AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 30, Number 8, 2014

DOI: 10.1089/aid.2014.0005

Short Communication: Effects of Omega-3 Fatty Acids on Triglycerides and High-Density Lipoprotein Subprofiles in HIV-Infected Persons with Hypertriglyceridemia

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Abstract

Hypertriglyceridemia and low high-density lipoprotein (HDL)-cholesterol (HDL-C) may contribute to a presumed accelerated risk for cardiovascular disease in HIV-infected individuals. We evaluated the effect of omega-3 fatty acid treatment on triglycerides, low-density lipoprotein (LDL)-C, HDL-C, and HDL subpopulations. Forty-one HIV-seropositive subjects with hypertriglyceridemia (≥ 150 mg/dl) on active antiretroviral therapy were enrolled in this placebo-controlled, double-blind, randomized, crossover trial comparing the effects of omega-3 fatty acid treatment (1.9 g EPA and 1.5 g DHA) on triglycerides, LDL-C, HDL-C, and HDL subpopulations. An independent sample *t*-test was used to assess the study start to posttreatment change for all components. After omega-3 fatty acid treatment, triglyceride levels decreased 63.2 ± 86.9 mg/dl ($p < 0.001$). No significant changes in total cholesterol, LDL-C, or HDL-C were found. Within HDL subpopulations, significant changes were seen in the most atheroprotective HDL particles, α-1, which increased by 2.5 ± 5.6 mg/dl ($p < 0.05$), and preα-1, which increased by 0.6 ± 1.0 mg/dl ($p < 0.001$). Preα-3, a presumably atherogenic HDL particle, decreased by 0.5 ± 0.9 mg/dl ($p < 0.01$). Omega-3 fatty acid treatment significantly lowered triglyceride levels in HIV-positive patients with moderate hypertriglyceridemia. While no study-wide improvements in LDL-C or HDL-C were detected, the HDL subpopulation profile changed in a beneficial way suggesting more cardioprotection after treatment.

Hypertriglyceridemia and low high-density lipoprotein-cholesterol (HDL-C) are the most common lipid abnormalities in HIV-infected individuals.[1] High triglyceride levels result in higher cholesteryl ester transfer protein (CETP) activity, which in turn results in increased levels of atherogenic, small, dense low-density lipoprotein (LDL) particles and decreased levels of the more atheroprotective, large high-density lipoprotein (HDL) particles.[2] Therefore, logically, these abnormalities potentially accelerate the risk for cardiovascular disease in the HIV population.[3,4] Hence, HIV clinicians have become involved in evaluating and treating dyslipidemia alongside HIV treatment. Omega-3 fatty acids have been shown to decrease triglyceride levels in HIV-infected patients with wasting and hypertriglyceridemia,[5] and are often included as part of dyslipidemia management.[1] However, their effects on HDL-C are not evident.[6–10] Over the past decade, research has shown that HDL-C is a dynamic entity with multiple components.[2,11] Some components, α-3 and preα-3, can be associated with atherogenesis and yet others, α-1 and preα-1, with a cardioprotective effect.[12,13] Additionally, certain lipid-lowering agents have been shown to alter these subtypes and presumably improve the cardioprotective nature of HDL-C.[14–16] Most of these studies have been conducted in the non-HIV-infected population. The effects of lipid-lowering treatment on HDL subtypes in the HIV population are still largely unknown. Only now are we beginning to describe the HDL subtype profile in HIV-infected patients, whether antiretroviral treatment (ART) naive or experienced. The primary objective of this study was to revalidate the effect of omega-3 fatty acid treatment on serum triglycerides in HIV-infected people. Secondarily, we also explored its effects on the HDL subtypes within this population.

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HIV-infected adults (18 years or older) with hypertriglyceridemia (fasting triglycerides ≥ 150 mg/dl) were recruited for this study. Recruitment methods included direct mail to participants in other HIV studies in our unit, craigslist, newspaper advertisements, and HIV community program outreach. Participants were required to be on a stable regimen of highly active antiretroviral therapy (HAART) for the previous 3 months and have plasma HIV-1 RNA less than 10,000 copies/ml. Participants were permitted to be on lipid-lowering medications provided they had been on a stable regimen for at least 8 weeks. All participants needed to anticipate no changes to their HAART regimen or lipid-lowering medication (if any) for the duration of the study, and female participants of reproductive age had to consent to using birth control, unless surgically sterilized or postmenopausal. Exclusion criteria included pregnancy, lactation, and a history of diabetes mellitus or atherosclerotic disease.

