OMEGA-3 FATTY ACIDS AND DYSLIPIDEMIC AGENT FOR REDUCTION OF APO-B LEVELS

[0001] The present application claims priority to U.S. Provisional Application Serial No. 60/850,280, filed October 10, 2006, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a method utilizing a single administration or a unit dosage of a combination of a dyslipidemic agent and omega-3 fatty acids for the reduction of apolipoprotein-B levels. This method is especially useful in the treatment of patients with primary hypertriglyceridemia or hypercholesterolemia or mixed dyslipidemia, coronary heart disease (CHD), vascular disease, atherosclerotic disease and related conditions, and for the prevention or reduction of cardiovascular and vascular events.

BACKGROUND OF THE INVENTION

[0003] In humans, cholesterol and triglycerides are part of lipoprotein complexes in the bloodstream, and can be separated via ultracentrifugation into high-density lipoprotein (HDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) fractions. Cholesterol and triglycerides are synthesized in the liver, incorporated into VLDL, and released into the plasma. High levels of total cholesterol (total-C), LDL-C, and apolipoproteinB (apo-B, a membrane complex for LDL-C and VLDL-C) promote

human atherosclerosis and decreased levels of HDL-C and its transport complex, apolipoprotein A (apo-A), which are associated with the development of atherosclerosis. Further, cardiovascular morbidity and mortality in humans can vary directly with the level of TC and LDL-C and inversely with the level of HDL-C. In addition, researchers have found that non-HDL cholesterol (non-HDL-C), which is determined by the subtraction of HDL-C from TC, is an important indicator of hypertriglyceridemia, vascular disease, artherosclerotic disease and related conditions. Non-HDL-C particles contain apo-B as the membrane-complexing apolipoprotein. Although non-HDL-C is a good measure for the total amount of cholesterol present in atherogenic apo-B-containing particles, a direct measure of apo-B may provide a better measure of the amount of atherogenic particles per unit of serum.

[0004] Although LDL-C remains the lipid value commonly used to assess cardiovascular risk, apo-B may better reflect lipid risk. Sniderman, *Am. J. Cardiol.* 90(suppl):48i-54l (2002), reviews the evidence supporting the value of apo-B in predicting coronary artery disease risk and its superiority over calculated LDL-C levels.

[0005] Cardiovascular disease (CVD) is a broad term that encompasses a variety of diseases and conditions. It refers to any disorder in any of the various parts of the cardiovascular system, which consists of the heart and all of the blood vessels found throughout the body. Diseases of the heart may include coronary artery disease, CHD, cardiomyopathy, valvular heart disease, pericardial disease, congenital heart disease (e.g., coarctation, atrial or

ventricular septal defects), and heart failure. Diseases of the blood vessels may include arteriosclerosis, atherosclerosis, hypertension, stroke, vascular dementia, aneurysm, peripheral arterial disease, intermittent claudication, vasculitis, venous incompetence, venous thrombosis, varicose veins, and lymphedema. Some patients may have received treatment for their CVD, such as vascular or coronary revascularizations (angioplasty with or without stent placement, or vascular grafting). Some types of cardiovascular disease are congenital, but many are acquired later in life and are attributable to unhealthy habits, such as a sedentary lifestyle and smoking. Some types of CVD can also lead to further heart problems, such as angina, major adverse cardiovascular events (MACEs) and/or major coronary events (MCEs) such as myocardial infarction (MI) or coronary intervention, or even death (cardiac or cardiovascular), which underscores the importance of efforts to treat and prevent CVD.

[0006] Primary prevention efforts are focused on reducing known risk factors for CVD, or preventing their development, with the aim of delaying or preventing the onset of CVD, MACEs or MCEs. Secondary prevention efforts are focused on reducing recurrent CVD and decreasing mortality, MACEs or MCEs in patients with established CVD.

[0007] MACEs include cardiac death, other cardiovascular death, MCEs (which include myocardial infarction (MI) and coronary intervention such as coronary revascularization, angioplasty, percutaneous transluminal coronary angioplasty (PTCA), percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG)), hospitalization for unstable angina, stroke, transient ischemic

attack (TIA) and hospitalization and/or intervention for peripheral artery disease (PAD).

[0008] The Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, NIH Publication No. 02-5215 (September 2002) (also known as the "NCEP ATP III"), hereby incorporated by reference, provides recommendations for cholesterol-lowering therapy in an effort to reduce risk of CHD. In the ATP III, CHD is defined as symptomatic ischemic heart disease, including MI, stable or unstable angina, demonstrated myocardial ischemia by noninvasive testing, and history of coronary artery procedures. The ATP III indicates that LDL-C is the primary target of lipid therapy, with other lipids to be controlled including triglycerides (TG), non-HDL-C and HDL-C. Apo-B is listed as an emerging risk factor. While the ATP III was not prepared to replace LDL-C as the primary target of lipid therapy, it noted that limited epidemiological and clinical trial evidence supports apo-B's superiority over LDL-C in risk prediction.

[0009] A guiding principle of ATP III is that the intensity of LDL-C lowering therapy is adjusted to the individual's absolute risk for CHD. Risk assessment is broken down into short term ($\leq$10-year) and long term (>10-year) risk of CHD, and the LDL-C goals are adjusted accordingly. In addition, ATP III identifies three categories of risk for CHD that modify LDL-C goals: established CHD and CHD risk equivalents, multiple (2+) risk factors, and 0-1 risk factor. Established CHD and CHD risk equivalents include CHD, other clinical atherosclerotic diseases, diabetes mellitus, and multiple risk factors and a 10-year risk for CHD

>20 percent. The major independent risk factors identified in risk factor counting include cigarette smoking, hypertension, low HDL-C, family history of premature CHD and age.

[00010] The LDL-C goals for the three categories of risk factors are as follows:

Risk Factors	LDL-C Goal
CHD and CHD Risk Equivalent	<100 mg/dl
Multiple (2+) Risk Factors	<130 mg/dl*
0-1 Risk Factor	<160 mg/dl

* LDL-C goal for multiple risk factor persons with 10-year risk >20 percent is <100 mg/dl.

The ATP III also outlines LDL-C goals for patients based on the percentage of 10-year risk for CHD:

10-Year Risk	LDL-C Goal
>20%	<100 mg/dl
10-20%	<130 mg/dl
<10% and Multiple (2+) Risk Factors	<130 mg/dl
<10% and 0-1 Risk Factor	<160 mg/dl

[00011] Agents, such as dyslipidemic agents and omega-3 fatty acids, have been used to treat post-myocardial infarction (MI) and adult endogenous hyperlipidemias of hypercholesterolemias and of hypertriglyceridemias, which are generally categorized as "cardiovascular events."

[00012] Dyslipidemic agents commonly include HMG CoA reductase inhibitors (statins), cholesterol absorption inhibitors, niacin and derivatives such as

nicotinamide, fibrates, bile acid sequestrants, MTP inhibitors, LXR agonists and/or antagonists and PPAR agonists and/or antagonists.

[00013] Statins, which are 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, have been used to treat hyperlipidemia and arthrosclerosis, for example. Typically, statin monotherapy has been used to treat cholesterol levels, particularly when a patient is not at an acceptable LDL-C level. Statins inhibit the enzyme HMG-CoA reductase, which controls the rate of cholesterol production in the body. Statins lower cholesterol by slowing down the production of cholesterol and by increasing the liver's ability to remove the LDL-cholesterol already in the blood. Accordingly, the major effect of the statins is to lower LDL-C levels. Statins have been shown to decrease CHD risk by about one-third. However, statins only appear to have a modest effect on the TG-HDL axis. It is also known that monotherapy with various statins significantly reduces apo-B levels, e.g., by about 25 to about 40% from baseline. See, e.g., Ballantyne et al., *Am. Heart J.* 151(5):975.e1-975.e9 (2006).

[00014] Cholesterol absorption inhibitors, such as ezetimibe and MD-0727, are a class of lipid-lowering compounds that selectively inhibit the intestinal absorption of cholesterol. Ezetimibe acts on brush border of the small intestine and decreases biliary and dietary cholesterol from the small intestine uptake into the enterocytes. Results from studies presented at the 2006 World Cardiology Congress show that a combination of ezetimibe and simvastatin has a more positive effect than simvastatin alone on apo-B levels in hypercholesterolemia patients. Orse et al., Effects Of Ezetimibe/simvastatin On Lipoprotein Subclasses

In Patients With Primary Hypercholesterolemia, 2006 World Cardiology Congress - poster presentation; Ballantyne et al., Effects of Ezetimibe/Simvastatin Compared to Simvastatin Monotherapy in Reducing C-Reactive Protein and Low Density Lipoprotein-Cholesterol, 2006 World Cardiology Congress - poster presentation.

