Published in final edited form as:
J Acquir Immune Defic Syndr. 2008 April 1; 47(4): 459–466. doi:10.1097/QAI.0b013e31815bace2.

Fish Oil and Fenofibrate for the Treatment of Hypertriglyceridemia in HIV-Infected Subjects on Antiretroviral Therapy:

Results of ACTG A5186

John G. Gerber, MD[*], **Douglas W. Kitch, MS**[†], **Carl J. Fichtenbaum, MD**[‡], **Robert A. Zackin, ScD**[†], **Stéphannie Charles, BA**[§], **Evelyn Hogg, BA**[||], **Edward P. Acosta, PharmD**[¶], **Elizabeth Connick, MD**[*], **David Wohl, MD**[#], **E. Milu Kojic, MD**[**], **Constance A. Benson, MD**[††], **Judith A. Aberg, MD**[‡‡], and **AIDS Clinical Trial Group A5186 Team**

[*]Department of Medicine, University of Colorado Health Sciences Center, Denver, CO

[†]Complications of HIV, Adult Division, Statistical and Data Analysis Center, Harvard School of Public Health, Boston, MA

[‡]Department of Medicine, University of Cincinnati Medical Center, Cincinnati, OH

[§]Frontier Science and Technical Research Foundation, Amherst, NY

[||]Social and Scientific Systems, Silver Spring, MD

[¶]Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL

[#]Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

[**]Department of Medicine, Brown Medical School, Providence, RI

[††]Department of Medicine, University of California, San Diego, San Diego, CA

[‡‡]Department of Medicine, New York University, New York, NY

Abstract

Introduction—Fish oil has been shown to reduce serum triglyceride (TG) concentrations. In HIV-infected patients on antiretroviral therapy, high TG concentrations likely contribute to increased risk of cardiovascular disease. AIDS Clinical Trials Group A5186 examined the safety and efficacy of fish oil plus fenofibrate in subjects not achieving serum TG levels ≤200 mg/dL with either agent alone.

Methods—One hundred subjects on highly active antiretroviral therapy with serum TG concentrations ≥400 mg/dL and low-density lipoprotein cholesterol ≤160 mg/dL were randomized to 3 g of fish oil twice daily or 160 mg of fenofibrate daily for 8 weeks. Subjects with a fasting TG

Correspondence to: Judith Aberg, MD, New York University, Bellevue C and D Building, Room 558, 550 First Avenue, New York, NY 10016-6481 (judith.aberg@med.nyu.edu)..

C. J. Fichtenbaum, C. A. Benson, and J. A. Aberg have served on the Advisory Board for Abbott Labs and have received honoraria and research grant support.

R. A. Zackin, ScD, is deceased.

Fenofibrate was donated by Abbott Laboratories (Abbott Park, IL), and fish oil was purchased from Advanced Nutritional Technologies, Inc. (Dublin, CA).

Data presented in part at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO, February 5–8, 2006 (abstract 146).

level >200 mg/dL at week 8 received a combination of fish oil and fenofibrate in the same doses from week 10 to week 18.

Results—Median baseline TG was 662 mg/dL in the fish oil group and 694 mg/dL in the fenofibrate group (*P* = not significant). Fish oil reduced TG levels by a median of 283 mg/dL (46%), fenofibrate reduced them by 367 mg/dL (58%), and combination therapy reduced them by 65.5%. Combination therapy achieved TG levels of ≤200 mg/dL in 22.7% subjects. Fish oil had no measurable effect on immunologic parameters or the pharmacokinetics of lopinavir.

Conclusions—Fish oil was safe when administered alone or combined with fenofibrate and significantly reduced TG levels in HIV-infected subjects with hypertriglyceridemia.

Keywords

fenofibrate; fish oil; hypertriglyceridemia; low-density lipoprotein cholesterol; omega-3 fatty acids

Before the introduction of highly active antiretroviral therapy (HAART), HIV-infected persons were noted to have elevated triglyceride (TG) levels with low total cholesterol and low high-density lipoprotein cholesterol (HDL-C), presumably attributable to a persistent inflammatory state. Furthermore, elevation of serum lipids is a frequent and serious complication associated with the use of HAART.[1-4] The most common lipid elevation observed is with the serum TG level.[5] Although ritonavir is the antiretroviral drug (ARV) most commonly associated with this lipid change, other ARVs have also been associated with serum TG perturbations.[6-8]

There is mounting evidence that the observed metabolic syndrome characterized by elevated TGs, low HDL-C, glucose intolerance, and body fat changes may accelerate the development of atherosclerosis, leading to higher rates of cerebrovascular and coronary heart disease (CHD).[9,10] HIV-infected patients with HAART-induced hypertriglyceridemia have been observed to have atherogenic dyslipidemia. Although there is not universal consensus, hypertriglyceridemia is increasingly being recognized as an independent risk factor in the development of CHD and is a secondary target for intervention in the most recent National Cholesterol Education Project (NCEP) Adult Treatment Panel III guidelines.[11-14] In addition to increased CHD risks, high serum TG is associated with the development of pancreatitis. Thus, the identification of effective therapy for drug-induced hypertriglyceridemia is an important clinical concern. Presently, the recommended approaches to the treatment of serum hypertriglyceridemia consist of diet, exercise, and the use of drugs like fibrates or niacin.[15] Neither diet nor exercise is consistently effective because of the difficulty in adhering to these lifestyle changes. Pharmacotherapy is somewhat effective, but in a large clinical trial in HIV-infected subjects with lipid abnormalities, fenofibrate alone reduced serum TG level to <200 mg/dL (the NCEP Treatment Panel III guidelines' suggested target level) in only 48% of the subjects, with a median baseline serum TG level of 336 mg/dL.[16]

Fish oil has been shown to reduce serum TGs in disparate populations, including those with congenital hypertriglyceridemia and drug-induced hypertriglyceridemia.[17-19] Two trials evaluating lower doses of fish oil than we used decreased the serum TG concentration by 25% to 30% in HIV-infected individuals on ARVs.[20,21] These studies were not designed to reduce serum TG to ≤200 mg/dL, however. Fish oil is an attractive compound because of its described effect in reducing atherogenic cardiovascular disease risk by means of a combination of anti-inflammatory and antiplatelet effects.[22] Epidemiologic studies have suggested that a diet rich in omega-3 fatty acids is associated with a low incidence of CHD.[23] In addition, the American Heart Association's dietary guidelines have recommended that healthy adults eat at least 2 servings of fish per week and that people who have elevated TGs need 2 to 4 g of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per day as a dietary supplement.