This was a randomized, placebo-controlled, double-blind, crossover study with 12-week treatment periods and a 4-week washout period. Treatment was provided as four omega-3 fatty acid (Lovaza) capsules daily, dosed as two capsules twice a day. Each capsule contained 1 g of omega-3 fatty acids with approximately 465 mg of eicosapentaenoic acid (EPA) and 375 mg of docosahexaenoic acid (DHA). Lovaza contains standardized, higher concentrations of EPA and DHA than over-the-counter formulations. The placebo was identical looking pills that contained corn oil (linoleic and oleic acids), which was considered appropriate for the placebo. Corn oil can be considered desirable for lipid abnormalities, as it may lower LDL and triglycerides and raise HDL when substituted for a similar amount of carbohydrate. Its use permitted the accentuation of the difference between this oil and omega-3 fatty acid if the hypothesis was true. Block randomization was done using a custom designed website, www.randomization.com, and only the pharmacy dispensation team knew the group assignments. Group ''A'' received omega-3 fatty acid treatment first and placebo last, whereas Group ''B'' received placebo first and omega-3 fatty acid treatment last. All capsules were provided by GlaxoSmithKline (Research Triangle Park, NC). This study was approved by the Ethics Review Committee at Tufts Medical Center/Tufts University School of Medicine.

Total cholesterol, HDL-C, and triglycerides were measured by the Beckman DXC800 (Beckman Coulter Inc, Fullerton, CA) in the Tufts Medical Center Clinical Laboratory. LDL-C was calculated by using the Friedewald equation unless triglycerides were over 400 mg/dl, in which case LDL was directly measured. Total plasma apoA-I concentrations were measured by automated immune-turbidimetric assay (Wako Diagnostics, Richmond, VA). Two-dimensional nondenaturing gel electrophoresis, immunoblotting, and image analysis were carried out on plasma previously stored at -80°C for determining the apoA-I-containing HDL subpopulations, as described.[17,18] The interassay and intraassay coefficients of variation were $< 10\%$ for the HDL subpopulation determinates. Individual HDL subpopulation apoA-I levels were calculated by multiplying plasma apoA-I levels by the subpopulation percentiles. $CD4^+$ cell counts were determined by flow cytometry. HIV RNA levels were quantified at the same time point using Roche Amplicor Version 1.5 (Roche Inc., Indianapolis, IN; limit of detection, 75 copies/ml).

Sample size was based on a power calculation with change in triglycerides as the primary outcome. Forty subjects (20 per group) were estimated to provide 80% power to detect a difference of 30% change in serum triglyceride levels pretreatment and posttreatment.[7] Serum triglycerides measured at both screening and baseline were averaged before analysis. Baseline demographic clinical and laboratory values were compared using Wilcoxon rank sums for continuous variables and Chi-square or Fisher's exact test for categorical variables. For serum lipids and HDL subpopulations, we assessed posttreatment change from study start for significance from zero using the independent *t*-test of treatment arms for Groups A and B combined, after confirming the normality of the sample distribution. Due to an inadequate washout period and sustained triglyceride improvements, we excluded Group A, which received placebo last, from independent *t*-test for the change between postplacebo from study start. Alpha was assumed to be 0.05. This study was analyzed using SAS system for Windows, Version 9.2 (SAS Institute, Cary, NC).

Baseline characteristics between the two treatment arms did not differ significantly (Table 1). The median age of the study population was 52 years (IQR 45.0, 58.0), and the median number of years with HIV was 17 (IQR 13, 20). Median body mass index (BMI) was 24.5 (IQR 21.8, 28.8). While half (51%) of the 35 men and six women had reported a previous diagnosis of AIDS at the time of recruitment, almost all (93%) had undetectable HIV viral load copies. Baseline lipid profiles were not significantly different with median triglyceride levels of 222 mg/dl (IQR 187, 304), LDL of 107 mg/dl (IQR 95, 136), and HDL of 36 mg/dl (IQR 30, 45). All participants were on stable antiretroviral therapy and roughly a third (32%) were on lipid-lowering medications. Thirty-six patients completed the protocol. Of the five patients who did not, one began treatment for alcoholism, one felt ''unwell'' without further elaboration, one stated that they were too busy, one quit due to ''heartburn,'' and, finally, one was lost to follow-up. Compliance with and tolerance of Lovaza omega-3 fatty acid treatment were excellent, similar to that of over-the-counter omega-3 fatty acid supplements, despite the higher concentrations of EPA and DHA. Possible drug-related effects were grade 1 or 2 adverse effects, and included loose stools, flatulence, hiccups, and eructation.

