visit during the randomized treatment period. The table below summarizes the duration for individual participants. Subject participation in the study will last 4 to 7 months depending on the length of the screening period and whether the subject is already taking a statin medication.

Study Duration for an Individual Subject

Interval	Minimum Duration
Since Screening (Visit 1A)	≥ 19 weeks
Since First Experimental Visit (Visit 2K)	≥ 14 weeks
Since Starting Experimental Treatment (Visit 2F)	≥ 13 weeks

3.1.5 Summary of Scientific Objectives

To our knowledge, this will be the first lipid kinetics study of EPA-rich oil, and therefore, the first to evaluate the concept that EPA preferentially shunts VLDL toward hepatic catabolism and away from LDL production. Moreover, this would be the first study to determine whether these effects account for improved postprandial triglyceride handling. Both phenomena would be considered favorable in that they mitigate atherogenic lipoproteins. Because these findings are so novel in their own right, the Pilot Stage itself is likely to yield publishable data without even progressing to the Confirmation Stage, especially since the interim analysis may be used to increase the sample size of the Pilot Stage to limit chances of a Type II error. If the Pilot Stage results in a solid null result (i.e. a null from a well-powered sample size), the results would be important as a way to rule out the expected effects. In either case, we believe there is sufficient scientific/clinical merit to justify the Pilot Stage alone, even if the Confirmation Stage turns out to not be justified. That said, if we do develop data to support progressing to the Confirmation Stage, the ability to compare EPA-rich oil to EPA-poor oil addresses another novel and impactful question, namely, is there a benefit to simply using EPA-rich oil? In summary, we believe the Pilot Stage alone is warranted based on the novel and important clinical and scientific issues it addresses, and the Confirmation Stage would then provide a more robust test of the results by subjecting them to a separate, positive control.

3.1.6 Position of Study in Drug Development Cycle

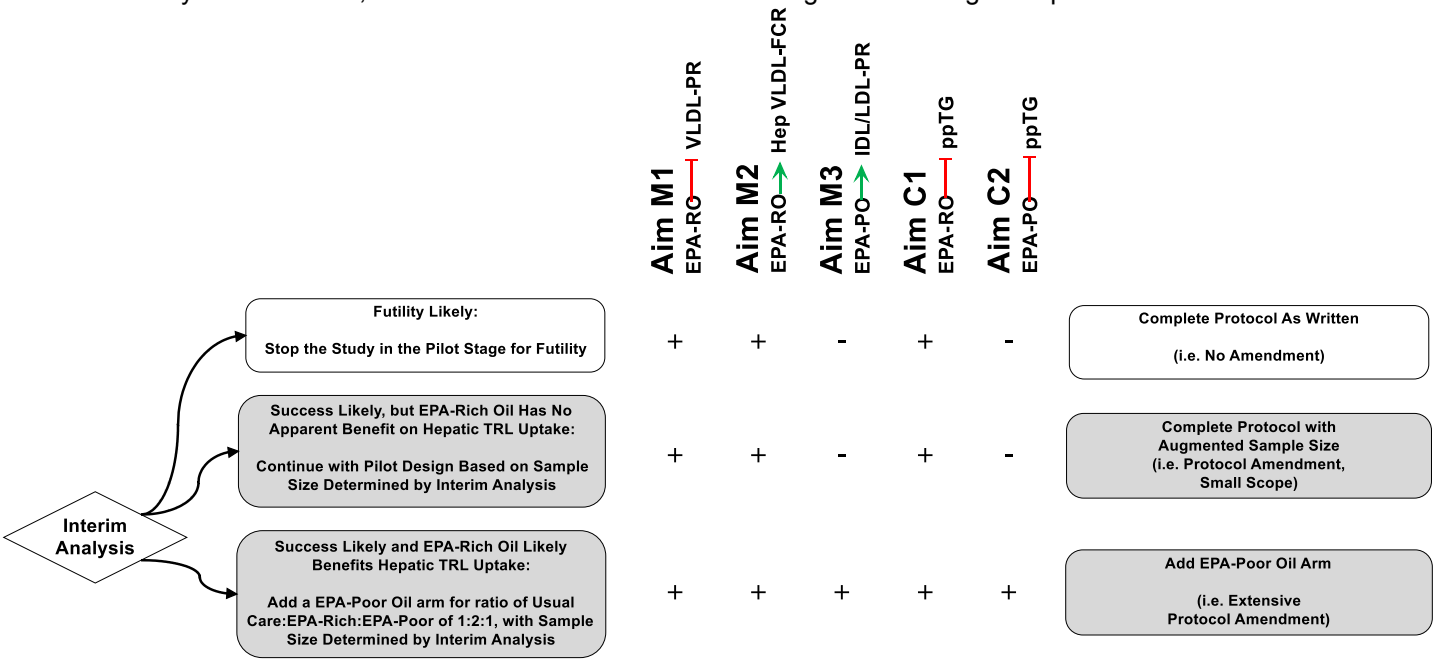
The experimental intervention of the Pilot Stage is marketed (i.e. Vascepa), thus the study takes place in the post-approval phase of Vascepa's development. Perhaps more importantly, the purpose of the study centers on drug metabolism and mechanism of

action in humans, and the investigational drug is used as a research tool to explore biological phenomena and disease processes, namely, lipid kinetics and fat metabolism among patients with abnormal triglycerides. Moreover, the modestly sized Pilot Phase is focused on developing sufficient information about the drug's pharmacological effects to permit the design of a well-controlled, scientifically-valid, definitively-powered test of the study hypotheses (hopefully in the Confirmation Stage). Despite taking place post-approval, the actual study features accord with the regulatory definition of Phase 1 (cf. 21 CFR § 312.21). Supporting that, there is no requirement that Phase 1 investigations match the sequence of the drug's development cycle; thus, the fact that Phase 2, 3, and 4 studies have been conducted with Vascepa is immaterial to the phase of this particular study.

3.1.7 Specific Aims and Relationship to Hypotheses

Each hypothesis has a corresponding specific Aim. Since there are not sufficient data to formally power a definitive study at this time, the purpose of each Aim is to develop relevant statistical parameters needed to design a definitive study to test the corresponding hypothesis (e.g. mean, standard deviation, and shape of distribution for outcomes). As an adaptive design, it is expected that the interim analysis will provide sufficient information to definitively power our tests of each hypothesis. At that time, the Aims would progress from developing pilot statistical parameters to formally testing the hypotheses, which would be specified in a protocol amendment.

The table below presents the specific Aims and how they relate to the results of the interim analysis, as well as the anticipated protocol amendments for each contingency. Note that Aims M1, M2, and C1 are universal aims, meaning that they will be evaluated in any circumstance, whereas Aims M3 and C2 are contingent on adding EPA-poor oil as an active control.



4 Study Endpoints

This section specifies the primary endpoints for each of the hypotheses described above.

4.1 Primary Study Endpoint

Consistent with the adaptive design approach, the primary aim at the Pilot Stage is to develop adequate data for each endpoint, including central tendency and variability, to design a complementary Confirmation Stage. The purpose of Confirmation Stage would be to definitively test each hypothesis with the corresponding endpoints.

4.1.1 EPM1: VLDL Production Rate

Hypothesis M1 (Pilot and Confirmation Stages): The endpoint for this hypothesis is the **VLDL-apoB100 production rate**.

4.2 Secondary Mechanistic Endpoints: VLDL Fate

4.2.1 EPM2: VLDL Catabolic Rate

Hypothesis M2 (Pilot and Confirmation Stages): The endpoint for this hypothesis is the **hepatic VLDL-apoB 100 catabolic rate.**

4.2.2 [EPM3: Production Rate of VLDL Derivatives: IDL and LDL]

[**Hypothesis M3 (Contingent, Confirmation Stage Only):** If an EPA-poor arm is added to the Confirmation Stage, the endpoints for this hypothesis would be the **IDL-apoB100 production rate** and the **LDL-apoB 100 production rate** as well as the **hepatic VLDL-apoB 100 catabolic rate**.]

4.3 Secondary Clinical Endpoints: Postprandial Triglyceridemia

4.3.1 EPC1: Postprandial Triglyceridemia on EPA-Rich Oil

Hypothesis C1 (Pilot and Confirmation Stages): The endpoint for this hypothesis is postprandial triglyceridemia, assessed as the incremental area under the curve for the triglyceride curve.

4.3.2 [EPC2: Postprandial Triglyceridemia on EPA-Poor Oil]

[**Hypothesis C2 (Contingent, Confirmation Stage Only):** If an EPA-poor arm is added to the Confirmation Stage, the endpoint for this hypothesis would be postprandial triglyceridemia, assessed as the incremental area under the curve for the triglyceride curve.]

4.4 Primary Safety Endpoints

4.4.1 Subjective Safety and Tolerability of Study Therapeutics

We will tabulate subjective adverse events (i.e. symptoms) as described in the section under Adverse Effects.

4.4.2 Objective Safety and Tolerability of Study Therapeutics

We will tabulate objective adverse events (i.e. clinically-significant objective outcomes, such as laboratory abnormalities), as described in the section under Adverse Effects.

4.5 Future Analyses

We will preserve specimens anticipating that the initial analyses will suggest additional mechanistic or clinical questions that could be addressed by further analysis. For example, we may store plasma and red blood cells, but may also store lipid fractions from ultracentrifugation as well as gels. This approach will not only allow us to quickly interrogate new questions to corroborate the specific aims, but would allow us to corroborate new information arising from other investigations into the mechanism of the study oils.

5 Subject Selection and Withdrawal

Regarding selection, subjects must meet all the following inclusion criteria to enroll in the study, and must not have any exclusion criteria set forth below. Enrollment begins at the first experimental visit, that is, the first prolonged visit involving the provocative physiologic challenges under Phase 2. After enrollment, separate rules govern early withdrawal of subjects.

5.1 Inclusion Criteria

5.1.1 Population of Reference

The population of reference is statin-treated Caucasian adults with triglyceridemia:

1. Triglyceridemia, defined as:

   a) statin-treated TG $\geq$ 200 mg/dL

   – and/or – b) statin-treated TG $\geq$ 150 mg/dL – plus– statin-treated HDL $\leq$ 45 [men] or $\leq$ 55 [women]

2. Self-reported Caucasian-majority race, defined as 3 out of 4 grandparents Caucasian

Up to three valid statin-treated lipid panels are allowable, and at least one individual triglyceride value and the mean of all valid triglycerides must meet the appropriate threshold. When required a single qualifying HDL-c value is sufficient. Subjects are excluded for an average of valid, statin-treated TGs > 500 mg/dL. Subjects with average statin-treated TG > 350 mg/dL will be randomized separately in an attempt to balance the groups with subjects whose TGs are on the higher end.