[00015] Cholesteryl ester transfer protein (CETP) inhibitors, such as torcetrapib, inhibit the CETP molecule which, among other things, moves cholesterol from the HDL form to the LDL form. Inhibiting this molecule is, therefore, a promising approach to increasing HDL cholesterol levels. It has been reported that torcetrapib also reduced the levels of LDL-C and apo-B, both when given as monotherapy and when administered in combination with a statin. Bhardwaj et al., *Indian J. Pharmacol.* 37:46 (2005).

[00016] Niacin (nicotinic acid or 3-pyridinecarboxylic acid) has previously been used to treat hyperlipidemia and atherosclerosis. Niacin is known to reduce total cholesterol, LDL-C and triglycerides and increase HDL-C. Niacin therapy is also known to decrease serum levels of apo-B. However, the magnitude of the individual lipid and lipoprotein response from niacin therapy may be influenced by the severity and type of underlying lipid abnormality. In one study, low-dose niacin plus either atorvastatin (20 mg) or rosuvastatin (10 mg) lowered apo-B levels comparably to an ezetimibe-simvastatin combination or rosuvastatin alone. McKenney et al. *Atherosclerosis* 7(suppl):174. Abstract Tu-W27:4 (2006).

[00017] Fibrates such as fenofibrate, bezafibrate, clofibrate and gemfibrozil, are PPAR-alpha agonists and are used in patients to decrease lipoproteins rich in

triglycerides, to increase HDL and to decrease atherogenic-dense LDL. Fibrates are typically orally administered to such patients.

[00018] Fenofibrate or 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester, which belongs to the fibrate family, has been known for many years as a medicinal active principle because of its efficacy in lowering blood triglyceride and cholesterol levels. Fenofibrate is an active principle which is very poorly soluble in water and the absorption of fenofibrate in the digestive tract is limited. A treatment of 40 to 300 mg of fenofibrate per day enables a 20 to 25% reduction of cholesterolemia and a 40 to 50% reduction of triglyceridemia to be obtained.

[00019] Bile acid sequestrants, such as cholestyramine, colestipol and colesevelam, are a class of drugs that binds bile acids, prevents their reabsorption from the digestive system, and reduces cholesterol levels. The usual effect of bile acid sequestrants is to lower LDL-cholesterol by about 10 to 20 percent. Small doses of sequestrants can produce useful reductions in LDL-cholesterol. One study reported that combination treatment with fluvastatin and cholestyramine produced a significant, dose-dependent reduction in apo-B levels. Bard et al., *Am. J. Cardiol.*, 76(2): 65A-70A (2005).

[00020] MTP inhibitors, such as implitapide, are known to inhibit the secretion of cholesterol and triglycerides.

[00021] Liver X receptors (LXRs) are "cholesterol sensors" that regulate the expression of genes involved in lipid metabolism in response to specific oxysterol ligands (Repa et al., *Annu. Rev. Cell Dev. Biol.* **16**: 459-481(2000)). LXR

agonists and antagonists are potential therapeutic agents for dyslipidemia and atherosclerosis.

[00022] PPAR-gamma agonists, such as the thiazolidinediones pioglitazone and rosiglitazone, have been shown to improve surrogate markers of cardiovascular risk and atherosclerosis. For example, thiazolidinediones decrease C-reactive protein and carotid intima-media thickness. Non-thiazolidinediones, such as tesaglitazar, naviglitizar and muraglitazar, are dual alpha/gamma PPAR agonists. These compounds are used for lowering glucose, insulin, triglycerides and free fatty acids. In one study, rosiglitazone was shown to significantly reduce apo-B levels. Goldberg et al., *Diabetes Care* 28(7):1547-1554 (2005).

[00023] Partial PPAR-gamma agonist/antagonists, such as metaglidasen, are used for the treatment of type II diabetes.

[00024] Marine oils, also commonly referred to as fish oils, are a good source of two omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been found to regulate lipid metabolism. Omega-3 fatty acids have been found to have beneficial effects on the risk factors for cardiovascular diseases, especially mild hypertension, hypertriglyceridemia and on the coagulation factor VII phospholipid complex activity. Omega-3 fatty acids lower serum triglycerides, increase serum HDL-cholesterol, lower systolic and diastolic blood pressure and the pulse rate, and lower the activity of the blood coagulation factor VII-phospholipid complex. Further, omega-3 fatty acids seem to be well tolerated, without giving rise to any severe side effects.

[00025] One such form of omega-3 fatty acid is a concentrate of omega-3, long chain, polyunsaturated fatty acids from fish oil containing DHA and EPA and was sold under the trademark Omacor®, and is now known as Lovaza™. Such a form of omega-3 fatty acid is described, for example, in U.S. Patent Nos. 5,502,077, 5,656,667 and 5,698,594, each incorporated herein by reference.

[00026] Patients with mixed dyslipidemia or hypercholesteremia often present with blood levels of LDL cholesterol greater than 190 mg/dl, triglyceride levels of 200 mg/dl or higher, and apo-B levels of greater than 0.9 g/l. In many patients with hypertriglyceridemia, hypercholesterolemia and/or mixed dyslipidemia, the use of diet and single-drug therapy does not always decrease LDL cholesterol, triglycerides and/or apo-B levels adequately enough to reach targeted values. In these patients, a complementary combination therapy of a dyslipidemic agent and omega-3 fatty acids may be desirable.

[00027] Many studies have examined the combined effects of omega-3 fatty acid and statin therapy on apo-B levels. While most of these studies confirm that statins significantly reduce apo-B levels, most studies also report a lack of significant further reduction of apo-B levels with added omega-3 fatty acid treatment.

[00028] Hong *et al.* investigated the effects of fish oil and simvastatin in patients with coronary heart disease and mixed dyslipidemia. Patients having baseline triglyceride levels of 292.8 mg/dl or 269.5 mg/dl were initially treated with 10-20 mg/day simvastatin for 6-12 weeks. Thereafter the patients were treated with simvastatin and placebo or simvastatin and 3 g/day fish oil

(Meilekang™). Combined treatment significantly reduced triglyceride levels, as compared to baseline and placebo. In addition, combined treatment numerically increased HDL-C levels, and numerically reduced LDL-C levels, as compared to baseline. However, the changes in HDL-C levels and LDL-C levels were not statistically significant. Levels of apo-B were raised in the combined treatment group, while the apo-B levels numerically decreased in the placebo group. Hong *et al.*, *Chin. Med. Sci. J.* 19:145-49 (2004).

[00029] Contacos *et al.* investigated the effects of fish oil and pravastatin on patients with mixed hyperlipidemia. Patients having baseline triglyceride levels of 4.6 to 5.5 mmol/l (404 to 483 mg/dl) were initially treated for 6 weeks with 40 mg/day pravastatin, 6 g/day fish oil (Himega™, containing 3 g of omega-3 fatty acids, with an EPA/DHA ratio of 2:1), or placebo. Thereafter, all patients were treated with pravastatin and fish oil for an additional 12 weeks. Initial treatment with pravastatin significantly reduced LDL-C levels. Combined treatment of pravastatin and fish oil also significantly reduced triglyceride and LDL-C levels. However, the addition of fish oil to pravastatin monotherapy resulted in only a numerical increase in LDL-C levels, which was not statistically significant. Treatment with fish oil alone significantly reduced triglyceride levels, but increased LDL-C levels. Combined treatment for this group significantly reduced LDL-C levels, as compared to fish oil alone (but not as compared to baseline). Apo-B levels were significantly reduced with pravastatin treatment. Combination treatment with fish oil further numerically reduced apo-B levels, however it was

reported that this further reduction was not significant as compared to pravastatin monotherapy. Contacos *et al.*, *Arterioscl. Thromb.* 13:1755-62 (1993).

[00030] Grekas *et al.* Reported on the combined treatment of low-dose pravastatin and fish oil in post-renal transplantation dislipidemia. Thirty renal transplant patients with persistent hypercholesterolemia (total cholesterol >200 mg/dl) and on immunosuppressive therapy were given a standard diet for 4 weeks, followed by 8 weeks of therapy with 20 mg pravastatin. Baseline triglyceride levels at the diet stage were 184 mg/dl. This period was followed by an additional 4 weeks of standard diet, then 8 weeks of therapy with 20 mg pravastatin plus 1 g fish oil (Prolipid). Baseline triglyceride levels at the diet stage were 169 mg/dl. Apo-B levels were not significantly reduced with diet + statin therapy. However, diet + statin + fish oil was reported to significantly reduce apo-B levels. Grekas *et al.*, *Nephron* (2001) 88: 329-333. The Grekas et al. study results seem dubious, given that the study did not show a significant reduction in apo-B levels with pravastatin therapy alone. PRAVACHOL® (pravastatin) is indicated as an adjunct to diet to reduce elevated apo-B levels in patients with primary hypercholesterolemia and mixed dyslipidemia. Thus, the fact that the Grekas et al. study did not see significant apo-B reduction with pravastatin makes the study results subject to doubt.