Changes from baseline to posttreatment were tested for significance (different from zero). Triglyceride levels were improved with omega-3 fatty acid treatment overall (Table 2). The 12-week treatment period reduced triglycerides by an average of 63 ± 87 mg/dl, $p < 0.001$ (23.5%). Total cholesterol, HDL-C, and LDL-C showed no significant change from study start to posttreatment. There was no significant change in the lipid profile postplacebo. As above, only the group receiving treatment second was included in the postplacebo analysis, as the group receiving treatment first exhibited prolonged low triglyceride levels through the placebo period, possibly due to long-term effects of omega-3 fatty acid treatment even after its suspension.

Of the HDL subpopulation profile, α-1, preα-1, and preα-3 show statistically significant changes posttreatment, by an average of 1.9%, 0.5%, and -0.4%, respectively. Alpha-1 and preα-1, considered antiatherogenic particles, increased by 2.5 ± 5.6 mg/dl ($p < 0.05$) and 0.6 ± 1.0 mg/dl ($p < 0.001$), respectively. Preα-3, a precursor to α-3, an atherogenic particle,

Table 1. Demographic, Clinical, and Laboratory Values of Groups at Baseline

		At randomization/baseline [median (25th, 75th quartiles) or n *(%)]*		
	Overall (n = *41*)	*Group A* (n = *20*)	*Group B* (n = *21*)	p-*value*
Demographic				
Age (years)	52.0 (45.0, 58.0)	51.5 (46.5, 55.0)	55.0 (44.0, 58.0)	0.70
Male, *N* (%)	35 (85)	17 (85)	18 (86)	0.95
Ethnicity				
Other (Hispanic/Asian/AmIndian)	5 (12)	1 (5)	4 (19)	0.39
African American	13 (32)	7 (35)	6 (29)	
White	23 (56)	12 (60)	11 (52)	
Clinical				
Weight (kg)	76.9 (67.5, 91.1)	81.5 (68.9, 88.5)	76.4 (66.7, 97.0)	0.75
BMI (kg/m^2)	24.5 (21.8, 28.8)	25.1 (22.6, 28.0)	23.9 (21.0, 30.9)	0.81
Waist circumference (cm)	96.4 (82.9, 104.9)	95.2 (81.5, 102.5)	96.4 (83.4, 104.9)	0.95
Systolic blood pressure (mm Hg)	122 (110, 137)	126 (114, 136)	120 (108, 137)	0.47
Diastolic blood pressure (mm Hg)	79 (72, 84)	81 (74, 86)	76 (69, 84)	0.17
Lipid-lowering medication (Y/N)	13 (32)	4 (20)	9 (43)	0.12
Blood pressure medication (Y/N)	15 (37)	7 (35)	8 (38)	0.84
Years with HIV	17 (13, 20)	16 (12, 21)	18 (13, 20)	0.43
AIDS (Y/N)	21 (51)	11 (55)	10 (48)	0.64
ART use				
PI	21 (51)	8 (40)	13 (62)	0.24*
NNRTI	13 (32)	11 (55)	2 (10)	
PI and NNRTI	6 (15)	1 (5)	5 (24)	
Other[a]	1 (2)	0 (0)	1 (5)	
Exercise (1 + times/week)	30 (73)	16 (80)	14 (67)	0.34
Cigarette smoker	14 (34)	8 (40)	6 (29)	0.44
Laboratory				
CD4 count (cells/ml)	553 (455, 829)	574 (456, 811)	549 (432, 900)	0.87
ALT (U/liter)[b]	28 (24, 44)	28 (24, 38)	29 (23, 45)	0.65
AST (U/liter)[b]	30 (24, 44)	40 (28, 55)	29 (24, 32)	0.12
Viral load < 75	38 (93)	19 (95)	19 (90)	0.99
HDL (mg/dl)	36 (30, 45)	38 (30, 46)	35 (30,45)	0.84
HDL < 40 men < 50 women, *n* (%)	28 (68)	14 (70)	14 (67)	0.82
LDL (mg/dl)	107 (95, 136)	111 (96, 137)	105 (86, 134)	0.40
Total cholesterol	197 (175, 222)	202 (172, 224)	194 (175, 213)	0.67
Triglycerides (mg/dl)	222 (187, 304)	214 (169, 248)	261 (190, 320)	0.11