Statins include the following:

Atorvastatin (Lipitor®)
Fluvastatin (Lescol®)
Lovastatin (Mevacor®)
Pitavastatin (Livalo®)
Pravastatin (Pravachol®)
Red Yeast Rice
Rosuvastatin (Crestor®)
Simvastatin (Zocor®)

The following statin combination drugs would necessitate a washout and switch to statin monotherapy because the non-statin component is exclusionary, as discussed below:

Lovastatin+Niacin (Advicor®)
Simvastatin+Ezetimibe (Vytorin®)
Simvastatin+Niacin (Simcor®)

Patients who are not taking statins may start statin therapy under the study, during a statin run-in phase prior to the enrollment visit. This is discussed below in the section titled “Starting Statins.” Since red yeast statins are marketed as supplements rather than prescription medications, they may not be manufactured according to GMP. Accordingly, to assure consistency of statin exposure, patients taking red yeast statins will be switched to a prescription statin, preferably at a comparable dose.

5.1.2 Other Inclusion Criteria

3. Subjects between the ages of 21 and 75 years of age inclusive

4. Ability to understand and agree to informed consent

5. Are reliable and willing to make themselves available for the duration of the study, comply with study procedures, agree not to participate in other clinical experiments, and agree not to donate blood products during the study

5.2 Exclusion Criteria

5.2.1 Exclusions to Limit Excessive Heterogeneity

5.2.1.1 Exclusionary History

1. Diagnosis of idiopathic or otherwise active diabetes: History of resolved gestational or drug-induced diabetes is acceptable, but history Type I or Type II diabetes are exclusionary.

2. Use of medications indicated for the treatment of diabetes within 6 weeks of the first experimental visit (see Prohibited Treatments)

3. History of a myocardial infarction (MI), unstable angina leading to hospitalization, coronary artery bypass graft surgery (CABG), percutaneous coronary intervention (PCI), uncontrolled cardiac arrhythmia, carotid surgery or stenting, stroke, transient ischemic attack, carotid revascularization, endovascular procedure or surgical intervention within 6 months of baseline.

4. Known inefficacy to TG-lowering doses of fish oils (e.g. >= 4 caps daily of prescription fish oil or >= 6 caps daily of supplemental fish oil).

5.2.1.2 Exclusionary Measurements

5. TG > 500 mg/dL as the average of valid, statin-treated values

6. BMI $\geq$ 40 kg/m2

7. BMI < 20 kg/m2

8. Evidence of previously undiagnosed diabetes: Average fasting glucose during screening > 125 mg/dL

9. Known familial lipoprotein lipase impairment or deficiency (Fredrickson Type I), Apo C II deficiency, or familial dysbetalipoproteinemia (Fredrickson Type III).

5.2.2 Exclusions for Conditions Rendering Participation Inadvisable

10. Severe allergy to fish, unless non-allergic response to fish oil is established (n.b. most fish allergies are to the proteins as opposed to the fats, so with highly-purified oils the risk of a true allergy is remote).

11. Known intolerance or contraindication to Vascepa, and if the former is unknown, known intolerance or contraindication to fish oil

12. Any surgical or medical condition that may interfere with absorption, distribution, metabolism, or excretion of EPA or DHA.

13. History of extreme triglyceridemia (TG > 1000 mg/dL) within the past 5 years or a history of pancreatitis from triglyceridemia, regardless of whether it is currently controlled.

14. Medical condition that would prohibit fasting (e.g. diagnosis of insulinoma or postabsorptive hypoglycemia).

15. Significant disinclination to dairy products (e.g. lactose intolerance, inviolable dietary restrictions). All participants will receive a test dose of the fat challenge during the screening visit, which consists of heavy cream and lactase enzyme. Many people with lactose intolerance successfully avert symptoms by correcting their lactase deficiency with lactase supplements. We will allow these people to participate because we will allow them to take their preferred brand and dose of lactase supplement beyond the lactase in the fat challenge if needed. However, we still require that they are able to tolerate the test dose given during screening.

16. History of a non-skin malignancy within the previous 5 years.

17. Uncontrolled thyroid disease.

18. Any major active rheumatologic, pulmonary, or dermatologic disease or inflammatory condition.

19. Major surgery within the previous 6 weeks.

20. Subjects who have undergone any organ transplant.

21. History of illicit drug use within the past 3 years, or regular alcohol use of greater than 14 drinks per week. For clarity, illicit substances are per Federal law or regulations in effect at the time of first approval of this protocol.

22. Women who are breast-feeding.

23. Women of childbearing potential must have a negative urine pregnancy test at screening and baseline visits and be willing to have additional urine pregnancy tests during the study.

24. Sexually active subjects (both women and men) must be willing to use a medically accepted method of contraception from screening visit until month after last dose of study drug

25. Significant or unstable medical or psychological conditions, including known or suspected personality disorders, that could compromise the subject's safety or successful participation in the study in the opinion of the investigator.

26. Subject-reported history of HIV and/or use of HIV medications, including the following

Abacavir (Ziagen)
Atazanavir (Reyataz)
Darunavir (TMC114, Prezista)
Delaviridine (Rescriptor)
Didanosine (ddI, Dideoxyinosine, Videx)
Efavirenz (Sustiva, Strocrin)
Epzicom (Abacavir/3TC)
Etravirine (Intelence, TMC125)
Fosamprenavir (Lexiva, Telzir)
FTC (Emtricitabine, Emtriva)
Indinavir Sulfate (Crixivan)
Kaletra (Lopinavir/Ritonavir)
Maraviroc (Selzentry, Celsentri)
Nelfinavir (Viracept)
Nevirapine (Viramune)
Raltegravir (Isentress, MK-0518)
Ritonavir (Norvir)
Saquinavir Mesylate (Fortovase, Invirase)
T-20 (Enfuvirtide, Fuzeon)
Tenofovir (Viread)
Tipranavir (Aptivus)
Trizivir (AZT/3TC/Abacavir)
Truvada (Tenofovir/FTC)
Zidovudine (AZT, ZDV, Retrovir, Combivir)

27. History of symptomatic gallstone disease unless definitively treated (e.g. condition successfully treated with cholecystectomy without recurrent or residual biliary disease).

28. History of bariatric surgery or other major gastrointestinal surgery associated with major disruptions to drug absorption.

29. Anticipation of major surgery during the screening or treatment periods of the study.

5.2.3 Exclusions to Heparin-Stimulated Lipase Determination

30. Participants with the following conditions will opt out of heparin exposure for lipase determinations, but will be allowed to participate in the overall protocol.

5.2.3.1 Exclusionary History

31. History of intolerance or adverse reaction to therapeutic or sub-therapeutic heparin regimens

32. History of intracerebral hemorrhage

33. History of significant GI bleed, unless definitively treated without recurrence

34. Women with dysfunctional uterine bleeding

5.2.3.2 Exclusionary Measurements

35. Individuals with clinically-significant coagulopathy at screening

36. Individuals with clinically-significant thrombocytopenia at screening

5.2.4 Exclusions to Limit Risks Related to Phlebotomy

5.2.4.1 Measurements

37. Hemoglobin < 12 g/dL at screening

38. Mean corpuscular volume (MCV) < 80 fL or > 100 fL at screening

5.2.4.2 History

39. Donation of whole blood within 8 weeks prior to the first experimental visit. Participation in the screening phase is permitted.

40. History of inherent unremediable risks for anemia, such as hemoglobinopathies, hemolytic disorders, and bleeding disorders, regardless of whether their hemoglobin is currently normal.

41. History of a large-volume gastrointestinal (GI) bleeding, such as a bleed that required ER evaluation or admission, required acute endoscopic or surgical management, was managed by blood transfusion, or caused anemia.

42. History of NSAID-mediated peptic ulcer disease is excluded, irrespective of current medical treatment. A history of peptic ulcer disease from *H. pylori* does not exclude participation provided *H. pylori* was successfully eradicated. Other causes of peptic ulcer disease may be allowable, especially if definitively treated or the risk of recurrence is otherwise low.

43. Chronic, untreated conditions that predispose to anemia, such as significant iron or B vitamin malabsorption, persistent menorrhagia, or other chronic or intermittent bleeding.

Allowance for Current Treatment
Individuals with these conditions could participate provided the condition is treated and no longer poses a significant risk for anemia. For example, menorrhagia could be controlled by surgery, hormonal therapy, or NSAIDs. Likewise, iron deficiency could be reversed by iron supplementation or macrocytic anemias by B vitamins.

Stipulations for Current Treatment
Therapy for their condition must remain operative for the duration of the study. Accordingly, surgical control would be operative and would not require further inquiry. On the other hand, medical therapies require further stipulations. Namely, medical therapy must be under the supervision of a non-study physician (e.g. the patient's PCP or specialist), and the subject must be willing to sign a release of information so that we can review records from the treating physician and confer. In order to participate, the treating physician would have to agree that their patient is treated to the point that they are not expected to become anemic given the volume and schedule of study phlebotomy. Put another way, if they feel their patient can donate a unit of whole blood every two months, this would provide the same assurance, since we are drawing a little less than a blood donation.

5.2.5 Exclusions for Current Medications or Supplements

44. Participation in an investigational drug study concurrently; participants who previously completed an investigational drug study cannot participate in the first experimental visit until at least 6 weeks after the final dose given during the previous investigational drug study. For the purposes of this exclusion, an investigational drug is a new chemical entity or a pharmaceutical investigated to support an initial new drug application. Furthermore, herbal or other supplements already deemed "generally regarded as safe" or legally sold in the U.S. would not be considered an investigational drug.