[00031] Huff et al. found that the combination of dietary fish oil and lovastatin reduces apo-B levels in both very low-density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions in miniature pigs. However, the study only compared combination treatment versus fish oil monotherapy, and did not compare

combination treatment versus statin monotherapy. Huff *et al.*, *Arteroscl. Thromb.*, 12(8): 901-910 (August 1992).

[00032] Jula et al. studied the effects of diet and simvastatin on various serum lipids in hypercholesterolemic men. After an open placebo period, subjects were allocated to a "habitual diet" or "dietary treatment" group. The dietary treatment consisted of a Mediterranean-type diet in which no more than 10% energy was from saturated and trans-unsaturated fatty acids; cholesterol intake was no more than 250 mg/day; omega-3 fatty acid intake of plant and marine origin was at least 4 g/day, and the ratio of omega-6 fatty acids to omega-3 fatty acids was less than 4; and intake of fruits, vegetables and soluble fiber was increased. Subjects were then also allocated to receive 20 mg/day simvastatin or placebo for 12 weeks in a double-blind, crossover fashion. Subjects in the dietary treatment group and the simvastatin group had significant reductions in apo-B levels. The interaction between the two variables was reported as significant. Jula et al., *JAMA* 287(5): 598-605 (2002).

[00033] U.S. Patent Application Publication No. 2003/0170643 embodiments a method of treating a patient, by administering a therapeutic which lowers plasma concentrations of apo-B and/or an apo-B-containing lipoprotein and/or a component of an atherogenic lipoprotein by stimulating post-ER pre-secretory proteolysis (PERPP).

[00034] Studies have investigated the effect of statins and Omacor® omega-3 fatty acids. For example, Hansen *et al.* investigated the effect of lovastatin (40 mg/day) in combination with 6 g/day Omacor® omega-3 fatty acids in patients

with hypercholesterolemia. Patients having baseline triglyceride levels of 1.66 mmol/l (about 146 mg/dl) were treated with 6 g/day Omacor® for 6 weeks, followed by 40 mg/day lovastatin for an additional 6 weeks, and a combination of both Omacor® and lovastatin for a final 6 weeks. Lovastatin monotherapy resulted in significant increases in HDL-C levels, and significant decreases in triglyceride and LDL-C levels. After combination treatment, triglyceride and LDL-C levels were further significantly decreased. Apo-B levels were significantly reduced with lovastatin monotherapy, and further numerically reduced with the addition of omega-3 fatty acids, although such further reduction was not indicated as being significant as compared to lovastatin monotherapy. Hansen *et al.*, *Arterioscl. Thromb.* 14(2): 223-229 (February 1994).

[00035] Nordoy *et al.* investigated the effect of atorvastatin and omega-3 fatty acids on patients with hyperlipemia. Patients having baseline triglyceride levels of 3.84 mmol/l (about 337 mg/dl) or 4.22 mmol/l (about 371 mg/dl) were treated with 10 mg/day atorvastatin for 5 weeks. Thereafter, for an additional 5 weeks, atorvastatin treatment was supplemented with 2 g/day Omacor® or placebo. Atorvastatin monotherapy, significantly increased HDL-C levels, and triglyceride, LDL-C and apo-B levels significantly decreased, as compared to baseline. Combination treatment further increased HDL-C levels, as compared to atorvastatin alone. Triglyceride, LDL-C and apo-B levels numerically further declined slightly with combination treatment, as compared to atorvastatin monotherapy; however, the decrease was not significant. Nordoy *et al.*, *Nutr. Metab. Cardiovasc. Dis.* (2001) 11:7-16.

[00036] Chan *et al.* studied the combined treatment of atorvastatin (40 mg/day) and 4 g/day 4 Omacor® on obese, insulin-resistant men with dyslipidemia studied in a fasted state. Patients having baseline triglyceride levels of 1.7 to 2.0 mmol/l (about 150 to 170 mg/dl) were treated for 6 weeks with: 40 mg/day atorvastatin and placebo; 4 g/day Omacor® and placebo; a combination of atorvastatin and Omacor®; or a combination of placebos. Atorvastatin monotherapy significantly decreased apo-B levels. Combination treatment also significantly decreased apo-B levels, as compared to the placebo group. However, the effects attributable to the Omacor® were not significant. Chan *et al.*, *Diabetes*, 51: 2377-2386 (Aug. 2002).

[00037] Nordoy *et al.* investigated the effectiveness of combination treatment of 40 mg/day lovastatin and 6 g/day Omacor® (identified as "K-85") in patients with familial hypercholesterolemia, but who were without cardiovascular disease. The study included three intervention periods, each 6 weeks long, interrupted by washout periods of 6 weeks. The final test was carried out 12 weeks after the last intervention. Apo-B levels numerically reduced slightly with omega-3 fatty acid monotherapy, and were significantly reduced with lovastatin monotherapy. The combination treatment also significantly reduced apo-B levels, as compared to baseline. However, the reduction was not indicated as being significant as compared to lovastatin monotherapy. Nordoy et al., *Essent. Fatty Acids Eicosanoids*, Invited Pap. Int'l Congr. 4th, 256-61 (1998).

[00038] Nordoy *et al.* also investigated the efficiency and the safety of treatment with simvastatin and omega-3 fatty acids in patients with

nyperlipidemia. Patients having baseline triglyceride levels of 2.76 mmol/l (about 243 mg/dl) or 3.03 mmol/l (about 266 mg/dl) were treated for 5 weeks with 20 mg/day simvastatin or placebo, then all patients were treated for an additional 5 weeks with 20 mg/day simvastatin. Thereafter, patients were additionally treated with 4 g/day Omacor® or placebo, for a further 5 weeks. The administration of omega-3 fatty acids with simvastatin resulted in moderate reductions in serum total cholesterol and reduction in triglycerol levels, and a small numerical decrease in apo-B levels. However, the effect attributable to the omega-3 fatty acids was not significant. Nordoy *et al.*, *J. of Internal Medicine*, 243:163-170 (1998).

[00039] Durrington *et al.* examined the effectiveness, safety, and tolerability of a combination of Omacor® omega-3 acids and simvastatin in patients with established coronary heart disease and persisting hypertriglyceridemia. Patients having an average baseline triglyceride levels >2.3 mmol/l (average patient serum triglyceride level was 4.6 mmol/l), were treated with 10-40 mg/day simvastatin and 2 g/day Omacor® or placebo, for 24 weeks in a double-blind trial, after which both groups were invited to receive Omacor® for a further 24 weeks in an open study. Combination treatment significantly decreased triglyceride levels within 12 weeks, as compared to baseline monotherapy. In addition, the VLDL cholesterol levels in these patients decreased by 30-40%. LDL-C levels significantly decreased, as compared to baseline monotherapy, only after 48 weeks, although there was a numerical (statistically insignificant) decrease at 12 and 24 weeks. Apo-B levels showed a slight numerical (statistically insignificant)

decrease with addition of omega-3 fatty acids to simvastatin monotherapy. Durrington *et al.*, *Heart*, 85:544-548 (2001).

SUMMARY OF THE INVENTION

[00040] There is an unmet need in the art for methods for the increased reduction of apo-B levels over monotherapy with a dyslipidemic agent alone. This method is especially useful in the treatment of one or more of the following: hypercholesterolemia, hypertriglyceridemia, mixed dyslipidemia, coronary heart disease (CHD), vascular disease, atherosclerotic disease and related conditions, and/or for the prevention or reduction of cardiovascular and/or vascular events, in subjects such as human subjects.

[00041] Some embodiments of the present invention provide for a method of utilizing a combination of a dyslipidemic agent and omega-3 fatty acids for the reduction of apo-B levels, which is suitable for the treatment of one or more of the following: hypercholesterolemia, hypertriglyceridemia, mixed dyslipidemia, coronary heart disease, vascular disease, atherosclerotic disease and related conditions, and/or for the prevention or reduction of cardiovascular and vascular events.

[00042] Some embodiments according to the present invention include a method of blood lipid therapy in a subject comprising administering to the subject an effective amount of a dyslipidemic agent and an omega-3 fatty acid, wherein an apo-B level in the subject is reduced as compared to treatment with the dyslipidemic agent alone.

[00043] In other embodiments, the present invention includes methods of reducing an apo-B level in a subject group, comprising providing a subject group, and reducing the apo-B level of the subject group by administering to the subject group a combination of a dyslipidemic agent and omega-3 fatty acids in an amount effective to reduce the apo-B level of the subject group as compared to treatment with a dyslipidemic agent alone. In preferred embodiments, the subject group has at least one of the following conditions: hypertriglyceridemia, hypercholesterolemia, mixed dyslipidemia, vascular disease, and/or atherosclerotic disease and related conditions.