[a]Four NRTIs.
[b]ALT and AST began in mid-January 2009, and so are missing for five Group A baselines and for five Group B baselines.
p-value: Wilcoxon rank sums for continuous variables and Chi-square or Fisher's exact test (*) for categorical variables.
BMI, body mass index; ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

decreased by -0.5 ± 0.9 mg/dl ($p < 0.01$). There was no difference from baseline seen among other HDL subpopulations, specifically α-2, α-3, α-4, preα-2, preα-4, preβ-1, preβ-2, and total apoA-1 posttreatment. There was no significant change in the HDL subparticle profile postplacebo.

Further analysis was done on a subset of patients with undetectable viral loads, as ongoing viral replication can result in changes in lipid metabolism. Ninety-three percent of patients were undetectable, and repeating all the analyses on these patients yielded findings similar to that of the entire cohort. For HDL subtypes, the analysis was repeated, excluding patients on established lipid-lowering drugs, due to their possible effect on subtypes, and those 25 patients showed similar, nonsignificant, changes in HDL subtypes.

In the present study, HIV-infected individuals had a significant posttreatment decrease in triglycerides of 23.5%. This is not unexpected as unequivocal decreases in triglycerides have been observed with omega-3 fatty acid treatment previously.[7,9,10,19–21] Generally, studies have found that higher baseline triglyceride levels yielded proportionally greater improvements with intervention.[20] This trend is also reflected in studies specifically addressing HIV-infected patients. In HIV-infected patients triglyceride levels in the mid-400 range showed a 25% decrease with 2–4 g of EPA and DHA combined daily,[7,10] while those with levels below 200 mg/dl showed only about a 10% reduction.[6] Despite this general trend, our study population achieved a much greater proportional decrease in triglycerides with the intervention than would have been expected given their baseline triglyceride levels. Our cohort achieved triglyceride levels analogous to the HIV-negative population, with just a 4 g dose of omega-3 fatty acid treatment.[20]

Our study showed no statistically significant change in LDL-C pretreatment and posttreatment. Our findings were confirmatory of studies that showed that LDL-C did not change with n-3 polyunsaturated fatty acids in HIV-infected

Table 2. Lipids and High Density Lipoprotein Subpopulations (mg/dl and % Total ApoA-1) Pre- and Post-Omega-3 Fatty Acid Treatment

