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CLINICAL TRIAL/CLINICAL STUDY

Omega-3 Fatty Acids Do Not Improve Endothelial Function In Virologically Suppressed HIV-Infected Men: A Randomized Placebo-Controlled Trial

Corrilynn O. Hileman,[1,2] Teresa L. Carman,[2,3] Norma J. Storer,[2,4] Danielle E. Labbato,[2,4] Cynthia A. White,[2,3] and Grace A. McComsey[2,4]

Abstract

Omega-3 fatty acids decrease cardiovascular disease (CVD) mortality possibly due to antiinflammatory effect. Inflammation and endothelial dysfunction likely play a role in the heightened CVD risk in HIV. Our goal was to evaluate the effect of omega-3 fatty acids primarily on endothelial function and inflammation in HIV-infected adults with moderate CVD risk on stable antiretroviral therapy. We conducted a 24-week, randomized, double-blind, placebo-controlled study to evaluate the effect of omega-3-acid ethyl esters 1 g twice a day. Flow-mediated dilation (FMD) of the brachial artery, lipoproteins and markers of inflammation, endothelial activation, coagulation, and insulin resistance were measured at entry and week 24. There were no within- or between-group differences in change in FMD over 24 weeks (mean change in FMD −0.13% vs. 1.5% for treatment vs. placebo; $p = 0.21$). There were no between-group differences in changes in lipoprotein levels or biomarkers tested, except soluble tumor necrosis factor receptor-I, which favored omega-3-acid ethyl esters. Omega-3 fatty acids did not improve endothelial function or activation, coagulation, or insulin resistance in virologically suppressed, HIV-infected men with moderate CVD risk; however, inflammation tended to improve. This suggests that omega-3 fatty acids may not be potent enough to counteract the enhanced inflammation and endothelial dysfunction due to HIV and antiretrovirals.

Introduction

Management of cardiovascular risk has become an increasingly important aspect of the care of HIV-infected adults due to a rise in proportionate morbidity and mortality attributed to cardiovascular disease (CVD).[1] It has been shown that traditional CVD risk factors,[2] antiretroviral therapy (ART),[3] and perhaps chronic immune activation due to HIV infection[4–8] contribute to the risk of CVD in this population. The complex interplay of these factors has yet to be unraveled; however, the consequences of CVD warrant studying targets to decrease this risk now.

High intake of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been associated with reduced risk of myocardial infarction (MI)[9] and CVD mortality.[10–12] Omega-3 fatty acids inhibit the activation of the proinflammatory nuclear factor-κB pathway[13,14] and reduce circulating proinflammatory cytokines[15,16] and expression of vascular adhesion molecules.[13,17,18] There is mounting evidence to support the role of chronic inflammation, oxidative stress, and endothelial cell dysfunction in the development of plaque and progression of atherosclerosis in the general population.[19] In HIV, we are beginning to understand the contribution of these factors as well.[4–8,20–22] Given that omega-3 fatty acids have been shown to modulate inflammation, we sought to test the efficacy of omega-3 fatty acids in improving endothelial function, activation, inflammation, coagulation, and insulin resistance in virologically suppressed, HIV-infected adults on stable ART with at least moderate CVD risk.

Materials and Methods

Study population and design

We conducted a 24-week, randomized, double-blind, placebo-controlled trial to evaluate the effect of omega-3 fatty

[1]Department of Medicine, Division of Infectious Diseases, MetroHealth Medical Center, Cleveland, Ohio.
[2]Case Western Reserve University, Cleveland, Ohio.
[3]Department of Medicine, University Hospitals Case Medical Center, Cleveland, Ohio.
[4]Departments of Pediatrics and Medicine, University Hospitals Case Medical Center, Cleveland, Ohio.