45. Use of medications indicated for the treatment of diabetes within 6 weeks of the first experimental visit:

ORAL DIABETES MEDICATIONS

Biguanides
Metformin (Glucophage)
Metformin liquid (Riomet)
Metformin extended release (Glucophage XR, Fortamet, Glumetza)

Sulfonylureas
Glimepiride (Amaryl)
Glyburide (Diabeta, Micronase)
Glipizide (Glucotrol, Glucotrol XL)
Micronized glyburide (Glynase)

Meglitinides
Repaglinide (Prandin)

D-Phenylalanine Derivatives
Nateglinide (Starlix)

Thiazolidinediones (TZDs)
Pioglitazone (Actos)

DPP-4 Inhibitors
Sitagliptin (Januvia)
Saxagliptin (Onglyza)
Linagliptin (Tradjenta)

Alpha-glucosidase Inhibitors
Acarbose (Precose)
Miglitol (Glyset)

Bile Acid Sequestrants

Colesevelam (Welchol)

Combination Pills
Pioglitazone & metformin) (Actoplus Met)
Glyburide & metformin (Glucovance)
Glipizide & metformin (Metaglip)
Sitagliptin & metformin (Janumet)
Saxagliptin & metformin (kombiglyze)
Repaglinide & metformin (Prandimet)
Pioglitazone & glimepiride (Duetact)

INJECTABLE DIABETES MEDICATIONS

Incretin Mimetics and Non-Insulin Synthetic Analogs
Exenatide (Byetta)
Pramlintide (Symlin)

Rapid-Acting Insulin
Insulin aspart analog (NovoLog)
Insulin glulisine analog (Apidra)
Insulin lispro analog (Humalog)

Short-Acting Insulin
Human Regular (Humulin R, Novolin R)

Intermediate-Acting Insulin
Human NPH insulin (Humulin N, Novolin N)

Long-Acting Insulin
Insulin detemir (Levemir)
Insulin glargine (Lantus)

Premixed Insulin Combinations
50% NPH; 50% Regular (Humulin 50/50)
70% NPH; 30% Regular (Humulin 70/30)
70% NPH; 30% Regular (Novolin 70/30)
50% lispro protamine suspension, 50% lispro (Humalog Mix 50/50)
50% aspart protamine suspension, 50% aspart (Novolog Mix 50/50)
75% lispro protamine suspension, 25% lispro (Humalog Mix 75/25)
70% aspart protamine suspension, 30% aspart (NovoLog Mix 70/30)

Source: Joslin Diabetes Center

46. Daily therapy with non-statin lipid-altering medications within 6 weeks of the first experimental visit is exclusionary, including long-term therapy with the agents listed below. Subjects may wash off these medications so long as the first experimental visit occurs at least 6 weeks after chronic therapy ceases. Following a washout period, fasting lipids will be repeated prior to the first experimental visit to assure that these are not exclusionary as defined above.

    a. Niacin > 100 mg/ day: (Niacor®, Slo-Niacin®, Niaspan®, Advicor®, Simcor®, Inositol Hexanicotinate, or supplemental niacin).
    b. Fibrates: gemfibrozil (Lopid®), fenofibrate (Antara®, Lofibra®, Tricor®, Triglide®), fenofibric acid (Trilipix®, Certriad®).
    c. Enterically active lipid altering drugs: colestipol (Colestid®), cholestyramine (Questran®), colesevelam (Welchol®), ezetimibe (Zetia®, Vytorin®), orlistat (Xenical®, Ali®).
    d. Prescription fish oil: Vascepa®, Epanova®, Lovaza® (nee Omacor®)
    e. Supplemental omega-3-enriched oils: flaxseed, fish, or algal oils
    f. Foods enriched with omega-3 fatty acids
    g. Consumption of up to 2 servings per week of fish is acceptable

47. Lipid-altering supplements

    a. Sterol/stanol products (e.g. CholestOff), policosanols
    b. Dietary fiber supplements, including >2 teaspoons of Metamucil® or psyllium containing supplements per day
    c. Garlic supplements or soy isoflavones supplements
    d. Supplemental vitamin B5 or related compounds unless part of a multi-vitamin
    e. As above, red yeast statin will be switched to a GMP prescription statin
    f. Any other medications, herbal products, or dietary supplements with known or potential lipid-altering effects

48. Anticoagulant therapy (except aspirin)

    a. Warfarin
    b. Rivaroxiban
    c. Apixaba
    d. Dabigantran

49. Anti-obesity medications or their components

    a. Orlistat (Xenical)
    b. Lorcaserin (Belviq)
    c. Phentermine plus extended-release topiramate (Qsymia)

5.3 Subject Recruitment and Screening

Subjects will be recruited from the Preventive Cardiology Research and Division of Translational Medicine and Human Genetics databases, comprised of subjects who have participated in previous research studies and who have asked to be contacted for future studies, and from subjects attending the lipid clinic. Potential subjects may also be recruited via local IRB-approved flyers, radio, internet, and newspaper advertisements targeting the Delaware Valley region. All ads will be submitted to the IRB for approval before use.

Subject recruitment will also be facilitated through querying and prescreening other Penn health system databases. The study team will come up with a list of patients who have been pre-screened and may qualify for the study. This list will include the treating physician name, patient name, contact information, diagnoses, visit dates, lab results and medical record numbers. The physicians for patients who will be sought for recruitment will be contacted and asked for their permission for the research team to approach the patient for recruitment into the study.

5.4 Early Withdrawal of Subjects

This study investigates chronic physiologic changes by fasting laboratory work as well as acutely provoked physiologic challenges. The two approaches are highly complementary, and whenever possible, we prefer to collect both kinds of information. It is conceivable that certain adverse events could motivate withdrawing a subject from selected study procedures without withdrawing from the entire study. Where appropriate, we prefer to withdraw subjects from specific procedures rather than then entire study. This section describes different foreseeable scenarios in which subjects would either be withdrawn from the entire study or withdrawn from selected procedures. For unforeseeable situations, the PI is at liberty to drop study procedures conducted for scientific reasons, but would continue separate procedures conducted for safety reasons.

5.4.1 When and How to Withdraw Subjects

The PI may withdraw a subject at any time for safety reasons or out of concern for subject adherence to the protocol requirements using clinical and scientific judgment. There are no expected adverse consequences to abrupt cessation of the study medications. Therefore, we don't anticipate any health consequences to major lapses in adherence, withdrawal or subject-initiated discontinuation. As a result, there is no need to titrate off of medications in the event a subject stops participation for any reason. If a subject is withdrawn for safety reasons, we may ask them to return for one or more brief outpatient visits following cessation of study medications to monitor for resolution.

In addition to withdrawing a subject outright, there may be circumstances where a subject may be withdrawn from a particular study procedure without being withdrawn from the entire study. For example, if a subject finds that they cannot tolerate the oral fat challenge, they can be withdrawn from that element of the study but participate in the entire study. Likewise, if a subject develops anemia during the study, the PI may delay or forego visits with heavy phlebotomy, thus averting large-volume phlebotomy. In this case, the subject could remain in the overall study but only have fasting blood draws (i.e. low-volume phlebotomy). Alternatively, since we do not expect the results to be influenced by a secular trend, such visits could be delayed.

This section provides a brief overview of specific stopping rules for individuals who develop 1) a clinically-significant elevation of liver-related clinical laboratory tests and 2) anemia, as detailed below.

5.4.1.1 Withdrawal for Clinically-Significant Changes in Liver-Related Laboratory Tests

We will monitor transaminases, bilirubin, and coagulation studies throughout the chronic therapy phase of the trial and will respond to elevations accordingly. We have developed a set of standard operating procedures (SOP) for making decisions about subjects with possible drug-induced liver injury, which appears in our SOP titled, "**Liver Monitoring Plan**." We adapted these procedures from FDA guidance to a standard operating procedure to be used across different studies, so that our group could develop a practical, evidence-based approach to liver test abnormalities. Consistent with the FDA guidance, the procedures in our SOP titled, "**Liver Monitoring Plan"** provide a range of weeks during which laboratory studies should generally be obtained, rather than a specific week. We provide the specific week the studies should occur for this study in the table in section 7 titled**, "Time and Events Chart for Visit Protocol."** In all other respects, we will adhere to management plan in our SOP titled, "**Liver Monitoring Plan"** when subjects present with evidence suggesting drug-induced liver injury.

5.4.1.2 Withdrawal or Dropping Large-Phlebotomy Visits for Low Post-Enrollment Hemoglobin

We present an overview of our approach to limiting the potential for anemia in our SOP titled, **"Hemoglobin Monitoring Plan."** That section also provides detailed stopping rules based on post-enrollment hemoglobin levels. Briefly, we will monitor hemoglobin during the study by periodic complete blood counts, and if the hemoglobin drops below 10 g/dL, we will delay upcoming visits with heavy phlebotomy (namely, the kinetics visit and oral fat tolerance test) until the hemoglobin is >= 10 g/dL, as detailed in our SOP titled, **"Hemoglobin Monitoring Plan."** If the hemoglobin does not rise to this level within 6 weeks, we will forego the next visits

involving large-volume phlebotomy and simply collect the fasting laboratory studies that would have occurred at that visit, thus averting large-volume phlebotomy, or drop the visit from the planned sequence.

5.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a subject is withdrawn for a safety-related reason that could be related to study participation, we may conduct additional follow-up visits. For example, we may follow them until resolution if medically appropriate, or until it becomes clear that the issue was not likely to be related to study participation. We will attempt to collect at least survival data on subjects who discontinue the study throughout the intended follow-up period.

6 Medications Used in the Study

6.1 Study Drug

6.1.1 Description

Per the Prescribing Information, "VASCEPA, a lipid-regulating agent, is supplied as a 1-gram amber-colored, liquid-filled soft gelatin capsule for oral administration. Each VASCEPA capsule contains 1 gram of icosapent ethyl. Icosapent ethyl is an ethyl ester of the omega-3 fatty acid eicosapentaenoic acid (EPA)."

6.1.2 Treatment Regimen

Subjects randomized to open-label EPA-rich oil will take Vascepa 4 grams daily, divided as 2 caps with the first daily meal and 2 caps with supper. We will encourage subjects to take EPA-rich oil at minimum with a calorically-substantial snack/light meal if they skip full meals.

6.1.3 Method for Assigning Subjects to Treatment Groups

The IDS will conduct the randomization for this study, and will assign subjects to the active experimental interventions vs usual care using a computerized randomization technique depending on the apolipoprotein E phenotype (see discussion in Section 7 below). Given that apoE2 and 4 mutations are far less common than apoE3, if we encounter subjects with mutations, we will randomize 1:1 to lessen chances they would cluster on one group or the other.

ApoE Phenotype	Randomization Ratio	Stratification
ApoE3	2:1	TG < 350 vs TG ≥ 350 mg/dL
ApoE2 or ApoE4	1:1	none

6.1.4 Preparation and Administration of Study Drug

IDS will handle IV preparations and tracers (one of which is oral). IDS would handle any blinded medications should the protocol be expanded to include an EPA-poor arm. In that case, IDS would conduct the randomization and dispense the masked, blinded oils. The study team will handle open-label oral medications, including open-label EPA-rich oil and iron supplements, with the option of delegating to IDS if we want to.

6.1.5 Subject Compliance Monitoring

At each study visit, compliance with study interventions will be reinforced. When study interventions are returned, compliance will be assessed by pill count for tablets. Study intervention bottles will be required to be returned at applicable visits throughout the study.

6.1.6 Prior and Concomitant Therapy

We will document concomitant prescription and OTC medications, supplements, nutraceuticals, and/or medicinal foods at baseline and at each subsequent visit, using the Concomitant Medications CRF with indication, to daily dose, and dates of drug administration. We will also record the use of dietary supplements. Starting an exclusionary medicine may result in discontinuation from the trial at the discretion of the principal investigator.

6.1.7 Packaging

The study therapeutics will be received and dispensed by the study team. Study therapeutics will be kept in a locked cabinet at ambient temperature and will be temperature will be monitored using a logger device.

6.1.8 Blinding of Study Drug

During the present Pilot Stage of this protocol, the intervention is open-label, so this section is moot. If the study progresses to the Confirmation Stage and an EPA-poor oil arm is added, we will supply additional information on investigational drug masking during a protocol amendment.