AIDS Clinical Trials Group (ACTG) A5186 was designed to test the hypothesis that we could reach a TG goal <200 mg/dL in a significant proportion of subjects using fenofibrate plus fish oil in those failing either agent alone. At the time this study was designed, these agents had not been used in combination in this population. Further, we proposed using fish oil containing higher doses of EPA and DHA than previously studied in persons with HIV. Thus, the study was designed as a phase 2 study that, if successful, would merit a larger randomized controlled trial. The primary objectives of ACTG A5186 were (1) to evaluate whether the combination of fish oil supplement and fenofibrate would decrease fasting serum TGs to ≤200 mg/dL in subjects not responding to either agent alone and (2) to evaluate the safety and tolerability of fish oil supplement alone and in combination with fenofibrate in an HIV-infected population with elevated fasting serum TGs.

The secondary objectives were (1) to evaluate the change in serum TG and low-density lipoprotein cholesterol (LDL-C) with the combination of fish oil supplement and fenofibrate and with each agent alone, (2) to describe the effect of fish oil supplement on lymphocyte proliferative responses to *Candida* and cytomegalovirus (CMV) antigens, (3) to describe the effect of fish oil supplement on C-reactive protein (CRP) concentrations, and (4) to describe the effect of fish oil supplement on the pharmacokinetics of concomitantly administered HIV protease inhibitors (PIs).

MATERIALS AND METHODS

Study Design

ACTG A5186 was a phase 2, randomized, open-label, multisite trial. Therapeutic response was defined as achieving a fasting serum TG concentration ≤200 mg/dL. Eligible subjects entered step 1 of ACTG A5186 and were randomized 1:1 into 1 of 2 treatment arms. Subjects in arm A received 3 g of fish oil supplement twice a day, to be taken with meals (Advanced Nutritional Technology, Dublin, CA; each gram of fish oil contains 500 mg of EPA, 310 mg of DHA, 190 mg of other omega-3 fatty acid, and 20 IU of d-alpha tocopherol). Subjects in arm B received 160 mg of fenofibrate once daily (Abbott Laboratories, Abbott Park, IL). Subjects were evaluated for response to treatment at week 8. At week 10, the subjects who had not responded were registered to step 2 and began combined therapy with fenofibrate and fish oil at the same doses through week 18. Subjects who had responded to their step 1 treatment (fasting serum TG ≤200 mg/dL) remained on their original single-agent therapy through week 18. All subjects returned for a follow-up safety visit at week 22. The primary goal of the study was to determine the proportion of subjects who responded to the combination of fish oil and fenofibrate when there was no response to monotherapy. The primary endpoint was response (TG ≤200 mg/dL) on step 2, with no statistical analysis comparing the step 2 response rate with the step 1 monotherapy response rate.

Inclusion and Exclusion Criteria

Subjects were at least 18 years of age with documented HIV infection who had been taking HAART for at least 3 months before study entry and stable HAART for at least 4 weeks before study entry. Serum lipid eligibility criteria included fasting serum TGs ≥400 mg/dL and LDL-C ≤160 mg/dL by ultracentrifugation within 28 days before study entry. Fasting was defined as no food or beverage (other than water) for 8 to 12 hours before blood draw. Subjects had to be willing to remain on their current HAART regimen for the study duration, to have adhered to a lipid-lowering diet and exercise program for at least 28 days before screening, and to be willing to continue both for the study duration. All female subjects of reproductive potential had to have a negative pregnancy test result at screening and again at study entry. Male subjects on testosterone replacement therapy had to have been on stable therapy for at least 3 months

before study entry and to remain on therapy for the study duration. A Karnofsky Performance Scale score ≥70 was required within 28 days before study entry.

Exclusion criteria for step 1 included fasting glucose >126 mg/dL, platelet count <50,000 mm^3, international normalized ration (INR) >1.5, absolute neutrophil count <750 mm^3, hemoglobin <9.1 g/dL for men and <8.9 g/dL for women, serum creatinine >1.5 times the upper limit of normal (ULN), total bilirubin >2.5 times the ULN, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 times the ULN, plasma HIV RNA level >10,000 copies/mL, documented CHD, congestive heart failure, uncontrolled hypertension, active bleeding disorder, active peptic ulcer disease, diabetes mellitus requiring pharmacologic therapy, untreated hypothyroidism, gallbladder disease, use of systemic cancer chemotherapy, pregnancy, or breast-feeding. Subjects could not have received any lipid-lowering agent, hormonal therapy except replacement testosterone, systemic corticosteroids, immune modulators, anticoagulants, or investigational antiretroviral drugs within 28 days before study entry. Subjects with allergy to study drugs, any active drug or alcohol dependence, mental incapacity, acute illness, or active AIDS-defining infection within 28 days before study entry that would have interfered with study participation were excluded. Exclusion criteria for step 2 included a week 8 INR >1.5.