acids in the form of omega-3-acid ethyl esters (Lovaza; GlaxoSmithKline, Research Triangle, NC), an FDA-approved, lipid-regulating capsule containing at least 900 mg of ethyl esters predominately EPA (approximately 465 mg) and DHA (approximately 365 mg) per package insert. Participants were recruited from the John T. Carey Special Immunology Unit at University Hospitals Case Medical Center (UHCMC), Cleveland, Ohio. Eligible individuals were HIV-1-infected adults with HIV-1 RNA ≤400 copies/ml, receiving stable ART for at least 12 weeks, with a cumulative duration of ART of at least 12 months. Additionally, participants had to have a 10-year Framingham risk score for hard coronary heart disease of at least 6% and a body mass index (BMI) between 19 and 35 kg/m^2. Active infection, inflammatory condition or malignancy, uncontrolled diabetes defined as hemoglobin $A_{1C} \geq 8.5\%$, or having changes made to hypoglycemic medications within 4 weeks of screening, hypothyroidism or hypertension, known CVD or arrhythmia, low-density lipoprotein (LDL) >160 mg/dl, triglyceride level >750 mg/dl, creatinine clearance <50 ml/min, hemoglobin <9.0 g/dl, AST or ALT ≥ 2× the upper limit of normal, pregnancy, lactation, consistent use of antiinflammatory or antioxidant medication, and serious diarrhea or vomiting were exclusionary. Each participant signed an informed written consent prior to study entry. The study protocol and consent were approved by the UHCMC Institutional Review Board.

At entry, participants were randomized 1:1 by an investigational pharmacist to receive (1) omega-3-acid ethyl esters 1 capsule (1000 mg) twice daily or (2) matching placebo. Randomization was stratified by triglyceride level (>250 and ≤250 mg/dl) and by protease inhibitor (PI) use (on a PI and not on a PI). The study medication and placebo where provided by GlaxoSmithKline. Our primary outcome was endothelial function determined by flow-mediated dilation (FMD) of the brachial artery. Secondary outcomes of interest included lipoprotein levels, markers of inflammation, endothelial cell activation and coagulation, and insulin resistance estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). All participants returned at weeks 6 and 12 to complete a symptom questionnaire, pill count, and blood draw for complete blood count and comprehensive metabolic panel.

Evaluation of endothelial function

Endothelial function was evaluated at entry and week 24 by measuring FMD with ultrasound.[23] Participants were instructed to come fasting, to hold antihypertensive medications, and to not use tobacco or caffeine-containing products for 12 h before the study. All studies were performed by a single technologist (C.W.) who was blinded to the treatment status of the participants using a Phillips iU22 Ultrasound and a L10-7 MHz linear array transducer. Participants were placed in a supine position in a temperature-controlled room for 15 min. The right arm was the preferred site for measurement and was used unless not anatomically feasible or if there was a blood pressure discrepancy suggesting arterial disease. A pediatric blood pressure cuff was applied to the forearm. The brachial artery was imaged approximately 4–5 cm above the antecubital fossa. Landmarks were identified to ensure accuracy within and between studies. Images were optimized to visualize near and far walls of the brachial artery focusing on the M-line. Baseline Doppler, cine-loop recording, and images were captured. Next, the cuff was inflated to >200 mm Hg or 50 mm Hg above the systolic pressure. Confirmation of vessel occlusion was documented by duplex. After exactly 5 min of occlusion, the cuff was rapidly deflated and Doppler was obtained within 10 s of release to confirm reactive hyperemia. Then, postrelease cine and still images were captured at 55–65 s following cuff release in the same location as the baseline images. All images were stored in DICOM format and submitted for analysis using JPEG and MPEG formats. Images were read using Brachial Artery Analyzer software (MIA Inc.), a semiautomated, border interfacing program. For FMD determination, brachial artery diameters were measured in triplicate and averaged from a 1 cm segment of the artery. Care was taken to ensure the same segment of the artery was used for all images in all studies of the same participant. The studies were analyzed by a single reader (T.C.) blinded to treatment status of the participants. FMD is expressed as a percent change from baseline brachial artery diameter to brachial artery diameter after reactive hyperemia.