6.1.9 Receiving, Storage, Dispensing and Return

The table below shows a summary of who is handling the study interventions.

Storage, Dispensing and Accountability for Study Medications			
Medication	**Storage**	**Dispensing**	**Accountability**
AMR101	Study Team	Study Team*	Study Team
Iron	Study Team	Study Team	Study Team
Heparin	IDS	IDS	IDS
Deuterated Glycerol	IDS	IDS	IDS
Deuterated Leucine	IDS	IDS	IDS
Deuterated Water	IDS	IDS	IDS

* IDS will conduct randomization of AMR101

6.1.9.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

6.1.9.2 Storage

The study team will ensure that all study interventions are stored in a secured area, under recommended storage conditions (around 77° F) and in accordance with applicable regulatory requirements, and will be dispensed by qualified staff members. The study team will maintain accurate records regarding study intervention administration and return.

6.1.9.3 Dispensing of Study Drug

The study team will maintain accurate logs of study intervention dispensing, and will conduct regular intervention reconciliation checks to document intervention assigned, intervention consumed, and intervention remaining. This reconciliation will be logged on the intervention reconciliation form, and signed and dated by the service.

6.1.9.4 Return or Destruction of Study Drug

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

6.2 Chronically-Administered Interventions Other Than the Study Drug

6.2.1 Statins

Statins are not necessarily indicated for triglyceridemics, and in fact are among the least effective drugs for that condition. Accordingly, we expect our population to include individuals who have not been placed on statins. Despite this, we will start statins in such patients for scientific reasons. Since statins are a dominating therapy, we wanted a population of reference that included a statin background. Accordingly, subjects not on a statin will start one during a statin run-in period after the consenting visit, and if their statin-treated lipids qualify, will remain on the statin throughout the study. The run-in period will be at least 4 weeks long. When starting a statin in a statin-naïve patient, we give preference to doses expected to lower LDL in the vicinity 30 to 40%, in accordance with the recommendation by the NIH ATP report. The table below provides average percent reductions from a meta-analysis of 164 trials. Given that statin intolerance or aversion is common, we allow some doses from the meta-analysis that round up to 30% to afford greater flexibility. The shaded cells indicate the doses that we may initiate. We feel it is neither necessary nor practical to mandate a particular statin, because patients sometimes have strong preferences for or aversions to specific statins. Thus, mandating specific statins would likely prove more trouble than it's worth, especially since statin use is for a "soft" indication in this population. That said, absent a strong preference from the subject, we would favor generic statins on the stronger end of the potency spectrum (e.g. atorvastatin, simvastatin). Though we would not initiate the doses that are stricken, we would not require a patient who is doing well on such a dose to back down (i.e. we will respect their doctor's decision to place them on that dose).

Percentage Reductions In LDL Cholesterol from Statins

Statin	10 mg/d	20 mg/d	40 mg/d	80 mg/d
Atorvastatin	**-37%**	**-43%**	**-49%**	**-55%**
Fluvastatin	-15%	-21%	**-27%**	**-33%**
Lovastatin	-21%	**-29%**	**-37%**	**-45%**
Pravastatin	-20%	-24%	**-29%**	**-33%**
Rosuvastatin	**-43%**	**-48%**	**-53%**	~~-58%~~
Simvastatin	**-27%**	**-32%**	**-37%**	~~-42%~~

As an exception, for subjects who have had trouble tolerating statins, we would re-challenge with the smallest starting dose or a lower dose. Titration downward or dropping below 7 doses per week is permissible for recurrent symptoms, but we will not titrate upward if the initial dose is tolerated.

6.2.2 Marketed Hematinics and Related Therapy

The rate of phlebotomy for this study is comparable to blood draws for whole blood donation. Recently, the American Red Cross has changed their recommendations for frequent blood donors, and now recommends that donors take low-dose iron replacement to replenish iron loss from donation. This is based on literature that some donors become iron deficient, and this is easily pre-empted with modest iron replacement doses. We decided to adopt a similar approach, and initiate low-dose iron therapy to proactively replace the iron we are removing from phlebotomy. Our approach is detailed our SOP's. Briefly, we initiate iron replacement, but do not obligate participants to continue it if they do not tolerate it, for example, in those who become constipated despite the lower doses than traditional replacement doses. We also do not obligate those to start who have a history of not tolerating iron or a strong disinclination to iron supplementation. Short of discontinuing it, subjects may opt to use therapies to enhance tolerability (e.g. to relieve constipation). Adopting a similar approach as the Red Cross helps assure than risk from phlebotomy *per se* is no more than blood donation.

6.3 Acutely-Administered Interventions

The deep phenotyping procedures used in the protocol involve several acutely-dosed medications beyond the study medications. Information on these are included in their respective SOP's.

6.4 Regulatory Status of Study Interventions

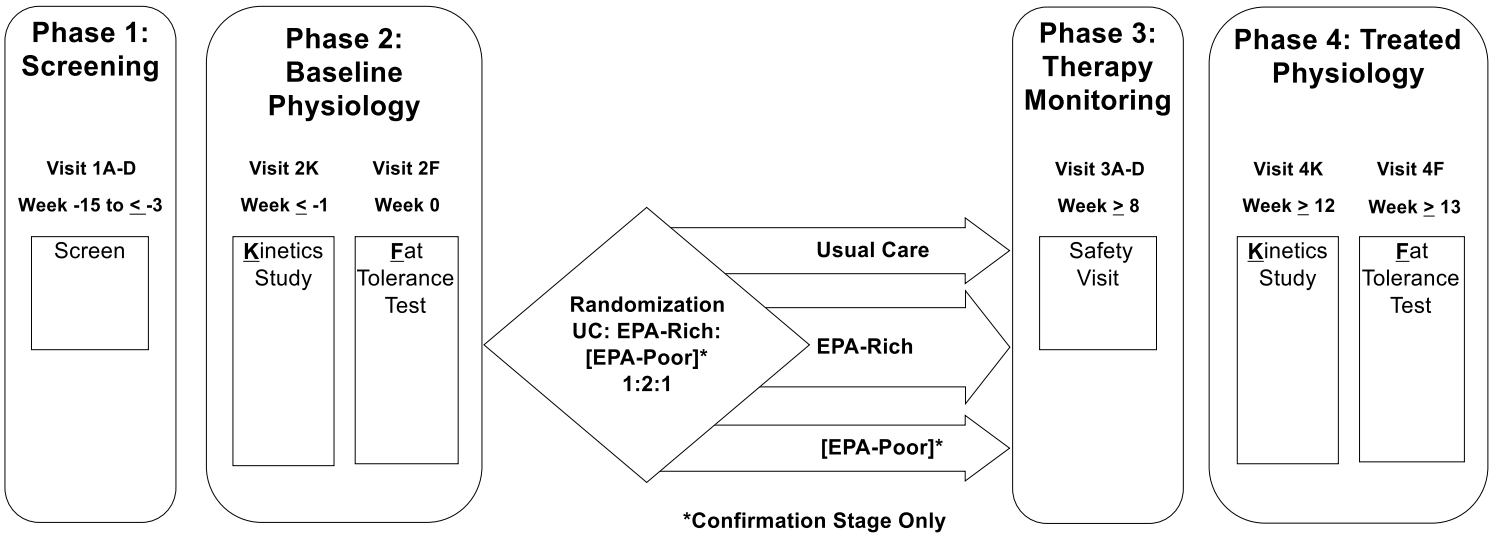
Summary of Study Interventions and Regulatory Status			
	Currently Marketed for Clinical Use	**Use Within Approved Labeling**	**IND or Exemption Required**
Chronically-Administered			
Lipid-Altering Therapy			
Omega-3 Oils			
EPA-Rich Oil (AMR101)	+	—	+
[future: EPA-Poor Oil (TBD)]	[TBD]	[TBD]	[TBD]
Statins	+	+	—
Hematinics and Related Therapy			
Supplemental Iron	+	+	—
Acutely-Administered Study Visits			
Intravenous Preparations			
Stable Isotopes for Lipid Kinetics			
Deuterated Water	—	—	+
Deuterated Leucine	—	—	+
Deuterated Glycerol	—	—	+
Lipoprotein Lipase Activity			
Heparin	+	—	+
Oral Preparations			
Foodstuffs			
Pediasure	+	+	—
Heavy cream	+	—	—
Lactase Enzyme Drops (LactAid, Lacteze)	+	+	—

TBD: to be determined; this intervention is not used in this protocol, but appears as a placeholder in anticipation of future amendments that might use this intervention, contingent on interim analysis

*This is not intended as a complete list of possible medications, since the investigators are at liberty to treat such symptoms per clinical judgment

7 Study Procedures

Schematic of Study Visits



Time and Events Chart for Visit Protocol

		Protocols					
Visit	Visit Name	Screening Procedures (SCREEN)	Longitudinal Fasting Assessments (LONGITUDINAL)	Pre-/Post-Therapy Assessments (PrePost)	Lipid Kinetics Studies (KINETICS)	Oral Fat Tolerance Test (FATLOAD)	Post-Heparin Lipase Activities (HEPARIN)
1A	Consenting Visit	+					
1B	Optional Repeat Assessment 1	+					
1C	Optional Repeat Assessment 2	+					
1D	Optional Repeat Assessment 3	+					
2K	Kinetics at Baseline		+	+	+		
2F	Fat Tolerance at Baseline		+			+	+
3A	Therapy Monitoring		+				
3B	Therapy Monitoring, Optional Re-evaluation 1		+				
3C	Therapy Monitoring, Optional Re-evaluation 2		+				
3D	Therapy Monitoring, Optional Re-evaluation 3		+				
4K	Kinetics on Randomized Intervention		+	+	+		
4F	Fat Tolerance on Randomized Intervention		+			+	+
–	Summary	4	8	2	2	2	2

This section provides details about the specific procedures that take place at study visits. The time and events charts that follow are for illustration purposes. In practice, we may decide to adjust time points (including repositioning the times, adding time points, or eliminating time points) without amending the full study protocol, provided such changes to not adversely affect safety.

7.1 Phase 1: Screening Visits

The screening phase commences with Visit 1A, which involves consent for the study and initial procedures, many of which are aimed at affirming eligibility. Visit 1A may be divided up into more than one session if needed. For example, we may opt to have prospective subjects consent to a reduced set of procedures aimed at preliminary assessment of eligibility (e.g. whole blood by point-of-care testing, venous blood for laboratory-based assays). Additional visits may occur to re-assess eligibility criteria, for example, when an assay is near the threshold or to re-assess after starting a statin, or when there is concern that an assay is not representative of the subject's baseline (e.g. suboptimal fast).

The table presents the procedures undertaken during screening. The order of procedures need not be followed, except that the consent must be performed before conducting procedures covered by the consent.