Evaluations

A fasting lipid panel was obtained at screening and at weeks 4, 8, and 18 (plus week 14 for step 2 subjects). In step 1, blood samples were collected from subjects in arm A at entry and week 8 for lymphocyte proliferation assays (LPAs) to evaluate the immunologic effect of fish oil. LPA samples were also collected in step 2 at weeks 10 and 18 from subjects who were formerly in arm B. For subjects who were on a PI that was taken twice a day, plasma samples were collected in arm A at entry and weeks 2 and 8 for trough PI concentrations. In step 2, plasma samples for PI trough concentrations were collected at weeks 10, 12, and 18 from subjects who were on a PI that was taken twice a day and who had been in arm B during step 1. Clinical and laboratory evaluations were assessed throughout the study. Adherence evaluations using the standardized 4-day recall questionnaire were administered during the study visits. In addition, all subjects had samples collected for measurement of serum high-sensitivity (hs) CRP concentrations at baseline and weeks 8 and 18. Subjects were not allowed to take any additional lipid-lowering therapies or over-the-counter lipid-lowering products during the conduct of the study.

Laboratory Analysis

Lipid Levels—Total cholesterol, TG, and HDL-C values[24] were determined by standard techniques on a Roche Hitachi 917 (Roche Diagnostics, Basel, Switzerland, and Hitachi Ltd., Tokyo, Japan), and lipoprotein fractionation was determined by ultracentrifugation on a Quest Diagnostics ultracentrifuge (Nicholls Institute, San Juan Capistrano, CA). LDL-C from ultracentrifugation was determined after ultracentrifugation using a TL120 tabletop ultracentrifuge (Beckman Coulter, Inc., Fullerton, CA). HDL-C and LDL-C were isolated together by spinning them into a low-salt solution (0.9% NaCl). HDL-C alone was obtained by centrifugation into a high-salt solution (16.7% NaCl). LDL-C was calculated by subtracting the HDL-C (16.7% bottom) from the LDL-C plus HDL-C (0.9% bottom).

Lymphocyte Proliferation Assays—Peripheral blood specimens were shipped overnight to a single laboratory, where peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density centrifugation. Lymphocyte proliferation was assessed in a standard 6-day ^{3}H-thymidine incorporation assay according to the consensus protocol of the ACTG (available at: http://aactg.s-3.com/pub/download/labmanual/29-ALM-Lymphocyte-Proliferation-

Assay.pdf) to antigens of *Candida albicans* (10 μg/mL; Greer Laboratories, Lenoir, NC) and CMV (1:1000; Bio Whittaker, Rockland, ME), the control CMV antigen (1:4000; Bio Whittaker), and the mitogen phytohemagglutinin (PHA; 5 μg/mL; Sigma, St. Louis, MO). ^{3}H-thymidine incorporation was determined, and the stimulation index (SI) was calculated by dividing the median counts per minute (cpm) of the antigen-containing wells by the median cpm of 8 control wells into which no antigens had been placed or the CMV antigen control. If this ratio was <1, the SI was defined as equal to 1.

Plasma PI Concentrations—Plasma concentrations of PIs were analyzed at the University of Alabama at Birmingham ACTG Pharmacology Core Laboratory using a modified version of a previously validated high-performance liquid chromatography (HPLC)-ultraviolet (UV) method.[25]

Statistical Considerations

A priori, it was anticipated that 25% of subjects would respond to step 2 therapy. If the addition of the second agent led to an incremental response rate of <10%, however, we determined that the combination would not be appropriate for further clinical study. We estimated that 55 subjects would be required to advance to step 2 combination therapy to detect a difference of 15% (25% vs. 10%) in the step 2 response rate, along with 90% power and a 1-sided type I error of 5%. We further estimated that an overall sample size of 100 subjects would be needed, assuming a step 1 response rate of 35% and an overall dropout rate of 15%.

The Fisher exact test was used to detect between-group differences in categoric outcomes. The Wilcoxon signed rank test was used to test within-group differences in continuous outcomes, and the Wilcoxon rank sum test was used to test between-group differences in continuous outcomes. Spearman rank coefficients were used to detect correlation between continuous endpoints and baseline variables.

A fasting lipid panel was not obtained at week 10 (initiation of combination therapy); thus, all step 2 lipid changes are calculated using week 8 and 18 data.

Approval, Support, and Conduct of the Study

The institutional review boards of the participating institutions approved the study, and each study subject gave written informed consent. The study was approved by the scientific committees of the ACTG and the National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS.

RESULTS

Baseline Characteristics

One hundred subjects were randomized to the 2 arms of the study (arm A, fish oil = 50; arm B, fenofibrate = 50). Baseline characteristics are outlined on Table 1. There were no baseline differences in the 2 arms except that more subjects receiving a nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimen were randomized to the fish oil arm of the study. The baseline median CD4 count was 457 cells/μL, and 94% of subjects had an HIV RNA level <200 copies/mL. The screening median fasting TGs were 668 mg/dL in arm A and 667 mg/dL in arm B. The screening median LDL-C, as measured by ultracentrifugation, was 85 mg/dL in arm A and 73 mg/dL in arm B. The median HDL-C was low, as previously described for HIV-infected subjects.[26] The median time between the screening lipid panel and randomization was 20 days (21 days in the fish oil arm and 19 days in the fenofibrate arm). The median difference from screening to entry TG level was −14 mg/dL ($P = 0.71$), which was not significantly different from 0.