Evaluation of glucose metabolism and biomarkers

Participants had blood drawn after a 12-h fast at entry and week 24. Real time measures of glucose, insulin, and lipoproteins were performed at these two time points. In addition, plasma from each participant was stored at −70°C until the completion of the study. Stored samples were then batched and tested for soluble vascular cell adhesion molecule-1 (VCAM-1), soluble intercellular adhesion molecule-1 (ICAM-1), high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), soluble tumor necrosis factor-α receptors (sTNFR-l and sTNFR-II), D-Dimer and fibrinogen. Soluble vascular cell adhesion molecule-1, ICAM-1, IL-6, and sTNFR-I and -II were determined by quantitative sandwich ELISAs (R&D Systems, Minneapolis, MN). Interassay variability ranged from 4.76–8.77%, 3.43–7.37%, 2.02–15.36%, 3.66–5.77%, and 2.13–3.79%, respectively. High sensitivity CRP and fibrinogen were determined by particle enhanced immunonephelometric assays on a BNII nephelometer (Siemens). Interassay variability ranged from 3.01–6.46% and 3.42–7.59%, respectively. D-Dimer was determined by immunoturbidometric assay on a STA-R Coagulation Analyzer (Diagnostica Stago). Interassay variability ranged from 1.54–9.03%. All biomarker assays were performed at the Laboratory for Clinical Biochemistry Research under the direction of Dr. Russell Tracy, Department of Pathology, University of Vermont. Insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR was calculated using the following formula: HOMA-IR = fasting glucose (mg/dl)× fasting insulin (μU/ml)/405.[24] $CD4^+$ cell counts and HIV-1 RNA levels were determined as part of clinical care. Measures closest to entry and week 24 were used for analysis.

Data analysis

The primary objective was to compare change in FMD over 24 weeks between the treatment and placebo groups. Secondary objectives were to compare changes in lipoprotein levels, markers of endothelial activation, inflammation and coagulation, and HOMA-IR over 24 weeks between groups.

Within-group comparisons were made as well. Because outcomes involved changes from baseline, only those participants with baseline and follow-up studies were used for the analysis of these objectives.

Baseline characteristics and laboratory values are described by mean and standard deviation for continuous variables following a Normal distribution, by median and interquartile range for continuous variables not following a Normal distribution, and by frequency and percent for categorical variables. Changes in FMD and laboratory tests are reported as absolute changes from baseline. Paired *t*-tests and Wilcoxon signed rank tests were used for within-group comparisons as distributionally appropriate. Unpaired *t*-tests and Wilcoxon rank sum tests were used for between-group comparisons as distributionally appropriate. To control for the effect of baseline FMD, triglyceride level, and PI status, multivariable analysis of covariance was performed. All statistical tests were two-sided with a 0.05 significance level. Percent adherence was calculated with the following equation: (Number pills dispensed – number pills returned)/expected number of pills taken × 100%.

In prior studies with omega-3 fatty acids, the treatment effect has ranged from 3–17%[25–27] and, in general, a 2–4% change in FMD is considered clinically relevant. In addition, we have previously shown that 3.5% is a reasonable choice for the standard deviation of this change.[28] Therefore, we based our sample size calculation on the assumption of a 4% change in the treated group and a 0% change in the control group with a 3.5% common standard deviation. Fourteen participants were needed per arm to achieve 80% power to detect this difference in change in FMD over time using an unpaired *t*-test with a 0.05 two-sided significance level. We planned to enroll 16 participants per group in case nonparametric tests were necessary.

All analyses were performed using SAS v. 9.2 (The SAS Institute, Carey, NC).

Results

Thirty-five individuals were enrolled and randomized between April 22, 2009 and September 1, 2009 (18 in the omega-3-acid ethyl esters group and 17 in the placebo group). Follow-up was complete by February 23, 2010.