[00044] In further embodiments, the dyslipidemic agent and the omega-3 fatty acid are administered as a single pharmaceutical composition as a combination product, for example, a unit dosage, comprising the dyslipidemic agent and the omega-3 fatty acids.

[00045] In preferred embodiments the pharmaceutical composition(s) comprise Lovaza™ omega-3 fatty acids, as described in U.S. Patent Nos. 5,502,077, 5,656,667 and 5,698,594. In other preferred embodiments the pharmaceutical composition(s) comprise omega-3 fatty acids present in a concentration of at least 40% by weight as compared to the total fatty acid content of the composition(s).

[00046] In still other preferred embodiments the omega-3 fatty acids comprise at least 50% by weight of EPA and DHA as compared to the total fatty acid content of the composition, and the EPA and DHA are in a weight ratio of EPA:DHA of from 99:1 to 1:99, preferably from 1:2 to 2:1.

[00047] In variations of the present invention, the dyslipidemic agent is a statin including, but not limited to, pitavastatin, simvastatin, rosuvastatin, pravastatin, atorvastatin, lovastatin and fluvastatin. In preferred embodiments, the statin used in combination with omega-3 fatty acids is simvastatin.

[00048] In one aspect of the invention, the combination product is used in the treatment of subjects with primary hypertriglyceridemia or hypercholesterolemia or mixed dyslipidemia.

[00049] In yet further preferred embodiments of the present invention the triglyceride levels in the serum of the subject (or the subject group) prior to the first administration of a combination of a dyslipidemic agent and omega-3 fatty acids, i.e., at baseline, is about 200 to about 499 mg/dl.

[00050] The invention also includes the use of an effective amount of a dyslipidemic agent and omega-3 fatty acids for the manufacture of a medicament useful for any of the treatment methods indicated herein.

[00051] Other features and advantages of the present invention will become apparent to those skilled in the art upon examination of the following or upon learning by practice of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00052] The present invention is directed to the utilization of dyslipidemic agents and omega-3 fatty acids for reduction of apo-B levels beyond that which is obtained with treatment of the dyslipidemic agent alone. The methods of the present invention are especially useful for the treatment of primary

hypertriglyceridemia or hypercholesteremia or mixed dyslipidemia, coronary heart disease, vascular disease, artherosclerotic disease and related conditions and/or the prevention or reduction of cardiovascular and/or vascular events. In a preferred embodiment, a subject has baseline apo-B levels of greater than 0.9 g/l, and the use of the invention reduces the apo-B levels to less than 0.9 g/l.

[00053] In some embodiments, a subject has non-HDL-C levels of at least 130 mg/dl, more preferably at least 160 mg/dl, and the use of the invention reduces the apo-B levels, preferably by at least 2% as compared to baseline and/or further than treatment with a dyslipidemic agent alone.

[00054] In some embodiments, a subject has elevated LDL-C levels (e.g., at least 100 mg/dl, at least 100 mg/dl and less than 130 mg/dl, at least 130 mg/dl, or at least 160 mg/dl) and/or elevated triglyceride levels (e.g., at least 150 mg/dl, at least 200 mg/dl, 200-499 mg/dl, or at least 500 mg/dl) and, if both, can be qualified as a subject with mixed dyslipidemia.

[00055] In some embodiments, the invention provides a novel combination. In a preferred embodiment, the combination comprises omega-3 fatty acids and a dyslipidemic agent, wherein the omega-3 fatty acids are administered simultaneous to administration of the dyslipidemic agent, *e.g.*, as a single fixed dosage pharmaceutical composition or as separate compositions administered at the same time.

[00056] In other preferred embodiments, the administration comprises omega-3 fatty acids and a dyslipidemic agent, wherein the omega-3 fatty acids are administered apart from the administration of the dyslipidemic agent, but in a

concomitant treatment regime. For example, the dyslipidemic agent may be administered once daily and the omega-3 fatty acids may be administered twice daily. One skilled in the art with the benefit of the present disclosure will understand that the precise dosage and schedule for the administration of the omega-3 fatty acids and the dyslipidemic agent will vary depending on numerous factors, such as, for example, the route of administration, the seriousness of the condition, other comorbidities, and the use of other medications.

[00057] In some embodiments, the claimed method of administration is a first-line therapy, meaning that it is the first type of therapy given for the condition or disease. In other embodiments, the claimed method of administration is a second-line therapy, meaning that the treatment is given when initial treatment (first-line therapy, e.g., dyslipidemic agent or omega-3 fatty acid treatment alone) does not work adequately with respect to treatment goals, or ceases to be adequate, e.g. due to physiological changes in the patient or changes in CHD risk factors.

[00058] In some embodiments, the invention is suitable for primary prevention. In other embodiments, the invention is suitable for secondary prevention.

[00059] In preferred embodiments, the selected subject group was receiving dyslipidemic agent therapy prior to the combination therapy of the dyslipidemic agent and the omega-3 fatty acids. Other active agents (other than omega-3 fatty acids) may also have been employed prior to the combination therapy of the dyslipidemic agent and the omega-3 fatty acids.

[00060] In preferred embodiments, the present invention includes a method of blood lipid therapy in a subject group comprising administering to the subject group an effective amount of a dyslipidemic agent and omega-3 fatty acids, and wherein after administration to the subject group the triglyceride level and an apo-B level of the subject group are reduced as compared to a control group treated with the dyslipidemic agent alone, and an HDL-C level of the subject group is increased as compared to a control group treated with the dyslipidemic agent alone and/or as compared to baseline. The subject group preferably has a baseline triglyceride level of 200 to 499 mg/dl.

[00061] In other preferred embodiments, the present invention includes a method of blood lipid therapy in a subject group comprising administering to the subject group an effective amount of a dyslipidemic agent and omega-3 fatty acids, and wherein after administration to the subject group the triglyceride level and an apo-B level of the subject group are reduced as compared to a control group treated with the dyslipidemic agent alone, without increasing LDL-C more than 1% as compared to baseline. The subject group preferably has a triglyceride level of 200 to 499 mg/dl.

[00062] In other preferred embodiments, the present invention includes a method of blood lipid therapy in a subject group, comprising administering to the subject group an effective amount of a dyslipidemic agent and omega-3 fatty acids, wherein after administration to the subject group a non-HDL-C level, a total cholesterol level, a triglyceride level, and an apo-B level of the subject group is reduced as compared to a control group treated with the dyslipidemic agent

alone, and an HDL-C level of the subject group is increased as compared to a control group treated with the dyslipidemic agent alone, without increasing LDL-C more than 1% as compared to baseline.

[00063] In other preferred embodiments, the present invention includes a method of blood lipid therapy in a subject group comprising administering to the subject group an effective amount of a dyslipidemic agent and omega-3 fatty acids, and wherein after administration to the subject group a non-HDL-C level of the subject group is reduced as compared to a control group treated with the dyslipidemic agent alone. The subject group preferably has a baseline triglyceride level of 200 to 499 mg/dl.

[00064] In other preferred embodiments, the invention includes a method of reducing a triglyceride level and an apo-B level in a subject group without increasing an LDL-C level in the subject group, comprising providing a subject group, and reducing the triglyceride level and the apo-B level of the subject group by administering to the subject group a combination of a dyslipidemic agent and omega-3 fatty acids in an amount effective to reduce the triglyceride level and the apo-B level of the subject group as compared to treatment with an HMG-CoA reductase inhibitor alone without increasing the LDL-C level.

[00065] The phrase "compared to treatment with dyslipidemic agent alone" can refer to treatment of the same subject or subject group, or treatment of a comparable subject or subject group (i.e., subject(s) within the same class with respect to a particular blood protein or lipid, such as a cholesterol or triglyceride level) in a different treatment group. The terms "reduce" and "increase" in

accordance with the embodimented methods are intended to mean a statistically significant reduction or increase in accordance with its general and customary meaning, i.e., a probability of chance of 5% or less (p=0.05 or less), more preferably 2.5% of less (p=0.025 or less). In some embodiments of the invention, the dyslipidemic agent alone statistically significantly reduces or increases certain levels (such as reducing apo-B levels), and the combination therapy of the dyslipidemic agent and the omega-3 fatty acids further statistically significantly reduces or increases the levels.

[00066] In addition to reducing apo-B levels, the methods and compositions of the invention may also be used to reduce any of the following lipid levels in a treated subject or subject group, as compared to treatment with the dyslipidemic agent alone: non-HDL-C levels, triglyceride levels, VLDL-C levels, total C levels, RLP-C levels, Lp-PLA2 levels and/or Apo-C3 levels. The methods and compositions of the invention may also be used to increase HDL-C levels, as compared to treatment with the dyslipidemic agent alone. Preferably, the methods and compositions of the invention are utilized without increasing LDL-C levels, as compared to baseline.