Safety and Follow-Up

The overall tolerability of the medications was excellent. During step 1 in arm A (fish oil), only 1 subject discontinued medication because of protocol-defined toxicity (grade 3 nausea and vomiting). Three other subjects in arm A discontinued medication for reasons unrelated to drug toxicity. In arm B (fenofibrate), 3 subjects prematurely discontinued study medication for toxicity; 2 of these cases were thought to be drug related, including grade 4 lipase and new-onset renal insufficiency with a glomerular filtration rate (GFR) <50 mL/min. One toxicity event was deemed unrelated to study drug (grade 2 muscle weakness). Five other subjects discontinued medication for reasons unrelated to drug toxicity. Seven of the 12 step 1 medication discontinuations occurred at or before week 8. Of the 91 subjects on treatment and with a TG result at week 8, 75 met criteria to advance to step 2. Of these subjects, 70 continued on study medications through week 18. Of the subjects who discontinued study drugs, 1 did so secondary to the development of grade 3 lipase elevation, which was a protocol-defined drug toxicity; 1 stopped because of the pill burden and a fishy taste; 1 stopped for severe headache that was not drug related; and 2 stopped for nonadherence to medications.

Primary Analysis of Data

The primary goal of the study was to determine the proportion of subjects who responded to the combination of fish oil and fenofibrate when there was no response to monotherapy (Tables 2, 3). We expected a response rate in step 1 of 35% to achieve a TG concentration <200 mg/dL, but the observed response rate was much lower: only 4 (8.5%) of 47 subjects on fish oil and 8 (16.7%) of 48 subjects on fenofibrate responded. Thus, more subjects advanced to combination therapy, improving the overall statistical power of the study. There were 75 subjects who went onto combination therapy. The primary analysis was intention to treat (ITT), in which subjects with missing week 18 TG levels were analyzed as nonresponders. Among the step 2 subjects, we observed a response rate of 22.7% (17 of 75 subjects). The lower limit of the 1-sided exact 95% confidence interval was 15%, demonstrating that the combination of fish oil and fenofibrate was deemed clinically appropriate for further study in subjects who did not respond to monotherapy. The response rate was not significantly different if fish oil was started first (21.4% for arm A) or fenofibrate first (24.2% for arm B).

By week 8, fish oil reduced TGs by a median of 283 mg/dL and fenofibrate by a median of 367 mg/dL, a difference that did not achieve statistical significance. When the data are expressed as percent decrease in TGs, however, fish oil reduced TGs a median of 46% compared with 58% for fenofibrate ($P = 0.039$). The median percent decrease in TGs from screening to week 18 for step 2 subjects was 65.5%.

The decrease in TG levels was accompanied by a significant increase in LDL-C during step 1 and step 2. This increase in LDL-C has been previously described with fish oil and fenofibrate.[27,28] During step 1, HDL-C increased slightly, but this increase was only statistically significant for arm B (median increase in arm A was 1 mg/dL and in arm B was 4 mg/dL). Fasting lipid values for step 1 and step 2 are detailed in Tables 2 and 3. There were no significant changes in hs-CRP in either arm or with combination therapy compared with baseline (data not shown).

Fish oil administration had no effect on absolute CD4 cell count or CD4 cell percent. The median absolute CD4 count of all subjects was 451 cells/μL. The median change in CD4 count at end of step 1 was an increase of 19 cells/μL (19 cells/μL in the fish oil arm and 18 cells/μL in the fenofibrate arm). An additional median increase of 9 cells/μL occurred during step 2 on combination therapy. There was no effect of fish oil administration on the lymphocyte SI to *Candida* or CMV antigens (data not shown).

In terms of pharmacokinetic effect of fish oil as evaluated by changes in trough concentrations of PIs, only fixed-dose lopinavir/ritonavir use was sufficient for analysis. There were 18 subjects who had lopinavir trough concentrations before and after fish oil initiation, with trough data 9 to 15 hours after lopinavir/ritonavir dosing. The median time of sample collection after lopinavir/ritonavir dosing was 11.85 hours before fish oil administration and 11.75 hours during fish oil administration. The median trough concentrations of lopinavir before and after fish oil administration were 6453 ng/mL (range: 389 to 9881 ng/mL) and 5881 ng/mL (range: 416 to 13,960 ng/mL), respectively, which were not significantly different, suggesting that fish oil had no effect on the pharmacokinetics of lopinavir.

DISCUSSION

Hypertriglyceridemia has been recognized as an independent risk factor in the development of CHD.[11] The most recent NCEP guidelines suggest that a more aggressive approach to therapy is warranted, although the exact goal concentration for this lipid is still unclear, because TGs, even in the range of 150 mg/dL, have been found to promote CHD in population-based studies.[14,29] HIV-infected patients on HAART frequently have elevated TGs and low HDL-C, similar to those levels found among persons with type 2 diabetes, which places them in the high-risk category for CHD.[30] In addition, HIV infection is associated with diminished vascular endothelial function[31] and elevated hs-CRP; thus, aggressive and effective lipid-lowering therapy is likely to be beneficial in reducing the overall risk of CHD, because these patients live longer.[32]

Fish oil has been used to treat hypertriglyceridemia for 2 decades. The first description was in the early 1980s, and fish oil was found to reduce TG concentration by as much as 80% in subjects with familial hypertriglyceridemia.[17] These subjects are phenotypically similar to subjects on HAART therapy in that they have extremely high very-low-density lipoprotein (VLDL) TG levels, normal to low LDL-C, and low HDL-C. In these subjects, an increase in LDL-C secondary to fish oil was described similar to what was observed in the present study. The increase in LDL-C was much less prominent in subjects with low baseline TGs. The effectiveness of fish oil in lowering TG levels has a clear dose response relation, as demonstrated by Harris et al.[33] In addition, fish oil has been shown to have efficacy in lowering TGs in subjects with drug-induced (retinoids) hypertriglyceridemia.[34]

We demonstrated that fish oil and fenofibrate alone could produce significant decreases in TG levels; however, few patients on either therapy achieved TG levels lower than the NCEP target of ≤200 mg/dL. Of note, most of our subjects were male and these results may not be generalizable to a female population, although we are unaware of any data that suggest a difference based on gender. The combination of both agents was found to reduce TG levels further and to increase the proportion of subjects with a TG level ≤200 mg/dL among those not achieving this target during monotherapy. These results demonstrate the relative ability of each agent and their combination to treat hypertriglyceridemia in HIV-infected persons. The modest effect of monotherapy on absolute TG levels suggests that for HIV-infected patients with extremely elevated TGs, dual therapy may be preferable.