Baseline characteristics

The two groups were well-balanced with regard to all important demographic, HIV and cardiovascular characteristics at baseline (see Table 1 for baseline characteristics by randomization group). Overall, the median (interquartile range or IQR) age was 51 years (48–53). All participants were men and most were caucasian (15/35 or 43%) or African-American (19/35 or 54%). The mean (standard deviation or SD) known duration of HIV infection and duration on ART were 161 months (78) and 107 months (50), respectively. Forty-three percent (15/35) were on a nonnucleoside reverse transcriptase inhibitor (NNRTI) and 60% (21/35) were on a PI. Seven participants were on abacavir (two in the treatment group and five in the placebo group). Median (IQR) nadir $CD4^+$ cell count and mean (SD) baseline $CD4^+$ cell count were 110 cells/ml^3 (32–172) and 602 cells/ml^3 (277), respectively. The median (IQR) Framingham risk score was 8% (6–10). Most participants were current (74%) or past (9%) smokers. Mean (SD) LDL level was 107 mg/dl (31), median (IQR) HDL level was 38 mg/dl (35–50), and median (IQR) triglyceride level was 126 mg/dl (83–180). Median (IQR) BMI was 25 kg/m^2 (22–28) and median (IQR) HOMA-IR was 1.45 (0.86–2.64). At baseline, 29% (10/35) were on an antihypertensive medication and 20% (7/35) were on an HMG-CoA reductase inhibitor. No participant initiated medications used to treat hypertension or hyperlipidemia during the study and only one participant, in the placebo group, had two antihypertensive medications discontinued during the study.

Table 1. Baseline Characteristics by Group

	Treatment group (n = 18)	*Placebo group (n = 17)*
Demographic characteristics		
Age, years	51 (47-53)	51 (49-54)
Male	18 (100)	17 (100)
Race		
Caucasian	9 (50)	6 (35)
African-American	9 (50)	10 (59)
Hispanic	0 (0)	1 (6)
HIV-related characteristics		
HIV duration, months	179 (71)	140 (83)
Nadir $CD4^+$, cells/mm^3	114 (32–240)	90 (17–171)
Current $CD4^+$, cells/mm^3	604 (270)	599 (293)
ART duration, months	112 (49)	101 (55)
On ART	18 (100)	17 (100)
PI	11 (61)	10 (59)
NNRTI	8 (44)	7 (41)
Cardiovascular risk factors		
Current smokers	14 (78)	12 (71)
On anti-HTN mediation	6 (33)	4 (24)
SBP, mm Hg	119 (110–132)	118 (112–140)
DBP, mm Hg	80 (78–88)	78 (76–82)
On lipid-lowering therapy	3 (17)	4 (24)
Total cholesterol, mg/dl[a]	180 (31)	177 (53)
LDL, mg/dl[a]	108 (29)	106 (34)
HDL, mg/dl[a]	42 (36–58)	37 (34–43)
Cholesterol/HDL ratio[a]	4 (3–5)	4 (4–5)
Triglycerides, mg/dl[a]	122 (75–158)	126 (96–180)
BMI, kg/m^2	25 (22–27)	25 (22–28)
Waist/hip ratio	0.96 (0.07)	0.96 (0.07)
Glucose, mg/ml[a]	81 (74–90)	84 (83–88)
Insulin, μU/ml[a]	6.5 (4–12)	7 (5–12)
HOMA-IR[a]	1.4 (0.8–2.5)	1.5 (0.9–2.6)
Framingham risk, %	7 (7–9)	8 (6–10)

[a]Fasting.

Continuous variables shown as mean (standard deviation) or median (interquartile range) as appropriate; nominal variables shown as frequency (percent).

ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside/nucleotide analogue reverse transcriptase inhibitor; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance.

Adherence

There was no difference between groups with regard to adherence to the study medication. Overall, median (IQR) percent adherence was 97% (91–99) based on pill counts. The study medication was well tolerated and there were no adverse events.