Time and Events Chart for Initial Procedures for Prospective Subjects

		Blood Drawn			
Protocol Event Name	Protocol Sequence Label	Apo E Genotyping (GENOTYPE_APOE4)	Chemistry and Lipid Panels (SST_PepperChemLipids)	Complete Blood Count (LavTop_PepperCBC)	Coagulation Panel (BlueTop_PepperCoags)
Brief consent for POC test, venous blood draw, Pediasure test, and Omega-3 Oil test	S00010				
Optional POC TG, HDL, and Glucose for early exclusion	S00020				
Pregnancy test for women of childbearing potential	S00030				
Full history	S00040				
Medications	S00050				
Venous blood draw in seated position	S00060	✓	✓	✓	✓
Pediasure Tolerance Test	S00070				
Heavy Cream Tolerance Test	S00080				
Omega-3 Oil Tolerance Test	S00090				
Vitals and dimensions (Ht, Wt, Girth)	S00100				
Full consent	S00110				

Note: Apo E Genotyping can occur at any visit prior to randomization; accordingly, it is not a deviation if Apo E genotyping is not after the Screening visit.

7.2 Procedures Common to Enrollment and Post-Enrollment Visits

7.2.1 Compliance Assessment

For subjects randomized to drug therapy, we will assess compliance by pill count at each post-enrollment visit.

7.2.2 Safety Outcomes

7.2.2.1 Adverse Events

As detailed in the safety section, we will record adverse events at each post-consenting visit.

7.2.2.2 Safety Laboratory Studies

We will assess safety by laboratory studies of complete blood count with differential and comprehensive chemistry. Ferritin may be run at the discretion of the investigator. We will run a urine pregnancy test on all women of child bearing potential at screening and the beginning of each phase (Phase 2, 3 & 4).

7.2.2.3 Scientific Studies

7.2.2.3.1 Laboratory Studies

At each study visit, we will also collect samples for fasting lipids. In addition, we may evaluate apoB-100, apoA-I, apoA-II, apoC-III, hydroxybutyrate, and free fatty acids. At the first and last kinetics visits we will also check hemoglobin A1c.

7.2.2.3.2 Fat-Free Body Mass Determination

We expect the analysis of physiologic challenges to benefit from incorporating fat-free body mass as determined by bioelectrical impedance analysis. This technique uses electrical impedance to estimate total body water, which estimates fat-free body mass. We will determine fat-free body mass at least one time during the experimental phases of the study: Phase 2 and Phase 4. We will optionally measure BIA at additional visits if practical (e.g. the machine is available during that visit).

7.2.2.4 Preparing for the Next Step in the Study

If needed, we will dispense medications. In preparation for the next study visit, we provide specific verbal and written instructions to follow the night before the next visit. We will specify the time supper should start and a hard stop for supper, snacks, and any drinks with calories. For selected visits, we provide a standardized meal, and when practical ask subjects to photograph the meal before they eat: meal, drinks, dessert, etc. We ask them to bring leftovers to the visit the next morning so we can weigh the uneaten food. We ask them to use the photograph to eat roughly the same amount at subsequent visits.

7.3 Phase 2: Baseline Physiology

Subjects found eligible during the screening visits will have a series of three deep-phenotyping procedures to determine their untreated physiology. These include an assessment of 1) lipoprotein **K**inetics (Visit 2K), 2) oral **F**at tolerance (Visit 2F), and 3) lipase activities determined at the end of the OFTT. All three procedures are repeated after the treatment period of Phase 4 (correspondingly, Visit 4K, and Visit 4F). Both of the visits must be conducted on a different week. Though we have presented these in a fixed sequence, and will endeavor to do them in this order, this is not obligatory, thus affording us flexibility in scheduling in case a subject has a week where they can commit to a shorter or longer visit. We do not expect any meaningful crossover effects to be in effect after a week's time.

7.3.1 Visit 2K: Kinetics Study

At least 7 days after measuring post-heparin lipase activities subjects will return for kinetics studies.

7.3.1.1 Kinetic Studies

We will administer deuterated water (D2O) and d-leucine to assess apolipoprotein kinetics, d-glycerol for triglyceride kinetics, and D2O for cholesterol kinetics. Labeling multiple substrates within a given lipid fraction enhances the overall model fit, improving sensitivity. This approach would permit the following assays to be measured.

Potential Kinetic Assays (Obligatory Assays Highlighted)							
Lipid Fraction	1. D20 & 2) d-Leucine					1. D20 & 2. d-Glycerol	D20
	ApoB-48	ApoB-100	ApoC-III	ApoA-I	ApoA-II	Triglyceride	Cholesterol
VLDL/CM	+	+	+			+	+
IDL (Remnant)	+	+	+			+	+
LDL		+	+			+	+
HDL			+	+	+	+	+

The shaded assays are the primary tracers that are definitely assayed during the Pilot Stage, and the remaining assays will be assayed contingent on the results of the Pilot Stage, since the utility of the contingent assays depends on the results of the primary assays. Some of the contingent assays are corroboratory, whereas others clarify mechanism by ruling out or affirming alternative pathways. Therefore, a strength of our approach is that by storing plasma and/or gels, we preserve the opportunity to interrogate the study specimens to answer questions that arise from the formal study aims. For example, emerging data supports combination EPA+DHA disrupts apoC-III metabolism, potentially explaining its mechanism of action. Our approach would allow us to answer this question comparing EPA-rich and EPA-poor oils to each other and to usual care, thus, refining the prior observation and perhaps distinguishing EPA from DHA. Similarly, we could evaluate HDL kinetics if the primary results suggest that EPA-rich and EPA-poor oils vary in their effects on the HDL pool. In summary, a distinct advantage of a multiple-substrate kinetics approach is the ability to respond quickly to new knowledge to ask new mechanistic questions without undergoing the expense of a new clinical experiment.

The overall visit commences with the following procedures including fasting lipids to assess the longitudinal response over the entire study.

Time and Events Chart for Longitudinal Plasma Lipid Assays

Protocol Event Name	Protocol Sequence Label	Blood Drawn: Chemistry Panel (SST_PepperChem)	Blood Drawn: Complete Blood Count (LavTop_PepperCBC)	Blood Drawn: Longitudinal Fasting Plasma (EDTA_Fasting_Archive)	Blood Drawn: Longitudinal Fasting Serum (SST_Fasting_Archive)
Sit in chair, feet touching floor	L00010				
Interval History	L00020				
Medication Update	L00030				
Discuss Last Meal	L00040				
Venous blood draw in seated position	L00050	✓	✓	✓	✓
Body composition by bioimpedence analysis	L00060				
Vitals and dimensions (Ht, Wt, Girth)	L00070				
Pregnancy test if not already done within a month	L00080				

We will also draw blood to assess changes to carbohydrate metabolism:

Time and Events Chart for Pre-Enrollment Procedures for Prospective Subjects

Protocol Event Name	Protocol Sequence Label	Blood Drawn: Pre/Post Intervention HgbA1c (LavTop_PepperHbA1c)	Blood Drawn: Pre/Post Intervention Carb Metabolism (EDTA_Carb_Metabolism)
Venous blood draw in seated position	P00010	✓	✓

The kinetics protocol itself includes the following procedures:

Time and Events Chart for Lipid Kinetics Procedures

						Intervention Doses					Blood Drawn			
Protocol Event Name	Protocol Sequence Label	Minute Post-Tracer Initiation	Elapsed Time Post-Tracer Initiation	Event Typical Time of Day	Omega-3 Oil (mg) vs Nothing (StudyMed)	Drink PediaSure (mL) (PediaSure)	Drink Deuterated Water (mL) (D2O_Bolus)	Inject D5-Glycerol IV Bolus (100 umol/kg) (D5Glyc_Bolus)	Inject D3-Leucine IV Bolus (umol/kg) (D3Leuc_Bolus)	ApoB Kinetics, Tube 1 (ProtINH_Kin_ApoB_Tube_1)	ApoB Kinetics, Tube 2 (ProtINH_Kin_ApoB_Tube_2)	Kinetics Archive (ProtINH_Kin_Archive)	Hgb/Hct (LavTop_Pepper_HandH)
Procedures in the lying position	KN300	-180	-03:00	06:00	-1000	237							
	KN200	-120	-02:00	07:00		237							
	KN100	-60	-01:00	08:00		237							
	KNNN1	-1	-00:01	08:59						✓	✓	✓	✓
Tracer Bolus/Infusion	K0000	0	00:00	09:00		237	52	100	10				
	K0020	20	00:20	09:20			52						
BIA±30min	K0030	30	00:30±00:30	09:30±00:30									
	K0040	40	00:40	09:40			52						
	K0100	60	01:00	10:00	-1000	237	52			✓	✓	✓	
	K0200	120	02:00	11:00		237				✓	✓	✓	
	K0300	180	03:00	12:00									
BIA±30min	K0330	210	03:30±00:30	12:30±00:30									
	K0400	240	04:00	13:00		237				✓	✓	✓	
	K0500	300	05:00	14:00	-1000								
	K0600	360	06:00	15:00		237				✓	✓	✓	✓
BIA±30min	K0630	390	06:30±00:30	15:30±00:30									
	K0700	420	07:00	16:00									
	K0800	480	08:00	17:00		237				✓	✓	✓	
	K0900	540	09:00	18:00	-1000								
	K1000	600	10:00	19:00		237				✓	✓	✓	✓
	K1100	660	11:00	20:00						✓	✓	✓	
D3-Leu Infusion Ends	K1200	720	12:00	21:00		237				✓	✓	✓	
	K1230	750	12:30	21:30						✓	✓	✓	
	K1300	780	13:00	22:00		237				✓	✓	✓	✓
Discharge for the Night	K1320	800	13:20	22:20									
Take Oil & Drink Pediasure at Home	KD2T1	1380	23:00±03:00	08:00±03:00	-1000	237							
Return in the Morning and Lie Down	KD2T2	1440	24:00±03:00	09:00±03:00									
	KD2T3	1460	24:20±03:00	09:20±03:00						✓	✓	✓	
	KD2T4	1490	24:50±03:00	09:50±03:00						✓	✓	✓	✓