Administration of fish oil has been shown to have a beneficial effect in lowering TGs in HIV-infected subjects in 2 other trials. De Truchis et al[20] used a dose of EPA plus DHA of 1.8 g/d and demonstrated a median decrease of 25.5% in TGs. The median baseline TG was 410 mg/dL in this group, which was lower than in our subjects. Wohl et al[21] administered 2.9 g/d of EPA plus DHA in HIV-infected subjects on HAART and demonstrated a mean decrease in fasting TGs of 25% in subjects with a mean baseline TG of 461 mg/dL. In both studies and in our study, the tolerability of fish oil was excellent. Our study used a higher dose of fish oil (4.86 g/d of EPA plus DHA), our subjects started with higher baseline TGs (median of 662

mg/dL) than in the other studies, and we demonstrated a 46% decrease in TGs. Thus, it seems, as in non-HIV-infected subjects, that the dose of fish oil correlates with the extent of response.

The combination of fish oil and fenofibrate was well tolerated with minimal toxicity. Although we did not evaluate LDL particle size, data from other studies suggest that the expected increase in LDL-C is secondary to an increase in larger more buoyant LDL, which is not as atherogenic as the dense small LDL-C.[19,27,35-38] In addition, initial LDL-C levels for subjects in our study were lower than those in the general population and only increased to levels that are considered normal for most subjects. It is unclear if the increase in LDL secondary to fenofibrate and fish oil is long term and what the clinical implications of this effect are. Standard of practice is to lower TGs first when TG levels exceed 500 mg/dL; after TGs are <400 mg/dL, the LDL can be calculated to determine if LDL-lowering therapy is indicated.[13,24] The small increase in HDL-C was also expected and may partially counteract the effect on LDL-C.

Fish oil has been previously shown to be immunosuppressive by altering cytokine production in PBMCs, reducing the percentage of helper T cells, and decreasing the mitogenic response to concanavalin A.[39] In contrast to what has been previously reported in healthy volunteers, we did not observe any change in CD4 cell percent or absolute CD4 cell count in subjects taking fish oil; in addition, the LPA responses to CMV and *Candida* antigens were not altered by the fish oil supplement. Thus, this dose of fish oil seems to be immunologically safe.

Aside from evaluating the immunologic parameters, it was important to demonstrate that fish oil administration had no adverse pharmacokinetic effect on boosted lopinavir—a PI in the ARV class most closely associated with elevated TGs. Fish oil did not alter the trough concentration of lopinavir, suggesting that fish oil would not be expected to modulate the antiviral efficacy of ritonavir-boosted PIs.

In summary, we have demonstrated that fish oil at the EPA plus DHA dose of 4.86 g/d is effective in combination with fenofibrate in lowering TGs in HIV-infected subjects with hypertriglyceridemia on HAARTwho fail to respond to single-drug lipid-lowering therapy. Most subjects failed to achieve the goal TG concentration of ≤200 mg/dL when treated with fenofibrate or fish oil alone. When fish oil was combined with fenofibrate, however, a further reduction in TGs was obtained without any safety concerns. HIV-associated dyslipidemia remains a difficult condition to treat, and our trial further demonstrates the need for evaluation of combination therapies or strategies to manage this comorbidity. Whether higher doses of omega-3 fatty acids would result in a more effective antilipid effect should be studied; however, immunologic monitoring should also be assessed.

Acknowledgments

The ACTG 5186 team acknowledges the tireless and creative contributions of their friend and colleague, Dr. Robert Zackin, who died before the study was completed.

Other members of the ACTG 5186 team were Beverly L. Alston-Smith, MD (Division of AIDS [DAIDS] Clinical Representative), Paul T. Tran, RPh (DAIDS Protocol Pharmacist), Debra DeMarco, BSN, ACRN (Field Representative), Stanford Steve Arrington, BS (CCG Representative), William Chris Woodward, DO (Abbott Laboratories), and Lori Mong-Kryspin, BS, MT (Laboratory Technologist).

Subject visits and laboratory assays were supported by the AIDS Clinical Trials Group (ACTG), funded by the National Institute of Allergy and Infectious Diseases (NIAID) and National Center for Complementary and Alternative Medicine. Supported by NIAID grants AI-38858 and AI-068636 to the ACTG; grants AI-32770, AI-69450, and RR00051 to the University of Colorado Health Sciences Center (J. G. Gerber, E. Connick); grants AI-38855 and AI-068636 to the Statistical and Data Analysis Center/Harvard School of Public Health (D. W. Kitch, R. A. Zackin); grants AI-25897 and AI-069513 to the University of Cincinnati (C. J. Fichtenbaum); grants M01RR-0032, 3U01AI32775-13S3, and AI-069452 to the University of Alabama (E. P. Acosta); grants AI-50410, AI-25868, AI-69423, and RR00046 to the University of North Carolina (D. Wohl); grant AI-46381 to The Miriam Hospital, Brown Medical School (E. M. Kojic); grants AI-27670 and AI-069432 to the University of California, San Diego

Antiviral Research Center (C. A. Benson); and grants AI-27665, AI-069532, and M01RR00096 to the New York University (J. A. Aberg).