[00067] Preferably, non-HDL-C levels may be reduced at least about 5%, preferably at least about 7%, from baseline and/or at least about 5%, preferably at least about 7%, further than treatment with the dyslipidemic agent alone.

[00068] Preferably, the triglyceride levels may be reduced by at least about 20%, preferably at least about 25%, as compared to baseline and/or at least

about 10%, preferably at least about 15%, more preferably at least about 20%, further than treatment with the dyslipidemic agent alone.

[00069] Preferably, the VLDL-C levels may be reduced by at least about 15%, preferably at least about 20%, more preferably at least about 25%, as compared to baseline and/or at least about 10%, preferably at least about 15%, more preferably at least about 20%, further than treatment with the dyslipidemic agent alone.

[00070] Preferably, the total C levels may be reduced by at least about 3%, preferably at least about 5%, as compared to baseline and/or at least about 2%, preferably at least about 3%, further than treatment with the dyslipidemic agent alone.

[00071] Preferably, the RLP-C levels may be reduced by at least about 15%, preferably at least about 20%, more preferably at least about 25%, as compared to baseline and/or at least about 10%, preferably at least about 15%, more preferably at least about 20%, further than treatment with the dyslipidemic agent alone.

[00072] Preferably, the Lp-PLA2 levels may be reduced by at least about 5%, preferably at least about 7%, more preferably at least about 10%, as compared to baseline and/or at least about 3%, preferably at least about 5%, more preferably at least about 7%, further than treatment with the dyslipidemic agent alone.

[00073] Preferably, the apo-B levels may be reduced by at least about 3%, preferably at least about 4%, as compared to baseline and/or at least about 1%,

preferably at least about 2%, further than treatment with the dyslipidemic agent alone.

[00074] Preferably, the Apo-C3 levels may be reduced by at least about 5%, preferably at least about 7%, as compared to baseline and/or at least about 8%, preferably at least about 10%, further than treatment with the dyslipidemic agent alone.

[00075] Preferably, the HDL-C levels may be increased by at least about 2%, preferably at least about 3%, as compared to baseline and/or at least about 3%, preferably at least about 5%, further than treatment with the dyslipidemic agent alone.

[00076] Preferably, the present invention also decreases the ratio of total cholesterol to HDL-C, preferably by at least about 5%, more preferably at least about 10%, as compared to baseline and/or at least about 5%, preferably at least about 10%, further than treatment with the dyslipidemic agent alone.

[00077] The present invention may incorporate now known or future known dyslipidemic agents in an amount generally recognized as safe. Preferred dyslipidemic agents include HMG CoA reductase inhibitors including statins, cholesterol absorption inhibitors such as but not limited to ezetimibe, niacin and derivatives such as nicotinamide, CETP inhibitors such as but not limited to torcetrapib, fibrates such as but not limited to fenofibrate, bezafibrate, clofibrate and gemfibrozil, bile acid sequestrants such as but not limited to cholestyramine, cholestipol and colesevelam, MTP inhibitors such as but not limited to those disclosed in WO 00/38725 and Science, 282, 23 October 1998, pp. 751-754,

herein incorporated by reference, LXR agonists and/or antagonists, and PPAR agonists and antagonists (such as but not limited to PPAR-alpha, PPAR-gamma, PPAR-delta, PPAR-alpha/gamma, PPAR-gamma/delta, PPAR-alpha/delta, and PPAR-alpha/gamma/delta agonists, antagonists and partial agonists and/or antagonists) such as but not limited to the thiazolidinediones, the non-thiazolidinediones and metaglidasen. There are currently six statins that are widely available: atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin. A seventh statin, pitavastatin, is in clinical trials. An eighth statin, cerivastatin, has been removed from the U.S. market at the time of this writing. However, it is conceivable to one skilled in the art that cerivastatin may be used in conjunction with some embodiments of the present invention if cerivastatin is ultimately determined to be safe and effective.

[00078] Generally, the effect of the dyslipidemic agent is dose dependent, i.e., the higher the dose, the greater the therapeutic affect. However, the effect of each dyslipidemic agent is different, and therefore the level of therapeutic effect of one dyslipidemic agent cannot be necessarily be directly correlated to the level of therapeutic effects of other dyslipidemic agents. However, those of ordinary skill in the art would understand the correct dosage to be given to a particular subject, based on experience and the seriousness of the condition.

[00079] As used herein, the term "omega-3 fatty acids" includes natural or synthetic omega-3 fatty acids, or pharmaceutically acceptable esters, derivatives, conjugates (see, e.g., Zaloga et al., U.S. Patent Application Publication No. 2004/0254357, and Horrobin et al., U.S. Patent No. 6,245,811, each hereby

incorporated by reference), precursors or salts thereof and mixtures thereof. Examples of omega-3 fatty acid oils include but are not limited to omega-3 polyunsaturated, long-chain fatty acids such as a eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid; esters of omega-3 fatty acids with glycerol such as mono-, di- and triglycerides; and esters of the omega-3 fatty acids and a primary, secondary or tertiary alcohol such as fatty acid methyl esters and fatty acid ethyl esters. Preferred omega-3 fatty acid oils are long-chain fatty acids such as EPA or DHA, triglycerides thereof, ethyl esters thereof and mixtures thereof. The omega-3 fatty acids or their esters, derivatives, conjugates, precursors, salts and mixtures thereof can be used either in their pure form or as a component of an oil such as fish oil, preferably purified fish oil concentrates. Commercial examples of omega-3 fatty acids suitable for use in the invention include Incromega F2250, F2628, E2251, F2573, TG2162, TG2779, TG2928, TG3525 and E5015 (Croda International PLC, Yorkshire, England), and EPAX6000FA, EPAX5000TG, EPAX4510TG, EPAX2050TG, K85TG, K85EE, K80EE and EPAX7010EE (Pronova Biocare a.s., 1327 Lysaker, Norway).

[00080] Preferred compositions include omega-3 fatty acids as recited in U.S. Patent Nos. 5,502,077, 5,656,667 and 5,698,694, which are hereby incorporated herein by reference in their entireties.

[00081] Another preferred composition includes omega-3 fatty acids present in a concentration of at least 40% by weight, preferably at least 50% by weight, more preferably at least 60% by weight, still more preferably at least 70% by

weight, most preferably at least 80% by weight, or even at least 90% by weight. Preferably, the omega-3 fatty acids comprise at least 50% by weight of EPA and DHA, more preferably at least 60% by weight, still more preferably at least 70% by weight, most preferably at least 80%, such as about 84% by weight. Preferably the omega-3 fatty acids comprise about 5 to about 100% by weight, more preferably about 25 to about 75% by weight, still more preferably about 40 to about 55% by weight, and most preferably about 46% by weight of EPA. Preferably the omega-3 fatty acids comprise about 5 to about 100% by weight, more preferably about 25 to about 75% by weight, still more preferably about 30 to about 60% by weight, and most preferably about 38% by weight of DHA. All percentages above are by weight as compared to the total fatty acid content in the composition, unless otherwise indicated. The percentage by weight may be based on the free acid or ester forms, although it is preferably based on the ethyl ester form of the omega-3 fatty acids even if other forms are utilized in accordance with the present invention.

[00082] The EPA:DHA ratio may be from 99:1 to 1:99, preferably 4:1 to 1:4, more preferably 3:1 to 1:3, most preferably 2:1 to 1:2. The omega-3 fatty acids may comprise pure EPA or pure DHA.

[00083] The omega-3 fatty acid composition optionally includes chemical antioxidants, such as alpha tocopherol, oils, such as soybean oil and partially hydrogenated vegetable oil, and lubricants such as fractionated coconut oil, lecithin and a mixture of the same.

[00084] The most preferred form of omega-3 fatty acids is the Lovaza™ omega-3 acid (K85EE, Pronova Biocare A.S., Lysaker, Norway) and preferably comprises the following characteristics (per dosage form):

Test	**Minimum Value**	**Maximum Value**
Eicosapentaenoic acid C20:5	430 mg/g	495 mg/g
Docosahexaenoic acid C22:6	347 mg/g	403 mg/g
EPA and DHA	800 mg/g	880 mg/g
Total n-3 fatty acids	90 % (w/w)	

[00085] The combination product of a dyslipidemic agent and concentrated omega-3 fatty acids may be administered in a capsule, a tablet, a powder that can be dispersed in a beverage, or another solid oral dosage form, a liquid, a soft gel capsule, a coated soft gel capsule (see U.S. Application Serial No. 11/716,020, hereby incorporated by reference) or other convenient dosage form such as oral liquid in a capsule, as known in the art. In some embodiments, the capsule comprises a hard gelatin. The combination product may also be contained in a liquid suitable for injection or infusion. The active ingredients of the present invention may also be administered with a combination of one or more non-active pharmaceutical ingredients (also known generally herein as "excipients"). Non-active ingredients, for example, serve to solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, protect, color, flavor, and fashion the

active ingredients into an applicable and efficacious preparation that is safe, convenient, and otherwise acceptable for use.