Time and Events Chart for Shortened Lipid Kinetics Procedures

					Intervention Doses					Blood Draws (mL)			
Protocol Event Name	Protocol Sequence Label	Minute Post-Tracer Initiation	Elapsed Time Post- Tracer Initiation	Event Typical Time of Day	Omega-3 Oil (mg) vs Nothing (StudyMed)	Drink Pediasure (mL) (Pediasure)	Drink Deuterated Water (mL) (D20_Bolus)	Inject D5-Glycerol IV Bolus (100 umol/kg) (D5Glyc_Bolus)	Inject D3-Leucine IV Bolus umol/kg) (D3Leuc_Bolus)	ApoB Kinetics, Tube 1 (ProtINH_Kin_ApoB_Tube_1)	ApoB Kinetics, Tube 2 (ProtINH_Kin_ApoB_Tube_2)	Kinetics Archive (ProtINH_Kin_Archive)	Hgb/Hct (LavTop_Pepper_HandH)
Procedures in the lying position	KN300	-180	-03:00	06:00	-1000	237							
	KN200	-120	-02:00	07:00		237							
	KN100	-60	-01:00	08:00		237							
	KNNN1	-1	-00.01	08:59						✓	✓	✓	✓
Tracer Bolus / Infusion	K0000	0	00:00	09:00		237	52	100	10				
	K0020	20	00:20	09:20			52						
BIA ± 30 min	K0030	30	00:30 ± 00:30	09:30±00:30									
	K0040	40	00:40	09:40			52						
	K0100	60	01:00	10:00	-1000	237	52			✓	✓	✓	
	K0200	120	02:00	11:00		237				✓	✓	✓	
	K0300	180	03:00	12:00									
BIA ± 30 min	K0330	210	03:30 ± 00:30	12:30 ± 00:30									
	K0400	240	04:00	13:00						✓	✓	✓	
	K0500	300	05:00	14:00	-1000								
	K0600	360	06:00	15:00		237				✓	✓	✓	✓
BIA ± 30 min	K0630	390	06:30 ± 00:30	15:30 ± 00:30									
	K0700	420	07:00	16:00									
	K0800	480	08:00	17:00		237				✓	✓	✓	
	K0900	540	09:00	18:00	-1000								
D3-Leu Infusion Ends	K1000	600	10:00	19:00		237				✓	✓	✓	✓
	K1030	630	10:30	19:30						✓	✓	✓	
	K1100	660	11:00	20:00						✓	✓	✓	
Discharge for the Night	K1200	720	12:00	21:00		237				✓	✓	✓	
Take Oil & Drink Pediasure at Home	KD2T1	1380	23:00±3:00	08:00±3:00	-1000	237							
Return in the Morning and Lie Down	KD2T2	1440	24:00±3:00	09:00±3:00									
	KD2T3	1460	24:20±3:00	09:20±3:00						✓	✓	✓	
	KD2T4	1490	23:50±3:00	09:50±3:00						✓	✓	✓	✓

Subjects will initially be treated with a statin for a minimum of 4 weeks prior to the first kinetic study. Subjects will undergo a lipoprotein kinetic study during Phase 2 and after treatment in Phase 4. Briefly, subjects will drink 4 doses (52mL per dose) of 70% deuterium oxide (aka “heavy water” or D2O) over a one hour period before a bolus injection of [1,1,2,3,3-^{2}H5]-glycerol (aka D5-glycerol) (100 µmol/kg body weight) and [5,5,5-^{2}H5]-L-leucine (also known as D3-leucine) (10 µmol/kg body weight). Both the glycerol and leucine boluses will be prepared in 20mL volumes. This is immediately followed by a constant infusion of D3-leucine (10 µmol/kg body weight/hour) (12 µmol/kg body weight/hour for shortened kinetics visit) over a 13-hour (10 hours for shortened kinetics visit) period under constantly-fed conditions: Subjects drink Pediasure (an iso-caloric drink consisting of ~33% fat) every hour for 5 hours and thereafter every other hour (12 servings total). We will collect blood samples before the tracer boluses and 1, 2, 4, 6, 8, 10, 11, 12, 12.5, and 13 hours post-bolus to determine the kinetics of apolipoprotein (apo) B100 in VLDL, very low-density lipoprotein, and low density lipoprotein (LDL). We will also collect 2 kinetics samples the next day, roughly between the hours of 6am to 12pm (indicated on the table as $\pm$03:00; the corresponding range for minutes post-tracer would be $\pm$180). Each sample will be separated by 15 to 30 minutes. Subjects will be instructed to drink a Pediasure (13th one) and take their study medication if applicable 1 hour before the first time-point. These Kinetics Day 2 samples are optional and set up this way at it may be difficult for subjects to return to the clinic the next day. The range of time points will not negatively impact the analysis, as long as the correct time of the shake and blood draws are documented. In general, we would strive to get more than one specimen at the end, so that the last part of the regression line doesn’t depend on a solitary data point. Archive samples will also be obtained for possible determination of VLDL Triglyceride (TG) as well as intermediate-density lipoprotein (IDL) apoB and LDL cholesterol kinetics. The kinetics models may be influenced by changes in plasma volume. Since plasma volume changes seasonally, and pre- and post-therapy kinetics may span seasonal transitions, we will estimate plasma volume and its changes throughout the duration of the study and within the prolonged kinetics visit, using BIA to measure various water compartments and Hgb/Hct at various times during the kinetics visit.

7.3.2 Visit 2F: Fat Tolerance Test

At least 7 days after the lipoprotein kinetic study, subjects will undergo their second metabolic study to determine the post-prandial lipoprotein response.

7.3.2.1 Oral Fat Tolerance Test

The results of the oral fat tolerance test depend on frequently-sampled total triglycerides, HDL-cholesterol, and apolipoprotein B-100. Other markers of interest may be performed, including apolipoprotein C-III, hydroxybutyrate, and free fatty acids as corroboratory assays or as assays of related physiology.

Time and Events Chart for Oral Fat Tolerance Test

						Intervention Doses			Blood Drawn				
Protocol Event Name	Protocol Sequence Label	Minute Post-Fat Load	Minute Post-Heparin	Elapsed Time Post-Fat Load	Event Typical Time of Day	Omega-3 Oil (mg) vs Nothing (StudyMed)	Drink Heavy Cream (g/m2) (FatLoad)	Heparin Injection (U/Kg) (HepINJ)	OFTT Lipids (mL) (EDTA_OFTT_Lipid)	OFTT Plasma Archive (mL) (EDTA_OFTT_Archive)	OFTT Serum Archive (mL) (Serum_OFTT_Archive)	RNA Expression (mL) (PAXRNA_OFTT)	Heparin Tube for Lipases (HepTube_LPL)
Procedures in the lying position	FNN15	-15		-00:15	07:45	-1000			✓				
	FNN11	-11		-00:11	07:49				✓	✓			
	FNNN6	-6		-00:06	07:54				✓				
	FNNN1	-1		-00:01	07:59				✓	✓	✓	✓	
Drink Fat Load	F0000	0		00:00	08:00		50						
	F0100	60		01:00	09:00				✓	✓			
	F0200	120		02:00	10:00				✓	✓	✓		
	F0300	180		03:00	11:00	-1000			✓	✓			
BIA±30min	F0330	210		03:30±00:30	11:30±00:30								
	F0400	240		04:00	12:00				✓	✓	✓	✓	
	F0500	300		05:00	13:00				✓				
	F0600	360		06:00	14:00	-1000			✓	✓	✓		
	F0700	420		07:00	15:00				✓				
BIA±30min	F0730	450		07:30±00:30	15:30±00:30								
	F0800	480		08:00	16:00				✓	✓	✓	✓	
	F0900	540		09:00	17:00	-1000			✓				
	F1000	600		10:00	18:00				✓	✓	✓		
	F1059	659		10:59	18:59				✓				
	HNNN1	659	-1										✓
Inject heparin after draw	H0000	660	0	11:00	19:00			60					
	H0004	664	4	11:04	19:04								✓
	H0008	668	8	11:08	19:08								✓
	H0012	672	12	11:12	19:12								✓
	H0016	676	16	11:16	19:16								✓
	H0020	680	20	11:20	19:20								✓
	H0030	690	30	11:30	19:30								✓
	H0045	705	45	11:45	19:45								✓
	H0100	720	60	12:00	20:00								✓
Eat general supper and d/c	H0101	721	61	12:01	20:01								

The oral fat tolerance test commences when the subject drinks the oral fat load of heavy cream. The CTRC metabolic kitchen staff will prepare the cream, and will add lactase drops. If the subject has lactose intolerance, we encourage them to take their preferred over-the-counter lactase enzymes. If they fail to bring lactase enzymes, we will also offer them additional lactase tablets if they so desire. The study team or the kitchen will maintain the stock of lactase tablets rather than the IDS. The Oral Fat Tolerance Test involves serial blood draws to evaluate pharmacodynamic effects of omega-3 oil on postprandial lipoproteins. Occasionally, subjects have dyspepsia, nausea, or uncomfortable hunger during the oral fat tolerance test visit. Some subjects respond to a sugar-free gelatin snack (Jell-O®, Kraft Foods or generic equivalent), which we provide when needed. At the conclusion of the Oral Fat Tolerance Test, we will give subjects a mixed meal prior to discharge.

Apart from the fat load itself and possibly gelatin, subjects do not receive calories from traditional meals until prior to discharge.

The Oral Fat Tolerance Test differs between Phase 2 and Phase 4 in that the latter two include dosing of omega-3 oil among those randomized to this group. Apart from study intervention administration and dispensing, the visits are identical.

7.3.2.2 Heparin-Stimulated Lipoprotein Lipase Activity

After the oral fat tolerance test concludes, we will assess heparin-stimulated LPL activity at baseline and on treatment, as changes in in LPL activity will aid the interpretation of the Mechanistic Aims (i.e. Aims M1, M2, and M3) and may improve the fit of the compartmental model. Moreover, LPL activity may aid the interpretation of the Clinical Aims (i.e. Aims C1 and C2).

Study staff will inject a small amount of heparin (60 units/kg body weight) as a one-time bolus. The table provides a schedule of subsequent lab draws to assess the post-heparin lipase activity curve.

7.4 Phase 3: Therapy Monitoring

Subjects return during the treatment phase of the study to review medications, assess for adverse events, check safely laboratory studies, and collect specimens for longitudinal pharmacodynamics responses and the like. Specific procedures are detailed above under “Procedures Common to Enrollment and Post-Enrollment Visits.” Subjects may return during this Phase if findings from the planned visit warrant close follow up.

We also assess therapy remotely by telephone call or other communications. For example, we will schedule a phone call to take place two to three weeks after starting therapy. We will ask about adverse effects during this call and interval changes to history or medications, and specifically ask about successful adherence to the regimen for those subjects assigned to an active intervention. We may ask the subject to count remaining capsules, or photograph the remaining capsules so we can conduct a pill-count without requiring a visit. If we learn that adherence is lower than 80%, we will counsel on the need to improve adherence, and initiate a compliance monitoring plan. The latter may involve scheduling additional calls to re-assess compliance before the next face-to-face visit.