APPENDIX

The following persons and institutions participated in the conduct of this trial: Karen Savage and Dana Green: University of Alabama (A5801) GCRC grant M01RR-0032, CTU grant 3U01AI32775-13S3 and AI069452-01. Molly Eaton, MD, and Ericka Patrick: Emory University (A5802) CFAR grant DA 12121. Daniel Lee, MD, and Linda Meixner, RN: University of California, San Diego Antiviral Research Center (A0701) CTU grant AI27670 and AI069432. Jeffery L. Meier and Barbara Ann Wiley: University of Iowa (A1504) CTU grant AI27661. Susan Swindells, MBBS, and Sarah Roth, RN: University of Nebraska Medical Center (A1505) CTU grant AI27661. Franette Hyc, SC, RN, BSN, and Tamar Canaan, RN, BSN: University of Cincinnati (A2401) CTU grant AI-25897 and AI-069513. Karen T. Tashima, MD, and Deborah Perez, RN: The Miriam Hospital (A2951) CTU grant AI46381. Olga Mendez, MD, and Jorge L. Santana, MD: University of Puerto Rico/Adults ACTU (A5401) CTU grant 3 U01 AI034832 and Al69415. Kim Whitely, RN, and Ann Conrad, RN: MetroHealth Medical Center (A2503) CTU grant AI069501. Michael Dubé, MD, and Deborah O'Connor, MSN, APRN-BC: Indiana University Hospital (A2601) grant MO1 RR000750 and CTU grant AI25859-19. Mary Shoemaker, RN, and Christine Hurley, RN: University of Rochester (A1101) CTU grant AI27658 and AI69411 and GCRC grant RR00044. Susan L. Koletar, MD, and Barbara Ehrgott, RN: The Ohio State University (A2301) CTU grant U01 A125924-18 and AI069474. Janet Forcht, RN, and Margie Vasquez, RN: NYU/NYC HHC at Bellevue (A0401) GCRC grant M01RR00096 and CTU grant AI27665 and AI069532-01. Sheila Dunaway, MD, and Beck Royer, PA-C: University of Washington (Seattle) (A1401) CTU grant AI27664. Nathan Thielman, MD, and Martha Silberman, RN: Duke University Medical Center (A1601) CTU grant AI069484. Donna Mildvan, MD, and Manuel Revuelta, MD: Beth Israel Medical Center (A2851) CTU grant AI46370. M. Graham Ray and Beverly Putnam: University of Colorado Health Sciences Center (A6101) GCRC grant RR00051, CTU grant AI32770 and AI069450. Jane Norris, PA-C, and Sylvia Stoudt, RN: Stanford University (A0501) CTU grant AI027666 and A1069556. Diane Havlir, MD, and Jody Lawrence, MD: University of California San Francisco/San Francisco General Hospital (A0801) CTU grant AI069502-01. David Wohl, MD, and David Currin, RN, CCRC: University of North Carolina (A3201) CFAR grant AI50410, CTU grant AI25868 and AI69423-01, GCRC grant RR00046. Timothy Lane, MD, and Kim Epperson: Moses Cone Health System (A3203). Mykyelle Crawford, RN, and Jolene Noel Connor, RN: Columbia University (A7802) grant UO1-AI46386 and AI069470-01. Hector Bolivar, MD, and Lillian Colon, RN, BSN: University of Miami (A0901) grant AI27675 and U01 AI069477. Karen Coleman, RN, and Baiba Berzins, MPH: Northwestern University (A2701) CTU grant AI25915 and AI69471. Marilou Marcelo, BS (University of Hawaii at Manoa), and Scott Souza, PharmD (Queen's Medical Center) (A5201): grant AI34853.

REFERENCES

1. Schmidt HH, Behrens G, Genschel J, et al. Lipid evaluations in HIV-1-positive patients treated with protease inhibitors. Antivir Ther 1999;4:163–170. [PubMed: 12731756]
2. Tsiodras S, Mantzoras C, Hammer S, et al. Effect of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5 year cohort study. Arch Intern Med 2000;160:2050–2056. [PubMed: 10888979]
3. Mooser V, Carr A. Antiretroviral therapy-associated hyperlipidemia in HIV disease. Curr Opin Lipidol 2001;12:313–319. [PubMed: 11353335]
4. Mulligan K, Grunfeld C, Tai VW, et al. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV-1 infection. J Acquir Immune Defic Syndr 2000;23:35–43. [PubMed: 10708054]