Brachial artery flow-mediated dilation

Of the participants, 31/35 had FMD performed at two time points. The four participants who did not have two studies were all in the treatment group. One participant inadvertently did not get a baseline study and three did not have follow-up studies performed as they prematurely withdrew from the study due to inability to adhere to the study protocol. One developed a non-study-related illness that prevented him from attending follow-up appointments, one moved, and one was not taking the study medication for reasons unrelated to intolerance or adverse events. In all FMD studies performed, baseline triphasic brachial artery waveforms and appropriate hyperemic responses were demonstrated. Table 2 shows the results of entry and week 24 FMD studies. Baseline brachial artery diameters and FMDs were similar in both groups; overall, the mean (SD) baseline FMD was 3.13% (2.86). This result is consistent with prior studies evaluating FMD on ART-treated, HIV-infected adults by our group.[28] The mean (SD) FMD change from baseline at 24 weeks in the treatment group was –0.13% (2.60) ($p=0.85$ within-group) and in the placebo group was 1.47% (4.05) ($p=0.15$ within-group). The changes were not significantly different between groups ($p=0.21$). Adjusting for baseline FMD and triglyceride values and for baseline FMD and PI status did not change these results (adjusted mean differences in FMD were 0.05% vs. 1.32% for treatment vs. placebo; $p=0.28$, and –0.02% vs. 1.38% for treatment vs. placebo; $p=0.21$, respectively).

Changes in endothelial activation, inflammation, and coagulation markers

Endothelial activation, inflammation, and coagulation markers measured at study entry were not significantly different between groups (Table 3). Follow-up markers were available for 32/35 participants. The three participants who withdrew early did not have follow-up markers performed. Changes in the markers from entry to week 24 for both groups are shown in Table 3. Changes were not statistically different between groups except for sTNFR-I [mean (SD) change in sTNFR-I was –83 pg/ml (136) vs. 35 pg/ml (128) for treatment vs. placebo groups, respectively; $p=0.02$]. Within-group changes are noted in Table 3.

Table 2. Ultrasound Measurements at Baseline and After 24 Weeks by Group

	Treatment group (n=14)	*Placebo group (n=17)*	p[a]
Entry			
Brachial artery diameter, mm	4.69 (0.50)	4.68 (0.54)	0.93
FMD, %	3.45 (2.97)	2.87 (2.83)	0.58
Week 24			
Brachial artery diameter, mm	4.63 (0.50)	4.66 (0.52)	0.88
FMD, %	3.32 (3.97)	4.34 (2.60)	0.39
Change			
FMD difference, %	–0.13 (2.60)	1.47 (4.05)	0.21

[a]Between groups.
All values are mean (standard deviation).
FMD, flow-mediated dilation.

Changes in insulin resistance and lipoproteins

Glucose, insulin, and HOMA-IR, LDL, HDL, and triglyceride levels were not statistically different at baseline. Changes in measures of glucose metabolism and lipoproteins are shown in Table 4. Over the 24 week study, glucose, insulin, and HOMA-IR did not change significantly within either group ($p=0.97$ and $p=0.09$ for change in treatment and placebo HOMA-IR, respectively). In addition, the changes in glucose, insulin, and HOMA-IR were not statistically different between groups ($p=0.30$ for HOMA-IR). There were no within-group changes in LDL, HDL, or triglycerides in either group over the study period with the exception of change in HDL in the placebo group ($p=0.01$). However, change in triglycerides neared significance in the treatment group [mean (SD) change in triglycerides was –27.7 mg/dl (58.3); $p=0.09$].

Changes in $CD4^+$ cell count and HIV-1 RNA level

All participants were on stable antiretroviral therapy at enrollment. Over the 24-week study, neither $CD4^+$ cell count nor HIV-1 RNA level changed significantly in either group (for the treatment group, $p=0.38$ for change in $CD4^+$ cell count and $p>0.99$ for change in HIV-1 RNA level; for the placebo group, $p=0.71$ for change in $CD4^+$ cell count and $p=0.38$ for change in HIV-1 RNA level).