[00086] Excipients include surfactants, such as propylene glycol monocaprylate, mixtures of glycerol and polyethylene glycol esters of long fatty acids, polyethoxylated castor oils, glycerol esters, oleoyl macrogol glycerides, propylene glycol monolaurate, propylene glycol dicaprylate/dicaprate, polyethylene-polypropylene glycol copolymer, and polyoxyethylene sorbitan monooleate, cosolvents such ethanol, glycerol, polyethylene glycol, and propylene glycol, and oils such as coconut, olive or safflower oils. The use of surfactants, cosolvents, oils or combinations thereof is generally known in the pharmaceutical arts, and as would be understood to one skilled in the art, any suitable surfactant may be used in conjunction with the present invention and embodiments thereof.

[00087] The concentrated omega-3 fatty acids can be administered in a daily amount of from about 0.1 g to about 10 g, more preferably about 1 g to about 8 g, and most preferably from about 2 g to about 6 g. In one embodiment, the omega-3 fatty acids are administered in an amount up to 4 g/day.

[00088] The dyslipidemic agent may be administered in an amount more than, equal to or less than the conventional full-strength dose as a single-administered product. For example, the dyslipidemic agent may be administered in an amount of from 10-100%, preferably about 25-100%, most preferably about 50-80%, of the conventional full-strength dose as a single-administered product. In one embodiment of the present invention, the dyslipidemic agent is a HMG-CoA

reductase inhibitor, or statin, which can generally be present in an amount from about 0.5 mg to 80 mg, more preferably from about 1 mg to about 40 mg, and most preferably from about 5 mg to about 20 mg, per gram of omega-3 fatty acid. The daily dose may range from about 2 mg to about 320 mg, preferably about 4 mg to about 160 mg.

[00089] In some variations of the present invention, the combination of dyslipidemic agent and the omega-3 fatty acids is formulated into a single administration or unit dosage. In preferred embodiments, the dyslipidemic agent is a statin selected from the following group: pitavastatin, atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin.

[00090] Pravastatin, which is known in the market as Pravachol® manufactured by Bristol-Myers Squibb, Princeton, NJ, is hydrophilic. Pravastatin is best absorbed without food, i.e., an empty stomach. The dosage of pravastatin, in the combined administration of concentrated omega-3 fatty acids is preferably from 2.5 to 80 mg, preferably 5 to 60, and more preferably from 10 to 40 mg per dosage of concentrated omega-3 fatty acids. In one variation, the combination product using pravastatin is taken at or around bedtime, *e.g.*, 10 pm.

[00091] Lovastatin, which is marketed under the name Mevacor® by Merck, Whitehouse Station, NJ, is hydrophobic. Unlike pravastatin, lovastatin should be taken with meals and accordingly, in some embodiments, the combination product of concentrated omega-3 fatty acids and lovastatin should be taken with food. The dosage of lovastatin, in the combined administration of concentrated omega-3 fatty acids is preferably from 2.5 to 100 mg, preferably 5 to 80 mg, and

more preferably from 10 to 40 mg per dosage of concentrated omega-3 fatty acids.

[00092] Simvastatin, which is marketed under the name Zocor® by Merck, Whitehouse Station, NJ, is hydrophobic. The dosage of simvastatin, in the combined administration of concentrated omega-3 fatty acids is preferably from 1 to 80 mg per day, preferably 2 to 60 mg, and more preferably from 5 to 40 mg per dosage of concentrated omega-3 fatty acids.

[00093] Atorvastatin, which is marketed under the name Lipitor® by Pfizer, New York, NY, is hydrophobic and is known as a synthetic statin. The dosage of atorvastatin, in the combined administration of concentrated omega-3 fatty acids is preferably from 2.5 to 100 mg, preferably 5 to 80 mg, and more preferably from 10 to 40 mg per dosage of concentrated omega-3 fatty acids.

[00094] Fluvastatin, which is marketed under the name Lescol® by Novartis, New York, NY, is hydrophilic and is known as a synthetic statin. The dosage of fluvastatin, in the combined administration of concentrated omega-3 fatty acids is from 5 to 160 mg, preferably 10 to 120 mg, and more preferably from 20 to 80 mg per dosage of concentrated omega-3 fatty acids.

[00095] Rosuvastatin is marketed under the name Crestor® by Astra Zeneca, Wilmington, DE. The dosage of rosuvastatin, in the combined administration of concentrated omega-3 fatty acids is from 1 to 80 mg, preferably 2 to 60 mg, and more preferably from 5 to 40 mg per dosage of concentrated omega-3 fatty acids.

[00096] The daily dosages of dyslipidemic agent and concentrated omega-3 fatty acids can be administered together in from 1 to 10 dosages, with the preferred number of dosages from 1 to 4 times a day, most preferred 1 to 2 times a day. The administration is preferably oral administration, although other forms of administration that provides a unit dosage of dyslipidemic agent and concentrated omega-3 fatty acids may be used.

[00097] In some embodiments, the formulations of the present invention allow for improved effectiveness of each active ingredient, with one or both administered as a conventional full-strength dose, as compared to the formulations in the prior art. In other embodiments, the formulations of the present invention may allow for reduced dosages of dyslipidemic agent and/or omega-3 fatty acids, as compared to the formulations in the prior art, while still maintaining or even improving upon the effectiveness of each active ingredient.

[00098] The present combination of a dyslipidemic agent and concentrated omega-3 fatty acids may allow for a greater effect than any expected combined or additive effect of the two drugs alone. Moreover, the combined or additive effect of the two drugs may depend on the initial level of triglycerides in the blood of a subject. For example, the triglyceride level of a subject is generally as normal if less than 150 mg/dL, borderline to high if within about 150-199 mg/dL, high if within about 200-499 mg/dL and very high if 500 mg/dL or higher. The present invention may be used to reduce the triglyceride level of a "very high" down to a "high" or "borderline to high" in less than 48 weeks, preferably within 24 weeks, more preferably within 12 weeks, and most preferably within 8 weeks.

The present invention may also be used to reduce the triglyceride level of a "high" down to a "borderline to high" or "normal" in less than 48 weeks, preferably within 24 weeks, more preferably within 12 weeks, and most preferably within 8 weeks.

EXAMPLE

[00099] Clinical study: A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of Combined Lovaza™ and Simvastatin Therapy in Hypertriglyceridemic Subjects

[000100] A randomized, double-blind, placebo-controlled clinical study was conducted to assess the efficacy and safety of combined treatment with Lovaza™ omega-3 fatty acids and simvastatin (Zocor®) in hypertriglyceridemic subjects. Patients were initially treated with 40 mg/day simvastatin for at least 8 weeks, whereupon baseline measurements were taken. Patients were eligible for enrollment and randomization if their baseline triglyceride levels were above normal (≥150 mg/dl) and their LDL-C at most 10% above the NCEP ATP III goal. A total of 259 patients were randomized and received at least one dose of either Lovaza™ omega-3 fatty acids or placebo, and 229 of these patients had baseline triglyceride levels between 200 and 499 mg/dl. Initial treatment was thereafter followed by an additional 8 week treatment with either 4 g/day Lovaza™ omega-3 fatty acids or placebo, while continuing statin therapy, in a double-blind fashion. 243 patients completed the study.

[000101] The following Table 1 shows the results obtained for changes in various lipid and inflammatory parameters and markers.

	Lovaza™ treatment: median % change from baseline	Lovaza™ treatment: mean % change from baseline	Placebo treatment: median % change from baseline	Placebo treatment: mean % change from baseline	Difference (% median)	p-value
Non-HDL-C	-9.0%	-7.9%	-2.2%	-1.5%	-6.8%	<0.0001
LDL-C	+0.7%	+3.1%	-2.8%	-1.9%	+3.5%	0.0522
Apo-B	-4.2%	-3.8%	-1.9%	-1.2%	-2.3%	0.0232
TG	-29.5%	-28.2%	-6.3%	-3.5%	-23.2%	<0.0001
VLDL-C	-27.5%	-23.8%	-7.2%	-4.8%	-20.3%	<0.0001
total C	-4.8%	-4.7%	-1.7%	-1.5%	-3.1%	0.0013
HDL-C	+3.4%	+4.1%	-1.2%	-1.1%	+4.6%	<0.0001
TC/HDL	-9.6%	-8.0%	-0.7%	+0.1%	-8.9%	<0.0001
RLP-C	-36.0%	-29.0%	-10.6%	+0.1%	-25.4%	<0.0001
Lp-PLA2	-12.8%	-10.7%	-4.7%	-1.4%	-8.1%	0.0019
Apo-C3	-7.8%	-7.1%	+3.9%	+3.8%	-11.7%	0.0002

[000102] The following Tables 2 and 3 show the LDL-C goal achievement experienced in the study by those on Lovaza™ treatment and placebo, respectively.