5. Sullivan AK, Feher MD, Nelson MR, et al. Marked hypertriglyceridemia associated with ritonavir therapy. AIDS 1998;12:1393–1394. [PubMed: 9708422]
6. Murphy RL, Sanne I, Cahn P, et al. Dose-ranging, randomized, clinical trials of atazanavir with lamivudine and stavudine in antiretroviral naïve subjects: 48-week results. AIDS 2003;17:2603–2614. [PubMed: 14685054]
7. Bonnet F, Bonarek M, deWitte S, et al. Efavirenz-associated severe hyperlipidemia. Clin Infect Dis 2002;35:776–777. [PubMed: 12203183]
8. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs. stavudine in combination therapy in antiretroviral naïve patients: a 3 year randomized trial. JAMA 2004;292:191–201. [PubMed: 15249568]
9. Friis-Möller N, Sabin C, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med 2003;349:1993–2003. [PubMed: 14627784]
10. Bergersen BM, Sandvik L, Bruun JN, et al. Elevated Framingham risk score in HIV-positive subjects on highly active antiretroviral therapy: results from a Norwegian study of 721 subjects. Eur J Clin Microbiol Infect Dis 2004;23:625–630. [PubMed: 15322938]
11. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–219. [PubMed: 8836866]
12. Cullen P. Evidence that triglycerides are an independent coronary artery disease risk factor. Am J Cardiol 2000;86:943–949. [PubMed: 11053704]
13. National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Final report. Circulation 2002;106:3143–3421. [PubMed: 12485966]
14. McBride PE. Triglycerides and risk factors for coronary heart disease: editorial. JAMA 2007;298:236–238.
15. Hunninghake DB. Pharmacologic management of triglycerides. Clin Cardiol 1999;22(Suppl II):44–48.
16. Aberg JA, Zackin RA, Brobst SW, et al. A randomized trial of the efficacy and safety of fenofibrate versus pravastatin in HIV-infected subjects with lipid abnormalities: AIDS Clinical Trials Group Study 5087. AIDS Res Hum Retroviruses 2005;21:757–767. [PubMed: 16218799]
17. Phillipson BE, Rothrock DW, Connor WE, et al. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 1985;312:1210–1216. [PubMed: 3990714]
18. Harris WS. N-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65(Suppl): 1645S–1654S. [PubMed: 9129504]
19. Calabresi L, Donati D, Pazzucconi F, et al. Omacor in familial combined hyperlipidemia: effects on low density lipoprotein subclasses. Atherosclerosis 2000;148:387–396. [PubMed: 10657575]
20. De Truchis P, Kirstetter M, Perier A, et al. VIH Study Group. Reduction in triglyceride levels with N-3 polyunsaturated fatty acids in HIV-infected patients taking potent antiretroviral therapy: a randomized prospective study. J Acquir Immune Defic Syndr 2007;44:278–285. [PubMed: 17179770]for the
21. Wohl DA, Tien H-C, Busby M, et al. Randomized study of the safety and efficacy of fish oil (omega-3 fatty acids) supplementation with dietary and exercise counseling for the treatment of antiretroviral therapy-associated hypertriglyceridemia. Clin Infect Dis 2005;41:1498–1504. [PubMed: 16231263]
22. Schmidt EB, Arnesen H, deCaterina R, et al. Marine n-3 polyunsaturated fatty acids and coronary heart disease. Part I. Background, epidemiology, animal data, effects on risk factors and safety. Thromb Res 2005;115:163–170. [PubMed: 15617737]
23. Bang HO, Dyerberg J. Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. Acta Med Scand 1972;192:85–94. [PubMed: 5052396]
24. Evans SR, Fichtenbaum CJ, Aberg JA. Comparison of direct and indirect measurement of LDL in HIV infected individuals: ACTG 5087. HIV Clin Trials 2007;8:45–52. [PubMed: 17434848]

25. Turner ML, Reed-Walker K, King JR, et al. Simultaneous determination of nine antiretroviral compounds in human plasma using liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2003;784:331–341.
26. Periard D, Telenti A, Sudre P, et al. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. Circulation 1999;100:700–705. [PubMed: 10449690]
27. Stalenhoef AFH, deGraaf J, Wittekoek ME, et al. The effect of concentrated n-3 fatty acids versus gemfibrozil on plasma lipoproteins, low density lipoprotein heterogeneity and oxidizability in patients with hypertriglyceridemia. Atherosclerosis 2000;153:129–138. [PubMed: 11058707]
28. Genest J Jr, Nguyen N-H, Theroux P, et al. Effect of micronized fenofibrate on plasma lipoprotein levels and homeostatic parameters of hypertriglyceridemic patients with low levels of high-density lipoprotein cholesterol in fed and fasted state. J Cardiovasc Pharmacol 2000;35:164–172. [PubMed: 10630748]
29. Miller M. The epidemiology of triglyceride as a coronary artery disease risk factor. Clin Cardiol 1999;22(Suppl II):1–6.
30. Calza L, Manfredi R, Chiodo F. Hyperlipidemia in patients with HIV-1 infection receiving highly active antiretroviral therapy: epidemiology, pathogenesis, clinical course and management. Int J Antimicrob Agents 2003;22:89–99. [PubMed: 12927947]
31. Solages A, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. Clin Infect Dis 2006;42:1325–1332. [PubMed: 16586393]
32. Sackoff JE, Hanna DB, Pfeiffer MR, et al. Underlying causes of death among persons with AIDS in the HAART era in New York City. Ann Intern Med 2006;145:397–406. [PubMed: 16983127]
33. Harris WS, Rothrock DW, Fanning A, et al. Fish oil in hypertriglyceridemia: a dose-response study. Am J Clin Nutr 1990;51:399–406. [PubMed: 2309646]
34. Ashley JM, Lowe NJ, Borok ME, et al. Fish oil supplementation results in decreased hypertriglyceridemia in patients with psoriasis undergoing etretinate or acitretin therapy. J Am Acad Dermatol 1988;19:76–82. [PubMed: 2969924]
35. Staels B, Dallongeville J, Auwerx J, et al. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 1998;98:2088–2093. [PubMed: 9808609]
36. Griffin BA. The effect of n-3 fatty acids on low density lipoprotein subfractions. Lipids 2001;36 (Suppl):S91–S97. [PubMed: 11837999]
37. Durrington PN, Bhatnagar D, Mackness MI, et al. An omega-3 polyunsaturated fatty acid concentrate administered for one year decreased triglycerides in simvastatin treated patients with coronary heart disease and persisting hypertriglyceridaemia. Heart 2001;85:544–548. [PubMed: 11303007]
38. Griffin MD, Sanders TAB, Davies IG, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45–70 y: the OPTILIP Study. Am J Clin Nutr 2006;84:1290–1298. [PubMed: 17158408]
39. Meydani SN, Lichtenstein AM, Cornwall S, et al. Immunologic effects of National Cholesterol Education Panel step-2 diets with and without fish-derived N-3 fatty acid enrichment. J Clin Invest 1993;92:105–113. [PubMed: 8325975]