Discussion

Few studies have investigated the use of omega-3 fatty acids in HIV-infected patients on ART and all studies reported to date focus on the effect of omega-3 fatty acids on triglyceride levels.[29–34] The primary goal of our study was to evaluate for the first time the effect of omega-3 fatty acids in the form of omega-3-acid ethyl esters on endothelial function and activation, as well as inflammation in virologically suppressed, HIV-infected adults on stable ART with at least moderate cardiovascular risk. We did not select patients based on elevated triglyceride levels because we wanted to focus on the effect of omega-3 fatty acids on endothelial function and inflammation independently of its effect on lipoprotein levels. Indeed, Murphy *et al*. in the SABAR study showed that improvement in lipid profiles by switching to atazanavir from other PI-based regimens did not improve endothelial function as measured by FMD of the brachial artery in virologically suppressed, HIV-infected participants.[35]

At the doses used in this study, omega-3-acid ethyl esters did not improve endothelial function measured by FMD of the brachial artery, endothelial activation measured by VCAM-1 and ICAM-1, coagulation measured by D-Dimer, and fibrinogen or insulin resistance measured by HOMA-IR. However, markers of inflammation did appear to improve with omega-3-acid ethyl esters, although only the change in sTNFR-I reached significance and was different between groups. Our findings are consistent with a study by Thusgaard *et al.* who showed that in HIV-infected participants on ART, omega-3 fatty acids did increase formation of the anti-inflammatory leukotriene B5, but did not have an effect on high sensitivity C-reactive protein (hsCRP) or adhesion molecules, VCAM-1 and ICAM-1.[31]

Studies have shown that ART initiation is likely the best strategy to decrease inflammation in ART-naïve patients.[36] In

Table 3. Biomarkers at Baseline and Changes After 24 Weeks by Group

	Treatment group (n = *18*)	*Placebo group* (n = *17*)	p[a]
Entry			
VCAM-1, ng/ml	662 (546–821)	693 (558–946)	
ICAM-1, ng/ml	313 (128)	297 (159)	
hs-CRP, μg/ml	2.03 (1.17–4.48)	2.24 (1.19–4.14)	
IL-6, pg/ml	3.25 (2.14–3.71)	3.61 (2.57–6.34)	
sTNFR-I, pg/ml	1309 (338)	1502 (426)	
sTNFR-II, pg/ml	2750 (647)	3133 (911)	
D-Dimer, μg/ml	0.22 (0.14–0.28)	0.18 (0.14–0.36)	
Fibrinogen, mg/dl	423 (85)	411 (79)	
Change			
VCAM-1, ng/ml	−54 (−182–5)[b]	−20 (−177–26)	0.24
ICAM-1, ng/ml	−20 (−26–5)	−14 (−50–3)[b]	0.95
hs-CRP, μg/ml	−0.63 (−1.08–1.21)	−0.01 (−1.60–1.22)	0.86
IL-6, pg/ml	−0.04 (−1.24–2.60)	0.06 (−1.36–0.76)	0.76
sTNFR-I, pg/ml	−83 (136)[b]	35 (128)	0.02
sTNFR-II, pg/ml	−149 (−330–126)	−91 (−301–570)	0.33
D-Dimer, μg/ml	0.05 (0.10)	0.03 (0.19)	0.63
Fibrinogen, mg/dl	39 (75)	28 (94)	0.71

[a]Between groups.
[b]$p < 0.05$ within group.
All values are mean (standard deviation) or median (interquartile range) as appropriate.
VCAM-1, soluble vascular cell adhesion molecule-1; ICAM-1, soluble intercellular adhesion molecule-1; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; sTNFR-I, soluble tumor necrosis factor-α receptor I; sTNFR-II, soluble tumor necrosis factor-α receptor II.

those on ART, the levels of inflammation are likely to be considerably lower than ART naïve. Therefore, identifying potent, non-ART antiinflammatory agents for patients on stable ART is an important challenge. In this study, we have shown that omega-3 fatty acids did reduce inflammation in virologically suppressed men on ART; however, the magnitude of this decrease is small. We speculate that this is why an effect was not seen in endothelial dysfunction or activation, coagulation, or insulin resistance. Perhaps the dose that was chosen in this study (2 g daily) was too low to see an effect. Prior studies have been conflicting with regard to the dose of omega-3 fatty acids required to see a cardiovascular effect, although in some populations, doses <2 g per day have been effective.[27,37] For example, in healthy volunteers, 1 g per day of omega-3 fatty acids for 14 days improved endothelium-dependent vasodilatation by a mean (SD) of 20.4% (13.2%), which was statistically significant ($p = 0.036$).[27] In addition, we chose this form of omega-3 fatty acids due to the high content of DHA and EPA. In 2 g of omega-3-acid ethyl esters, there are approximately 1680 mg of DHA and EPA combined per package insert.