7.5 Phase 4: Treated Physiology

Additional Visits for Safety Assessments

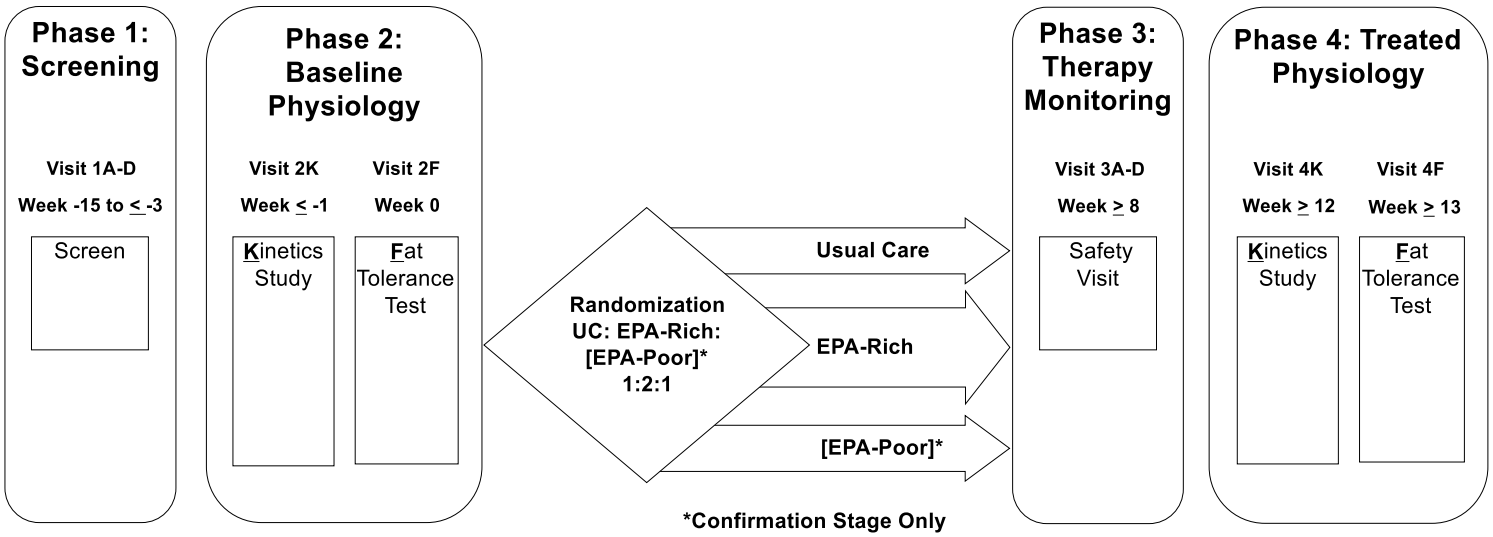
We may arrange additional visits with the subject if there are safety or tolerability concerns, during which we would conduct appropriate safety laboratory studies including BIA. The studies obtained at these visits are at the discretion of the investigators and would depend on the clinical circumstances.

Early Termination Visit

If a subject drops from the trial, we will encourage them to participate in an optional early termination visit, at which we will repeat safety laboratory studies, scientific laboratory studies as appropriate, and the optional BIA measurement of fat-free body mass.

7.6 Total Phlebotomy Across All Planned Visits

The figure below presents projected phlebotomy requirements for each phase of the study. The totals for Phase 1 and Phase 3 assume multiple visits (4 Phase 1 visits and 4 Phase 3 visits). In practice, subjects may only require 1 or 2 visits for screening and therapy monitoring, so this represents a "worst case scenario." Phase 2 and 4 involve phlebotomy on the order of a standard blood donation by the Red Cross, which allows a 550 mL draw as often as every 56 days. An important difference is that the Red Cross draws a comparable blood volume over 8-10 minutes, whereas we draw over at least a week, limiting potential for hemodynamic adverse events. Another important distinction is that we require at least 3 months from the last visit of Phase 2 and the first visit of Phase 4. Thus, we mandate more time between large-volume draws than the minimum time the Red Cross allows between donations. The time between phlebotomy is important to replenish iron stores removed by phlebotomy, which we aid by providing iron supplements. The maximal amount drawn for the study is 1300 mL spread out over at least 17 weeks; future changes that would involve exceeding that amount would require a protocol amendment.



7.7 Laboratory Evaluations

Laboratory assays for this study may either be 1) stored for subsequent assay in runs by subject or 2) contemporaneously (i.e. the assay is conducted as we go with continuous evaluation). By necessity, the safety assays are run in real time. Note the real time does not mean "same day." We decided against requiring contemporaneous assays to be conducted, reported, or reviewed on the same day, because this would likely require stat accession for all assays. Since this was not felt to be medically necessary, contemporaneous safety assays do not require same-day evaluation.

7.8 Laboratory Tests on Stored Samples

The pharmacodynamic outcomes based on laboratory analyses will be conducted on frozen plasma samples after a subject has completed a given experiment, so that the tests may be run in batches. Since this is a chronic therapy study, we prefer to analyze a given subject's samples in the same run or session.

7.8.1 Plasma Processing and Storage

Blood for plasma analysis will be collected into EDTA tubes that are pre-chilled on ice, and are again placed on ice immediately after collection. Within ½ hour of collection, plasma is separated using a centrifuge chilled to 4°C for 15 minutes at 3,000 rpm. The plasma is collected into cryovials for long-term storage in a –70°C freezer until laboratory analysis. Because FFA and insulin are especially sensitive to multiple freeze/thaw cycles, when assayed, we will make every attempt to analyze these on the first thaw. Whenever possible, we will use clinical chemistry analyzers with multiplexing capabilities so conserve plasma and limit multiple freeze/thaw cycles.

7.8.2 Additional Analyses of Unused Specimens

Unused specimens may be used for future assays related to atherosclerosis, lipoprotein metabolism, metabolism of administered interventions, inflammation, energy metabolism and/or intervention safety. Because red cell lipids are especially stable over time, we may isolate red cells for future analyses. At the investigator and Sponsor's discretion, any additional serum, plasma, or red blood cell isolates that remains following the protocol-required tests performed by University of Pennsylvania laboratories may be stored and analyzed, and custody of these samples will remain with investigator until destruction.

8 Statistical Plan

8.1 Sample Size Determination

8.1.1 Sample Size

Since data specific to EPA are not available, the study is formally designed as a pilot study to determine relevant statistical parameters to design a definitive study (e.g. mean, standard deviation, and shape of distribution for outcomes). Since African Americans differ substantially from Caucasians in triglyceride metabolism, at this stage we will limit participation to Caucasians to limit effect modification. This is especially important at the Pilot Stage because effect modification could disproportionately bias the estimates of statistical parameters, and thus, potentially misinform the anticipated Confirmation Stage. If this study affirms expected benefits, it would warrant future study of African Americans, but at this point, doing so might actually undermine the study objectives. One of the adaptive elements of the study design involves the sample size, which may be increased based on interim data under a protocol amendment. The minimum sample size is based on 19 enrollees, 12 assigned to open-label EPA-rich oil and 7 to usual care sans omega-3 oil. Assuming 15% drops from EPA-rich oil and 10% drops from usual care, we anticipate 10 completers assigned to EPA-rich oil and 6 to usual care (Table). The 10 observations on EPA-rich oil should allow us to assess distributional shape.

Screen Fail Rate:	**50%**	**Ratio:**	**2**	**1**	**Attrition Rate:**	**15%**	**10%**

Screened Population	**Enrolled Population**			**Completer Population**		
	Total Subjects	**On Drug**	**No Treatment**	**Total Subjects**	**On Drug**	**No Treatment**
80	40	26	14	34	22	12
78	39	26	13	33	22	11
76	38	25	13	32	21	11
74	37	24	13	31	20	11
72	36	24	12	30	20	10
70	35	23	12	29	19	10
68	34	22	12	28	18	10
66	33	22	11	27	18	9
64	32	21	11	26	17	9
62	31	20	11	26	17	9
60	30	20	10	26	17	9
58	29	19	10	25	16	9
56	28	18	10	24	15	9
54	27	18	9	23	15	8
52	26	17	9	22	14	8
50	25	16	9	21	13	8
48	24	16	8	20	13	7
46	23	15	8	19	12	7
44	22	14	8	18	11	7
42	21	14	7	17	11	6
40	20	13	7	17	11	6
38	**19**	**12**	**7**	**16**	**10**	**6**
36	18	12	6	15	10	5
34	17	11	6	14	9	5
32	16	10	6	13	8	5
30	15	10	5	12	8	4

In summary, the essential purpose of the Pilot Stage is to estimate statistical parameters to guide the design of the eventual Confirmation Stage. The sampling plan described above provides a reasonable sample to infer variability and central tendency, especially important to the EPA-rich oil group, for whom there are no data from the lipid kinetics literature.

Our ability to double-label certain substrates is expected to reduce variability compared to prior studies of fish oil kinetics, as is comparison to usual care sans fish oil rather than a metabolically-active oil. Moreover, the use of an EPA-rich oil may inherently

reduce variability if we are correct that EPA and DHA work at cross purposes somewhat. For these reasons, our empirical standard deviation may be smaller than prior studies. On the other hand, we may have more variability based on heterogeneity of our study population. To mitigate the effect of this uncertainty on power, we will estimate the pooled standard deviation after 6 subjects complete the study to determine the effect size that would be detectable, and may use this information to increase the sample size under a protocol amendment if appropriate. We will begin preliminary tests of the applicable outcomes after 13 subjects are enrolled (that is, both standard deviation *and* effect size), allowing 6 more subjects to accrue while analyzing the interim data. We may use this information to increase the sample size under a protocol amendment (cf Table). We will expend the type I error rate accordingly, so that significance would require a cutoff lower than the customary 0.05 level. This approach will let us adapt the sample size to improve the chances of affirming outcomes that would benefit from additional observations.

Again, the interim analysis would also inform the design of the eventual Confirmation Stage, which again would be submitted as a substantial protocol amendment should it be warranted by the Pilot Stage.

8.1.2 Altered Enrollment Ratio for Apo E Variants

Triglyceride handling varies by apolipoprotein E (apoE) status. There are 3 common alleles for apoE: ApoE2, ApoE3, and ApoE4. The normal allele is ApoE3, so most individuals have ApoE3/E3. However, the other alleles are sufficiently common that we will likely have alternative genotypes in the study. Certain functional variations in the ApoE gene appear to cause effect modification on 1. fish oil kinetics and 2. postprandial triglyceridemia. Thus, it is especially important that people with phenotypes influenced by ApoE variants do not inadvertently cluster into one group or the other. Since alternative phenotypes are common enough to be expected, but still remain in the minority, stratification by a 2:1 ratio would be problematic because we may or may not get a match for a patient with a given phenotype. Therefore, if we enroll a person with an ApoE2 phenotype (genotypes 2/2 or 2/3) or ApoE4 phenotype (genotypes 3/4 or 4/4), we will attempt to balance them 1:1 between usual care and active EPA-rich oil. This is an exception to the targeted 2:1 ratio for most subjects, who will have the Apo E3 phenotype (genotypes 3/3 or 2/4). We will revisit the issue of ApoE variants during the interim analysis, which may motivate the alternative approach of designing a substudy to allow crossover for people with ApoE variants. Doing so would assure balance, but also improve power through statistical efficiencies of the crossover design for this rarer population. If the pilot data support a crossover approach for the variants, we would do so as a substantial protocol amendment. At this stage, we will simply gather data under the parallel design to inform that decision.