Table 2

Omacor treatment	Baseline		End of Treatment	
			At or below goal	Above goal
	At or below goal	113 (92.62%)	110 (97.35%)	3 (2.65%)
	Above goal	9 (7.38%)	3 (33.33%)	6 (66.67%)
	Total	122 (100%)	113 (92.62%)	9 (7.38%)

Table 3

Placebo treatment	Baseline		End of Treatment	
			At or below goal	Above goal
	At or below goal	120 (90.91%)	117 (97.50%)	3 (2.50%)
	Above goal	12 (9.09%)	2 (16.67%)	10 (83.33%)
	Total	132 (100%)	119 (90.15%)	13 (9.85%)

[000103] A more detailed analysis of apo-B reduction as function of baseline LDL-C and Non-HDL-C levels demonstrates the significant and increasing ability of Lovaza™ treatment to decrease apo-B levels at increasing LDL-C and Non-HDL-C baseline levels, whereas placebo treatment results in random and insignificant changes in apo-B levels.

[000104] Tables 4A, 4B and 5 show the apo-B reduction and other lipid parameter changes with Lovaza™ or placebo treatment for specific LDL-C and Non-HDL-C patient subgroups. At higher LDL-C (≥100 mg/dL) and Non-HDL-C (≥130 mg/dL baseline levels, Lovaza™ reduces apo-B while at lower baseline levels, apo-B changes by Lovaza™ versus placebo are insignificant. Table 4B shows that the Apo-B reducing effect is even more profound at higher LDL-C baseline levels, and seems to be accompanied by a reduction in LDL-C levels.

Table 4A

Patients with LDL-C < 100 mg/dL	Lipid Parameters†	LOVAZA (n=87)		Placebo (n=89)		P-value
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)	126.7	-8.6	126.3	-2.5	0.0002
	Total-C (mg/dL)	173.7	-4.7	172.3	-1.7	0.0289
	Triglycerides (mg/dL)	270.0	-29.1	273.0	-7.0	<0.0001
	VLDL-C (mg/dL)	51.7	-27.5	52.3	-7.8	<0.0001
	LDL-C (mg/dL)	82.0	2.4	81.0	-1.8	0.0108
	HDL-C (mg/dL)	45.0	3.3	40.7	-0.9	<0.0001
	Apo-B (mg/dL)	80.3	-3.2	80.3	-2.8	0.4220
Patients with LDL-C ≥100 mg/dL	**Lipid Parameters†**	**LOVAZA (n=35)**		**Placebo (n=43)**		**P-value**
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)*	159.5	-10.2	167.1	-1.0	0.0005
	Total-C (mg/dL)*	208.2	-7.1	215.7	-1.2	0.0066
	Triglycerides (mg/dL)*	270.6	-28.1	269.4	-1.8	<0.0001
	VLDL-C (mg/dL)*	51.4	-25.4	52.1	-2.2	<0.0001
	LDL-C (mg/dL)*	114.0	-3.6	118.8	-2.3	0.6503
	HDL-C (mg/dL)*	48.7	3.5	48.6	-1.4	0.0218
	Apo-B (mg/dL)*	98.9	-6.5	100.0	0.7	0.0016

Table 4B

Patients with LDL-C ≥100 and < 130 mg/dL	Lipid Parameters†	LOVAZA (n=30)		Placebo (n=33)		P-value
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)*	153.9	-8.3	159.3	-1.2	0.0138
	Total-C (mg/dL)*	201.0	-5.8	207.3	-1.2	0.0566
	Triglycerides (mg/dL)*	259.0	-24.8	272.8	-2.0	<0.0001
	VLDL-C (mg/dL)*	50.6	-23.5	52.7	-3.2	<0.0001
	LDL-C (mg/dL)*	110.3	-2.3	111.6	-2.3	0.9961
	HDL-C (mg/dL)*	47.1	3.0	48.0	-0.6	0.1391
	Apo-B (mg/dL)*	96.0	-5.4	96.6	0.4	0.0214
Patients with LDL-C ≥130 mg/dL	**Lipid Parameters†**	**LOVAZA (n=5)**		**Placebo (n=10)**		**P-value**
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)*	193.3	-21.3	192.8	-0.2	0.0017
	Total-C (mg/dL)*	251.4	-14.8	243.4	-1.0	0.0098
	Triglycerides (mg/dL)*	340.3	-47.8	258.2	-1.0	0.0010
	VLDL-C (mg/dL)*	56.4	-36.7	50.3	1.0	0.0087
	LDL-C (mg/dL)*	136.3	-11.1	142.7	-2.3	0.2180
	HDL-C (mg/dL)*	58.1	6.8	50.5	-3.9	0.0258
	Apo-B (mg/dL)*	116.5	-13.2	111.3	2.0	0.0127

† Variables typically not normally distributed, therefore statistical analyses were based on median values unless otherwise indicated

* Statistical analyses based on mean values due to normal distribution of the variables within the Subgroup

Table 5

Patients with Non-HDL-C < 130 mg/dL	Lipid Parameters†	LOVAZA (n=47) Baseline	% change	Placebo (n=52) Baseline	% change	P-value
	Non-HDL-C (mg/dL)	112.0	-7.7	116.0	-1.2	0.0066
	Total-C (mg/dL)	158.7	-3.4	158.3	-0.4	0.2453
	Triglycerides (mg/dL)*	272.1	-30.1	255.4	-4.1	<0.0001
	VLDL-C (mg/dL)	47.7	-28.5	48.0	-7.7	<0.0001
	LDL-C (mg/dL)	72.3	3.6	76.0	-1.1	0.0056
	HDL-C (mg/dL)*	47.3	6.4	43.6	-0.8	0.0003
	Apo-B (mg/dL)	73.7	-1.7	75.2	-0.9	0.8675
Patients with Non-HDL-C ≥130 mg/dL	**Lipid Parameters†**	**Omacor (n=75)** Baseline	% change	**Placebo (n=80)** Baseline	% change	**P-value**
	Non-HDL-C (mg/dL)*	150.5	-10.0	159.7	-2.1	<0.0001
	Total-C (mg/dL)*	197.9	-6.9	205.1	-2.0	0.0004
	Triglycerides (mg/dL)	272.3	-29.1	286.8	-5.0	<0.0001
	VLDL-C (mg/dL)*	53.6	-24.5	55.1	-3.7	<0.0001
	LDL-C (mg/dL)*	100.6	-0.7	104.3	-1.9	0.5476
	HDL-C (mg/dL)	46.7	2.0	44.8	-1.0	0.0153
	Apo-B (mg/dL)*	92.6	-5.9	95.4	-0.4	0.0005

† Variables typically not normally distributed, therefore statistical analyses were based on median values unless otherwise indicated

* Statistical analyses based on mean values due to normal distribution of the variables within the subgroup

[000105] Table 6 shows the apo-B reduction and other lipid parameter changes with Lovaza™ or placebo treatment for above 200 mg/dL triglyceride baseline levels versus below this level. At higher triglyceride baseline levels (≥200 mg/dL), Lovaza™ reduces apo-B while at lower baseline triglyceride levels, Apo-B changes by Lovaza™ versus placebo are insignificant.