TABLE 1

Baseline Characteristics of the 2 Treatment Groups

	Treatment A: Fish Oil (Range)	Treatment B: Fenofibrate (Range)
No. subjects	50	50
Age (y)	43.5 (26 to 61)	43 (23 to 58)
Gender	90% male	96% male
Ethnicity	58% white 16% black 26% Hispanic	56% white 12% black 30% Hispanic
CD4 count (cells/μL)	429.5 (114 to 1264)	528.5 (109 to 1111)
HIV RNA level (copies/mL)	<50 (<50 to 1483)	<50 (<50 to 4210)
Screening serum lipids		
TG (mg/dL)	668 (402 to 2980)	667 (401 to 1922)
LDL-C (mg/dL)	85 (31 to 152)	73 (30 to 160)
HDL-C (mg/dL)	31 (21 to 43)	30 (21 to 42)
TC (mg/dL)	243.5 (147 to 457)	240.5 (158 to 447)
Non-HDL-C (mg/dL)	209 (125 to 436)	209.5 (132 to 421)
Medications		
Subjects on lopinavir/ritonavir (any ritonavir-containing regimen)	18 (26)	21 (32)
Subjects on EFV (on NVP)	29 (4)	20 (0)*
Subjects on d4T	6	9
Subjects on TDF	21	14

All values for age, CD4 cell count, HIV RNA level, and serum lipids are medians and ranges.

d4T indicates stavudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

*$P < 0.05$, proportion on EFV or NVP, treatment A versus treatment B.

TABLE 2

Step 1 Median (Range) Fasting Lipid Values (mg/dL)

	Screening*	Week 8	Absolute Change	P†	Percent Change	P†
Fish oil (n = 47)						
TG	665 (402 to 2980)	362 (133 to 1278)	−283 (−2019 to 682)	<0.001	−46% (−87 to 114)	<0.001
LDL-C	86 (31 to 152)	108 (60 to 227)	30 (−48 to 120)	<0.001	37% (−44 to 348)	<0.001
HDL-C	31 (21 to 43)	32 (22 to 51)	1 (−9 to 18)	0.198	4% (−24 to 55)	0.074
TC	246 (147 to 457)	236 (128 to 450)	−15 (−204 to 105)	0.028	−7% (−45 to 42)	0.043
Non-HDL-C	210 (125 to 436)	204 (100 to 414)	−13 (−207 to 87)	0.018	−7% (−47 to 40)	0.026
Fenofibrate (n = 48)						
Serum TG	694.5 (401 to 1922)	338.5 (103 to 1476)	−367 (−1466 to 319)	<0.001	−58% (−90 to 67)	<0.001
LDL-C	73 (30 to 160)	110.5 (63 to 191)	31.5 (−45 to 101)	<0.001	46% (−33 to 233)	<0.001
HDL-C	30 (21 to 42)	33.5 (7 to 53)	4 (−17 to 24)	<0.001	13% (−71 to 110)	<0.001
TC	243 (158 to 447)	211.5 (140 to 321)	−21.5 (−289 to 60)	<0.001	−10% (−65 to 28)	<0.001
Non-HDL-C	210.5 (132 to 421)	178.5 (112 to 314)	−27 (−300 to 57)	<0.001	−13% (−71 to 31)	<0.001

TC indicates total cholesterol.

*Note that screening values differ from Table 1 because not all subjects had week 8 evaluations and the numbers of subjects differ from the baseline assessment.

†Signed rank test.

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2009 December 3.

TABLE 3

Step 2 Median (Range) Fasting Serum Lipid Values (mg/dL) on Combined Fenofibrate and Fish Oil

	Step 2 Entry	Week 18	Absolute Change	*P**	Percent Change	*P**
Combined group overall (n = 67)						
TG	377 (205 to 1476)	279 (97 to 1458)	−108 (−788 to 871)	<0.001	−30% (−78 to 231)	<0.001
LDL-C	106 (60 to 214)	120 (49 to 216)	13 (−64 to 102)	0.002	13% (−45 to 117)	0.001
HDL-C	32 (7 to 53)	33 (16 to 63)	2 (−13 to 27)	0.019	6% (−27 to 129)	0.019
TC	237 (128 to 355)	220 (133 to 370)	−5 (−87 to 59)	0.091	−2% (−33 to 27)	0.135
Non-HDL-C	207 (100 to 314)	184 (98 to 318)	−6 (−90 to 54)	0.034	−4% (−38 to 23)	0.054
Fish oil initiated first in step 1, adding fenofibrate at week 10 (n = 36)						
TG	369.5 (205 to 1278)	280 (97 to 1458)	−92 (−616 to 871)	<0.001	−28% (−71 to 231)	<0.001
LDL-C	101 (60 to 214)	119.5 (49 to 216)	11.5 (−64 to 102)	0.131	11% (−45 to 117)	0.026
HDL-C	30.5 (22 to 51)	32.5 (21 to 63)	2 (−8 to 27)	0.057	8% (−25 to 75)	0.032
TC	241 (128 to 355)	219.5 (133 to 342)	−3 (−75 to 50)	0.247	−1% (−27 to 27)	0.314
Non-HDL-C	211.5 (100 to 307)	183 (102 to 318)	−7 (−69 to 49)	0.081	−4% (−32 to 23)	0.110
Fenofibrate initiated first in step 1, adding fish oil at week 10 (n = 31)						
TG	414 (206 to 1476)	279 (105 to 970)	−117 (−788 to 176)	<0.001	−36% (−78 to 49)	<0.001
LDL-C	116 (70 to 191)	120 (69 to 203)	13 (−29 to 61)	0.003	13% (−26 to 76)	0.004
HDL-C	33 (7 to 53)	34 (16 to 58)	1 (−13 to 10)	0.170	3% (−27 to 129)	0.262
TC	231 (166 to 321)	222 (137 to 370)	−5 (−87 to 59)	0.239	−2% (233 to 19)	0.297
Non-HDL-C	199 (136 to 314)	186 (98 to 312)	−5 (−90 to 54)	0.236	−3% (−38 to 21)	0.302

TC indicates total cholesterol.

*Signed rank test.

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2009 December 3.