	Study start n = *41* *[mean (SD)]*	*Posttreatment Groups A and B*[a] n = *36* *[mean (SD)]*	*Posttreatment change from start Groups A and B*[a] n = *36* *[mean (SD)]*	*Postplacebo Group B only*[a] n = *19* *[mean (SD)]*	*Postplacebo change from start Group B only*[a] n = *19* *[mean (SD)]*
Triglycerides (mg/dl)[b]	269 ± 145	213 ± 134	−63.2 ± 86.9***	335 ± 314	29 ± 174
Total cholesterol (mg/dl)	196 ± 37	195 ± 38	−1.8 ± 28.2	205 ± 49	5 ± 42
HDL-cholesterol (mg/dl)	40 ± 17	39 ± 12	−0.1 ± 6.6	36 ± 10	−2.9 ± 7.7
LDL-cholesterol (mg/dl)	109 ± 37	116 ± 36	6.0 ± 23.3	113 ± 40	5.6 ± 33.1
	Study start[c] n = *35*	*Posttreatment Groups A and B* n = *35*	*Posttreatment change from start Groups A and B* n = *35*	*Postplacebo Group B only* n = *18*	*Postplacebo change from start Group B only* n = *18*
HDL subparticles mg/dl					
α-1	17.4 ± 9.8	19.9 ± 12.2	2.5 ± 5.6*	19.0 ± 9.7	−1.2 ± 4.5
α-2	40.4 ± 10.5	38.8 ± 8.2	−1.6 ± 7.9	37.9 ± 5.6	−0.6 ± 7.6
α-3	23.9 ± 4.9	23.0 ± 5.3	−0.9 ± 6.0	21.4 ± 4.8	1.1 ± 6.0
α-4	9.7 ± 3.9	10.5 ± 3.0	0.8 ± 4.0	10.4 ± 2.6	0.0 ± 2.8
Preα-1	2.4 ± 1.5	3.0 ± 1.8	0.6 ± 1.0***	3.2 ± 1.9	−0.2 ± 0.6
Preα-2	3.3 ± 1.2	3.1 ± 1.2	−0.2 ± 1.2	3.1 ± 1.1	0.0 ± 0.8
Preα-3	2.2 ± 0.9	1.8 ± 0.7	−0.5 ± 0.9**	1.8 ± 0.7	−0.4 ± 0.9
Preα-4	1.0 ± 0.5	1.0 ± 0.5	−0.0 ± 0.6	1.1 ± 0.5	−0.2 ± 0.4
Pre*β*-1	14.9 ± 5.4	13.5 ± 5.6	−1.4 ± 5.6	14.0 ± 4.7	0.7 ± 6.7
Pre*β*-2	6.0 ± 2.6	6.1 ± 3.3	0.2 ± 2.1	6.2 ± 3.9	0.1 ± 1.5
Total apoA-1	121.2 ± 27.4	120.6 ± 26.4	−0.5 ± 12.4	118.1 ± 17.5	−0.6 ± 11.9
HDL subparticles %					
α-1	13.6 ± 5.1	15.6 ± 6.4	1.9 ± 4.4*	15.6 ± 6.3	−0.7 ± 3.5
α-2	33.2 ± 4.1	32.5 ± 4.7	−0.8 ± 5.2	32.5 ± 4.7	−0.0 ± 4.8
α-3	20.5 ± 5.3	19.7 ± 5.7	−0.8 ± 4.5	18.3 ± 3.7	0.7 ± 4.2
α-4	8.0 ± 2.5	8.8 ± 2.2	0.8 ± 3.0	8.9 ± 2.1	−0.1 ± 2.4
Preα-1	1.9 ± 1.0	2.3 ± 1.2	0.5 ± 0.9**	2.6 ± 1.4	−0.1 ± 0.4
Preα-2	2.7 ± 0.8	2.6 ± 0.9	−0.2 ± 1.1	2.6 ± 0.8	0.0 ± 0.8
Preα-3	1.9 ± 0.8	1.5 ± 0.5	−0.4 ± 0.8**	1.5 ± 0.5	−0.4 ± 0.8
Preα-4	0.9 ± 0.4	0.8 ± 0.4	−0.0 ± 0.5	0.9 ± 0.4	−0.2 ± 0.4
Pre*β*-1	12.2 ± 3.2	11.0 ± 3.7	−1.2 ± 4.3	11.9 ± 3.5	0.5 ± 4.7
Pre*β*-2	5.1 ± 2.7	5.2 ± 2.5	0.1 ± 1.9	5.2 ± 3.0	0.1 ± 1.5

[a]Group A received 12w treatment, 4w washout, 12w placebo, and is excluded from placebo analysis due to an insufficient washout period. Group B received 12w placebo, 4w washout, 12w treatment.
[b]Average of screening and baseline for study start.
[c]HDL subpopulations not done for baseline only visits; $n = 35$ overall, $n = 17$ for Group A, $n = 18$ for Group B.
*Change is significantly different from zero, $p < 0.05$ by *t*-test.
**Change is significantly different from zero, $p < 0.01$ by *t*-test.
***Change is significantly different from zero, $p < 0.001$ by *t*-test.

individuals on antiretroviral therapy, regardless of the dose.[6,9]

In terms of HDL-C and its component apoA-1, there was no statistically significant change post-omega-3 fatty acid treatment. Baseline HDL-C levels in our cohort are similar to previously studied HIV-infected populations, whether HAART naive or on protease inhibitor (PI) treatment.[22] Several studies examining the effects of omega-3 fatty acids on HDL-C report no differences or small, nonsignificant increases in HDL-C levels with omega-3 fatty acid supplementation.[6–8,10]