Table 6

Patients with TG < 200 mg/dL	Lipid Parameters†	LOVAZA (n=11) Baseline	LOVAZA (n=11) % change	Placebo (n=10) Baseline	Placebo (n=10) % change	P-value
	Non-HDL-C (mg/dL)*	130.5	-7.8	135.6	-3.9	0.4757
	Total-C (mg/dL)*	183.0	-4.0	190.4	-4.6	0.8834
	Triglycerides (mg/dL)*	186.2	-25.2	189.1	4.6	0.0183
	VLDL-C (mg/dL)*	37.2	-24.9	37.9	-1.1	0.0268
	LDL-C (mg/dL)*	99.1	-3.3	102.8	-7.4	0.4498
	HDL-C (mg/dL)*	52.6	5.2	54.8	-5.8	0.0135
	Apo-B (mg/dL)*	82.6	-2.4	84.5	-2.8	0.9353
Patients with TG ≥200 mg/dL	**Lipid Parameters†**	**LOVAZA (n=111)** Baseline	**LOVAZA (n=111)** % change	**Placebo (n=122)** Baseline	**Placebo (n=122)** % change	**P-value**
	Non-HDL-C (mg/dL)	137.7	-9.3	141.7	-1.9	<0.0001
	Total-C (mg/dL)	184.3	-5.3	183.5	-1.1	0.0007
	Triglycerides (mg/dL)	272.3	-30.2	274.7	-6.3	<0.0001
	VLDL-C (mg/dL)	53.0	-27.8	53.7	-7.2	<0.0001
	LDL-C (mg/dL)	89.3	1.6	87.5	-1.8	0.0587
	HDL-C (mg/dL)	45.0	2.9	42.3	-0.9	0.0001
	Apo-B (mg/dL)	85.7	-4.7	87.0	-1.4	0.0117

† Variables typically not normally distributed, therefore statistical analyses were based on median values unless otherwise indicated
* Statistical analyses based on mean values due to normal distribution of the variables within the subgroup

[000106] Tables 7 and 8 show the apo-B reduction and other lipid parameter changes with Lovaza™ or placebo treatment for specific LDL-C/Triglyceride and Non-HDL-C/Triglyceride patient subgroups. At combined higher LDL-C (≥100 mg/dL) and triglyceride (≥200 mg/dL) baseline levels and at combined Non-HDL-C (≥130 mg/dL and triglyceride (≥200 mg/dL) baseline levels, Lovaza™ reduces apo-B while at lower baseline levels, apo-B changes by Lovaza™ versus placebo are insignificant.

Table 7

Patients with LDL-C < 100 mg/dL and/or TG < 200 mg/dL	**Lipid Parameters†**	**LOVAZA (n=93)** Baseline	% change	**Placebo (n=93)** Baseline	% change	**P-value**
	Non-HDL-C (mg/dL)	129.7	-7.9	128.3	-2.3	0.0001
	Total-C (mg/dL)	178.0	-4.7	174.0	-1.7	0.0171
	Triglycerides (mg/dL)	266.3	-29.1	269.0	-6.3	<0.0001
	VLDL-C (mg/dL)	50.3	-27.5	52.0	-7.7	<0.0001
	LDL-C (mg/dL)	83.7	1.6	82.3	-2.6	0.0177
	HDL-C (mg/dL)	45.3	3.3	42.7	-0.9	<0.0001
	Apo-B (mg/dL)	80.3	-2.7	80.3	-2.5	0.4178
Patients with LDL-C ≥100 mg/dL and TG ≥200 mg/dL	**Lipid Parameters†**	**LOVAZA (n=29)** Baseline	% change	**Placebo (n=39)** Baseline	% change	**P-value**
	Non-HDL-C (mg/dL)*	162.2	-10.7	167.0	-1.3	0.0012
	Total-C (mg/dL)*	210.9	-7.4	215.3	-1.5	0.0130
	Triglycerides (mg/dL)*	287.4	-28.8	278.1	-3.7	<0.0001
	VLDL-C (mg/dL)*	54.2	-25.6	53.7	-4.2	<0.0001
	LDL-C (mg/dL)*	114.1	-3.1	117.4	-2.4	0.8053
	HDL-C (mg/dL)*	48.6	3.9	48.3	-1.4	0.0204
	Apo-B (mg/dL)*	100.8	-7.5	100.1	0.4	0.0013

† Variables typically not normally distributed, therefore statistical analyses were based on median values unless otherwise indicated

* Statistical analyses based on mean values due to normal distribution of the variables within the subgroup

Table 8

Patients with Non-HDL-C < 130 mg/Dl and TG < 200 mg/dl	**Lipid Parameters†**	**LOVAZA (n=54)**		**Placebo (n=56)**		**P-value**
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)	116.2	-7.6	117.7	-0.9	0.0020
	Total-C (mg/dL)	162.2	-3.4	161.0	-0.7	0.1170
	Triglycerides (mg/dL)*	261.0	-29.2	250.4	-2.6	<0.0001
	VLDL-C (mg/dL)	45.3	-28.2	46.5	-7.2	<0.0001
	LDL-C (mg/dL)	73.7	2.5	76.8	-1.6	0.0213
	HDL-C (mg/dL)*	47.9	5.9	44.2	-0.9	0.0003
	Apo-B (mg/dL)	75.2	-1.7	75.8	-0.9	0.9953
Patients with Non-HDL-C ≥ 130 mg /dL and TG ≥200 mg/dL	**Lipid Parameters†**	**LOVAZA (n=68)**		**Placebo (n=76)**		**P-value**
		Baseline	% change	Baseline	% change	
	Non-HDL-C (mg/dL)*	151.2	-10.0	159.3	-2.3	<0.0001
	Total-C (mg/dL)*	198.1	-7.0	204.4	-2.2	0.0020
	Triglycerides (mg/dL)	280.5	-29.5	290.2	-5.9	<0.0001
	VLDL-C (mg/dL)*	55.4	-24.6	56.1	-4.9	<0.0001
	LDL-C (mg/dL)*	99.6	-0.1	102.8	-2.0	0.3863
	HDL-C (mg/dL)	46.2	1.8	44.5	-1.0	0.0348
	Apo-B (mg/dL)	90.7	-6.8	93.7	-2.4	0.0025

† Variables typically not normally distributed, therefore statistical analyses were based on median values unless otherwise indicated

* Statistical analyses based on mean values due to normal distribution of the variables within the subgroup

[000107] All references cited herein are hereby incorporated by reference in their entirety.

What is claimed is:

1. A method of reducing an apo-B level in a subject group, comprising providing a subject group, and reducing the apo-B level of the subject group by administering to the subject group a combination of a dyslipidemic agent and omega-3 fatty acids in an amount effective to reduce the apo-B level of the subject group as compared to treatment with a dyslipidemic agent alone.

2. The method of claim 1, wherein the dyslipidemic agent is selected from the group consisting of HMG CoA reductase inhibitors, cholesterol absorption inhibitors, CETP inhibitors, niacin and derivatives, fibrates, bile acid sequestrants, MTP inhibitors, LXR agonists and/or antagonists, and PPAR agonists, antagonists and/or partial agonists/antagonists.

3. The method of claim 1, wherein the subject group has at least one of the following conditions or diseases: hypertriglyceridemia, hypercholesterolemia, mixed dyslipidemia, vascular disease, and atherosclerotic disease.

4. The method of claim 1, wherein the subject group has an LDL-cholesterol level of at least 100 mg/dL.

5. The method of claim 1, wherein the subject group has an LDL-cholesterol level of at least 100 mg/dL and less than 130 mg/dL.

6. The method of claim 1, wherein the subject group has an LDL-cholesterol level of at least 130 mg/dL.

7. The method of claim 1, wherein the subject group has a non-HDL-cholesterol level of at least 130 mg/dL.

8. The method of claim 1, wherein the subject group has an LDL-cholesterol level of at least 100 mg/dL and a triglyceride level of at least 200 mg/dL.

9. The method of claim 1, wherein the subject group has a non-HDL-cholesterol level of at least 130 mg/dL and a triglyceride level of at least 200 mg/dL

10. The method of claim 1, wherein the omega-3 fatty acids are present in a concentration of at least 40% by weight as compared to the total fatty acid content of the composition.

11. The method of claim 1, wherein the omega-3 fatty acids are present in a concentration of at least 80% by weight as compared to the total fatty acid content of the composition.

12. The method of claim 1, wherein the omega-3 fatty acids comprise at least 80% by weight of EPA and DHA as compared to the total fatty acid content of the composition.

13. The method of claim 1, wherein the omega-3 fatty acids comprise about 40% to about 55% by weight of EPA as compared to the total fatty acid content of the composition.

14. The method of claim 1, wherein the omega-3 fatty acids comprise about 30% to about 60% by weight of DHA as compared to the total fatty acid content of the composition.

15. The method of claim 1, wherein omega-3 fatty acids comprise omega-3 polyunsaturated, long-chain fatty acids, esters of omega-3 fatty acids with glycerol, esters of omega-3 fatty acids and a primary, secondary or tertiary

alcohol, or mixtures thereof.

16. The method of claim 1, wherein the omega-3 fatty acids comprise EPA and DHA in a ratio of EPA:DHA from 2:1 to 1:2.

17. The method of claim 1, wherein the omega-3 fatty acids are administered apart from administration of the dyslipidemic agent.

18. The method of claim 1, wherein the omega-3 fatty acids are administered simultaneous to the administration of the dyslipidemic agent.

19. The method of claim 1, wherein the omega-3 fatty acids and the dyslipidemic agent are administered in a concomitant treatment regime.

20. The method of claim 1, wherein the omega-3 fatty acids and the dyslipidemic agent are administered together in a unit dose form.

21. The method of claim 1, wherein the subject group has a baseline triglyceride level of 200 to 499 mg/dl.

22. The method of claim 1, wherein an increase of an LDL-C level is avoided.