The strength of this study is the randomized, double-blind, placebo-controlled design; however, limitations include the small sample size and use of pill counts to monitor adherence. We did intend to have 16 participants in each group and fell short of this in the treatment group with regard to our main study endpoint (FMD) due to premature study discontinuation by three participants. However, given the apparent effect of this dose of omega-3-acid ethyl esters, having two additional participants would not likely have changed the outcome. Also, the sample size selected for this study was such that a small effect may have been missed. Although detecting a small effect may have been meaningful with regard to answering questions about the pathogenesis of the outcomes under study in this population, the clinical use of omega-3-acid ethyl esters for this indication would have been

Table 4. Changes in Glucose Metabolism and Lipoproteins over 24 Weeks by Group

	Treatment group	*Placebo group*	p[a]
Glucose, mg/ml	1.7 (13.0)	3.1 (9.2)	0.73
Insulin, μU/ml	0.07(6.41)	1.53 (5.39)	0.49
HOMA-IR	0.02 (1.61)	0.56 (1.28)	0.30
Total cholesterol, mg/dl	0.93 (24.88)	4.76 (21.20)	0.64
LDL, mg/dl	−4.5 (−14–5)	−3 (−12.5–8.5)	0.85
HDL, mg/dl	1.07 (5.55)	4.19 (6.34)[b]	0.17
Triglycerides, mg/dl	−27.73 (58.30)	1.59 (85.52)	0.27

[a]Between groups.
[b]$p < 0.05$ within group.
All values are mean (standard deviation) or median (interquartile range) as appropriate.
All tests fasting.
HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

questionable. Second, given the negative results of this study, demonstrating adherence with the study medication is of utmost importance. There are known limitations in measuring adherence by pill counts and this study is without exception. However, we chose to include only individuals with undetectable levels of HIV-1 RNA who were known to be adherent to their antiretrovirals over a long duration in order to increase the chances of obtaining a high level of adherence to the study drugs. Also, a trend toward improvement in triglyceride levels was seen in the treatment group [mean (SD) change in triglycerides was −27.7 mg/dl (58.3); $p = 0.09$], which is a known effect of omega-3 fatty acids in this population. This trend was not seen in the placebo group [mean (SD) change in triglycerides was 1.6 mg/dl (85.6); $p = 0.94$]. Given this, we are confident that the participants did adhere to the study medication.

In conclusion, omega-3 fatty acids in the form of omega-3-acid ethyl esters did not improve endothelial function or activation, coagulation, or insulin resistance in virologically suppressed, HIV-infected men on stable ART with moderate CVD risk and normal triglyceride levels. Inflammation did appear to improve modestly; however, sTNFR-I was the only marker that decreased significantly over the study period and was different between groups. This suggests that omega-3 fatty acids may not be potent enough to counteract the heightened inflammation and endothelial dysfunction due to HIV and antiretrovirals. Well-designed studies utilizing other more potent antiinflammatory medications such as HMG-CoA reductase inhibitors, anti-TNF-α agents, or higher doses of omega-3 fatty acids are needed in HIV-infected, virologically suppressed adults on ART.

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The trial is registered at www.clinicaltrials.gov; NCT01001767

Author Disclosure Statement

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Address correspondence to:
Grace A. McComsey
Department of Pediatrics and Medicine
Case Western Reserve University
11100 Euclid Avenue
Cleveland, Ohio 44106

E-mail: grace.mccomsey@case.edu