	Apo E2 Response		Apo E3 Response		Apo E4 Response	
Genotype	2/2	2/3	3/3	2/4	3/4	4/4
Population Frequency	1%	10%	62%	2%	20%	5%
Fish Oil[1]	↓↓ TG ↓ small dense LDL ↑ HDL		↓ TG ↓ small dense LDL ↑ HDL		↓ TG ↓↓ small dense LDL ↓ HDL ↑↑ LDL	
Low Fat Diet[2,3]	↓ LDL ↑ small dense LDL		↓↓ LDL ↔ small dense LDL		↓↓↓ LDL ↓ small dense LDL	
Moderate Fat Diet[3]	↔ LDL ↔ small dense LDL		↓ LDL ↓ small dense LDL		↓ LDL ↑↑ small dense LDL	
Moderate Alcohol[4]	↑ HDL ↓ LDL		↑ HDL		↓ HDL ↑ LDL	
Effective Drug Response	Atorvastatin Pravastatin Lovastatin		No distinction		Probucol Simvastatin	

1. Minihane AM *et al. Arterioscler Thromb Vasc Biol.* 2000 Aug; 20(8):1990-7.
2. Masson LF *et al. Am J Clin Nutr.* 2003;77:1098-111.
3. Moreno JA *et al. J Nutr.* 2004;134:2517-2522.
4. a) Corella D *et al. Am J Clin Nutr.* 2001 Apr; 73(4):736-45 b) Marques-Vidal P *et al. Obes Res.* 2003 Oct;11(10):1200-6. c) Mukamal KJ *et al. Atherosclerosis.* 2004 Mar:173(1):79-87 d) Bleich S *et al. J Neural Trans.* 2003 Apr;110(4):401-11. e) Proc Nutri Soc 2004(65):5-10 f) Lussier-Cacan S *et al. Arterioscler Thromb Vasc Biol.* 2002 May 1:22(5):824-31.

Figure 1 Source: Berkley Heart Lab

8.1.3 Stratification

To limit potential for confounding by triglycerides, we will attempt to balance randomization by TG > 350 mg/dL, so that very triglyceridemic people do not cluster in one group by chance alone. This stratification applies to people without functional Apo E variants (i.e. 3/3 and 2/4), but is not practical for those with functional variants because of their relative scarcity.

8.2 Techniques to Limit Variability

Intensive techniques such as lipoprotein kinetics offer the opportunity to control sources of variability. In this study, we plan to test several methods aimed at reducing measurement variability. For example, we will collect blood at more time points than is often done for kinetic studies, and in particular, we will over-sample the ends. In theory, this could help to mitigate the effect of an outlier at the start or end of the tracer enrichment period, which might otherwise be disproportionately influential. Likewise, we are oversampling regions of the postprandial curve when we anticipate rapid changes to reduce variability around critical points.

8.3 Statistical Methods

We will develop a separate Statistical Analysis Plan (SAP), but describe our general approach here. We will begin the analysis by fully describing data, including aspects of data quality, and will summarize outcomes and explanatory variables, using graphical methods (e.g. Tukey's notch plots and "spaghetti" plots) in order to evaluate data quality and examine distributional parameters (especially normality). We will evaluate analytes over time to assess patterns of change, and will examine subject-specific values and treatment group summaries over time. Interval variables will be summarized using means and standard deviations as well as medians and interquartile ranges. Categorical variables, including dichotomous factors, will be summarized by proportions. We will characterize the amount and patterns of missing data, if any, and will use transformations if needed to yield variables that conform to the distributional assumptions underlying the analytic techniques. For instance, some continuous variables may be transformed to log scales, as needed to reduce skew, or when multiple continuous variables are in the same model, optimized multivariable Box-Cox transformation.

Our general modeling approach is to use mixed or random effects panel regression (e.g. mixed or gsem in Stata), with treatment assignment and sex as categorical variables and subject as a random intercept (and/or slope), to model each outcome as the dependent variable. Initially we will model period as a categorical variable as well as a period x treatment interaction. On an exploratory basis we will include terms for apoE phenotype and/or baseline triglycerides. In any models with random slopes, we will determine the optimal covariance pattern empirically as that with the lowest Bayesian information criterion (BIC). Hypotheses are two-tailed, and statistical significance for hypothesis testing is initially set at an alpha < 0.05, but expended after interim analysis. If any of the assumptions underlying the analysis methods are violated, we will perform the analyses using alternative statistical methods as appropriate.

Our statistical approach to the adaptive elements accords with the FDA's draft "Guidance for Industry: Adaptive Design Clinical Trials for Drugs and Biologics," v2010. Specifically, the early look at pooled variability will not utilize information about treatment assignment, so as to limit alpha expenditure. On the other hand, the later look will utilize treatment assignment, thus inflating the Type I error rate. Accordingly, we will then re-set the customary alpha from p<0.05 to a "tighter" limit as recommended, per the SAP.

8.4 *Subject Population(s) for Analysis*

The purpose of this study is mechanistic in nature, rather than to inform clinical practice *per se.* Accordingly, outcomes assessment is per protocol, with the exception of clinical safety outcomes. If a subject assigned to an active intervention does not maintain >= 80% compliance, this presents more than one possibility for analysis. The primary analysis and perhaps most straightforward is to exclude them from the efficacy analysis outright. As an alternative sensitivity analysis, we may include the compliance rate as a covariate or a weight. If we measure specific levels of fat (e.g. RBC omega-3 index, or EPA/DHA levels), we could also consider the change in that outcome from baseline as a functional assay of drug exposure. For safety outcomes, we will conservatively analyze the intention to treat population, defined as all individuals who took at least one dose of at least one study medication during the chronic therapy phase of the study.

9 Safety and Adverse Events

9.1 Definitions

9.1.1 Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, prescribing information, the investigators brochure, Standard Operating Procedures and the like).
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

9.1.2 Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

9.1.3 Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

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

9.2 Intensity

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

Mild: associated with no limitation of usual activities or only slight discomfort
Moderate: associated with limitation of usual activities or significant discomfort
Severe: associated with inability to carry out usual activities or very marked discomfort
Life-Threatening: represents an immediate threat to life

9.3 Relationship to Study Procedure

The relationship of AEs to study procedures including medications will be assigned by the investigator according to the following guidance. Prior versions of this protocol used less specific guidance to rate the relationship. We feel the rating system below improves upon this because the guidance is more specific, lays out the different questions more clearly, and requires a specific number of criteria to be met.

1. **Unrelated**: The adverse event is clearly attributable to extraneous causes (e.g., underlying disease, environment)
2. **Unlikely Related** (must have 2)
   (A) Does not have temporal relationship to the study procedure.
   (B) Could readily have been produced by the subject's clinical state.
   (C) Could have been attributable to environmental or other interventions.
   (D) Does not follow known pattern of response to study procedure.
   (E) Does not reappear or worsen with reintroduction or continued use of the study procedure.
3. **Possibly Related** (must have 2)
   (A) Has a reasonable temporal relationship to study procedure.
   (B) Could not readily have been produced by the subject's clinical state.
   (C) Could not readily have been attributable to environmental or other interventions.
   (D) Follows a known pattern of response to study procedure.
4. **Probably Related** (must have 3)
   (A) Has a reasonable temporal relationship to study procedure.
   (B) Could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions.
   (C) Follows a known pattern of response to study procedure.
   (D) Disappears or decreases on cessation of the study procedure or following a remediating intervention (i.e. a reasonable temporal relationship to resolution of the adverse effect).
5. **Definitely Related** (must have all 4)
   (A) Has a reasonable temporal relationship to study procedure.
   (B) Could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions.
   (C) Follows a known pattern of response to study procedure.
   (D) Disappears or decreases on cessation of the study procedure or following a remediating intervention (i.e. a reasonable temporal relationship to resolution of the adverse effect).

9.4 Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

9.5 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

9.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

9.7 Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

9.8 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, referral to the patient's primary care physician for further evaluation and management, and the like.

9.9 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.10 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.11 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

9.11.1 Investigator reporting: notifying the funding sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and emailed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to:

Amarin Safety Contact Information:
Safety reporting hotline: 1-855-VAS-CEPA or 1-855-827-2327
E-mail: amarinmi@druginfo.com

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

Note: Pregnancy is not considered a serious adverse event. Pregnancy must however, be reported immediately in the same manner as an SAE.
Note: Overdose, regardless of adverse outcome, must be reported immediately in the manner as an SAE.

9.11.2 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, Standard Operating Procedures, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.

  AND

  Related to the research procedures: An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures. For clarity, events that are rated "probably related" or "definitely related" are considered more probable than not by definition (cf Relationship to Study Procedure above).

Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.

- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.12 Unblinding Procedures

The present Pilot Stage of the study does not involved blinded medication, so this section is moot. If the study progresses to the Confirmation Stage, unblinding procedures will be added under a protocol amendment.

9.13 Stopping Rules

Based on prior literature and experience with fish oil in general, and EPA-rich oil in particular, we think serious adverse events from the investigational medications are not likely. Safety and tolerability will be evaluated by the incidence, severity, and relationship to study intervention of any adverse events and changes from baseline in laboratory test results, physical examination findings, and vital sign measurements, at any time after the subject has received study intervention. If continuous safety analysis reveals an adverse trend, the Principal Investigator may halt the study independently, or in consultation with the CTRC or IRB, as appropriate. Furthermore, if three or more subjects develop the same serious adverse event related to a study medication or procedure, we will suspend the trial pending a safety review, in consultation with the CTRC and the IRB.

9.14 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. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

10 Data Handling and Record Keeping

10.1 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 (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 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.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

11 Study Monitoring, Auditing, and Inspecting

11.1 Study Monitoring Plan

The financial sponsor of the study may monitor the study according to a separate monitoring plan. 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 above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups 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 University compliance and quality assurance offices.

12 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

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. See the separate Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

13 Study Finances

13.1 Funding Source

The funding source for this trial is Amarin Pharma, Inc.

13.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

14 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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(2) Grundy SM. An International Atherosclerosis Society Position Paper: Global recommendations for the management of dyslipidemia. Journal of Clinical Lipidology 7[6], 561-565. 11-1-2013.
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(4) Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007 July 18;298(3):309-16.

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(6) Wong AT, Chan DC, Ooi EM, Ng TW, Watts GF, Barrett PH. Omega-3 fatty acid ethyl ester supplementation decreases very-low-density lipoprotein triacylglycerol secretion in obese men. *Clin Sci (Lond)* 2013 July;125(1):45-51.

(7) Chan DC, Watts GF, Barrett PH, Beilin LJ, Redgrave TG, Mori TA. Regulatory effects of HMG CoA reductase inhibitor and fish oils on apolipoprotein B-100 kinetics in insulin-resistant obese male subjects with dyslipidemia. *Diabetes* 2002 August;51(8):2377-86.

(8) Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *The Lancet* 1931 March;369(9567):1090-8.