Our study did show significant changes in the HDL subpopulation profile, more specifically, significant increases in α-1 and preα-1 and a decrease in preα-3 levels after omega-3 fatty acid therapy. This study, to our knowledge, is the first to use omega-3 fatty acid treatment in HIV-infected persons to determine the effects on HDL subpopulations. Whether this translates to a definite clinical benefit by lowering cardiovascular disease (CVD) events is yet to be determined. Studies have characterized certain HDL subtypes as being atherogenic or antiatherogenic.[12,13,16,23] HIV-negative subjects with either a history of or new CVD events were observed to have higher pre*β*-1 and α-3 levels and lower α-1 and α-2 levels at baseline.[12,13,22] Moreover, the increase of α-1 HDL level was significantly associated with a decrease in progression of coronary artery stenosis measured by angiography.[12] In HIV-infected, HAART-naive individuals without CVD, HDL subpopulation profiles resembled that of non-HIV persons with CVD, showing decreased α-1, α-2, preα-1, and preα-2 levels.[22] After PI therapy, in addition to the previous derangements, the profile worsened to include an increase in the pre*β*-1 level.[22] By improving the HDL

subpopulation profile as seen in our trial, omega-3 treatment may help decrease the risk of cardiovascular disease. This beneficial effect is likely caused by the indirect effects of the decrease in triglycerides. With high triglyceride levels, CETP activity causes HDL to become enriched in triglycerides, forming a good substrate for hepatic lipases.[24] When hepatic lipases hydrolyze the triglyceride-enriched HDL particle, the breakdown results in increased levels of small preβ-1 and decreased large α-1.[24] The same is reflected in the HIV-infected population, where increases in triglycerides lead to increased atherogenic preβ-1 and decreased atheroprotective α-1.[22] As an aside, although not directly related to our study, recent trials in CETP inhibition, which resulted in increased HDL levels, did not translate into a decrease in cardiovascular risk.[25] The reason for this is that CETP inhibitors worsen HDL functionality and minimize any improvement in cardiovascular risk.[25]

The percent change seen in any HDL subtype post-intervention, despite reaching statistical significance, was more modest than in previous trials that involved non-HIV populations treated with statins, fibrates, or niacin, alone or in combination.[14–16] Although other lipid-lowering agents may have more of an impact on HDL subtypes, our more modest change could be due to the higher baseline antiatherogenic α-1 and α-2 and lower atherogenic α-3 levels in our cohort compared to other HAART-naive and PI-treated HIV-infected individuals.[22] In fact, our cohort more closely resembled values of healthy subjects.[22] The presumably favorable baseline in our cohort may be the result of including subjects on well-established lipid-lowering therapy, leaving little room for improvement with omega-3 fatty acid treatment. Other possibilities are less advanced HIV disease, or more lipid friendly (NNRTI), non-PI-based HAART regimens in our cohort, which could have modified HDL subpopulations to reflect a less atherogenic baseline.

There were a number of limitations within our study. First, more research is required to validate our findings, especially in terms of HDL subtypes, as we had low numbers of participants and multiple comparisons. Second, we lacked HIV-negative controls, and could not note differences in the effects of omega-3 fatty acid treatment on lipids between the two groups. Third, we were unable to compare the posttreatment versus true postplacebo effects for half the group as the 4-week washout period between intervention and placebo was likely too short, resulting in the second placebo group having lower than anticipated triglyceride levels at the start of the placebo period. This alludes to a possible prolonged treatment effect of omega-3 fatty acids, and will likely need further characterization in future research. Finally, it could be argued that 32% of our cohort was on established lipid-lowering therapy, which may have blunted the degree of improvement seen in HDL subtypes. However, analysis conducted on those not using lipid-lowering drugs yielded results similar to that of the larger cohort.

In conclusion, omega-3 fatty acid treatment (1.9 g EPA, 1.5 g DHA) over 12 weeks significantly lowered triglycerides in HIV-positive patients with moderate hypertriglyceridemia ($\geq$ 150 mg/dl). Neither LDL-C nor HDL-C changed. However, certain HDL subpopulations, particularly α-1, were significantly improved, suggesting a cardioprotective benefit of omega-3 fatty acid treatment.

Acknowledgments

This study was supported by the Lifespan/Tufts/Brown Center for AIDS Research, NIH/NHLBI 5R01 HL065947-10, NIH 5T32 A1007438-19, and CFAR grant 1 P30 AI42853-12. Data collection, statistical analysis plan, and analyses were designed and performed by the Tufts University School of Medicine. The article was prepared by Dr. Wanke and the study team members.

The trial was registered at clinicaltrials.gov as NCT00795717.

Author Disclosure Statement

This study was supported by GlaxoSmithKline Pharmaceuticals (Research Triangle Park, NC). GlaxoSmithKline was not involved in the design of the study.

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