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Dose Response Effects of Marine Omega-3 Fatty Acids on Apolipoproteins, Apolipoprotein-Defined Lipoprotein Subclasses, and Lp-PLA₂ in Individuals with Moderate Hypertriglyceridemia

Ann C. Skulas-Ray, PhD^{1,*}, Petar Alaupovic, PhD², Penny M. Kris-Etherton, PhD, RD¹, and Sheila G. West, PhD^{1,3}

¹The Pennsylvania State University, Department of Nutritional Sciences, University Park, PA, USA

²Lipid and Lipoprotein Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

³Department of Biobehavioral Health, University Park, PA, USA

Abstract

Background—Apolipoprotein (apo) distribution and lipoprotein (Lp)-associated markers of inflammation, such as lipoprotein-associated phospholipase A₂ (Lp-PLA₂), influence the atherogenicity of circulating lipids and lipoproteins. Little evidence exists regarding the dose-response effects of the marine omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids on apos, apo-defined Lps, and Lp-PLA₂.

Objective—The purpose of this study was to compare the effects of 0 g/d, 0.85 g/d and 3.4 g/d of EPA + DHA on Lp-PLA₂ mass and activity in individuals with moderate hypertriglyceridemia. We also measured effects on concentrations of apo AI, AII, B, C, D, and E-defined Lp subclasses.

Methods—The study was a randomized, double-blind crossover design with 8-week treatment periods and 6-week washout periods. During the 3 treatment periods, subjects (n = 25) received 0 g/d EPA + DHA, 0.85 g/d EPA + DHA (low dose), and 3.4 g/d EPA + DHA (high dose) in random order.

Results—Apo B and apo C-III were significantly decreased by the high dose relative to placebo and low dose (p < 0.01), as was very low density lipoprotein-cholesterol (VLDL-C, p < 0.005). The low dose had no effect on Lp outcomes compared to placebo. The high and low dose effects differed significantly for heparin-precipitated apo C-III, LpB, LpA-I, and apo B/apo A-I ratio (p < 0.05). There was a trend for a decreased Lp-PLA₂ mass with the high dose (p = 0.1).

*To whom manuscript correspondence should be addressed: 110 Chandlee Lab, University Park, PA 16802, aus164@psu.edu, Phone: 814-863-8622, Fax: 814-863-6103.

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Conclusion—The effects of 3.4 g/d EPA + DHA on apo B and apo C-III may reduce atherosclerotic plaque progression in individuals with elevated triglycerides.

Keywords

apolipoproteins; lipoproteins; Lp-PLA₂; omega-3; docosahexaenoic acid; eicosapentaenoic acid; triglycerides; fish oil

Introduction

Triglyceride (TG) concentrations > 150 mg/dL are a risk factor for coronary heart disease (CHD)¹⁻³ and treatment with omega-3 fatty acids (> 3 g/d) is an established intervention for reducing TG. Few studies have evaluated the effects of omega-3 fatty acids on other co-existing CHD risk factors, such as the atherogenic lipoprotein (Lp) and apolipoprotein (apo) profile, as well as vascular inflammation, all of which may drive the progression of atherosclerotic plaque. There is concern, however, that high dose omega-3 fatty acids—specifically those containing docosahexaenoic acid (DHA)—increase low density lipoprotein-cholesterol (LDL-C), the primary target for reducing atherogenic risk⁴. Thus, a more comprehensive assessment of the effects of omega 3 fatty acids on apos and other Lp-associated markers, such as Lp-associated phospholipase A₂ (Lp-PLA₂) is needed, and would aid in determining how to balance any potential risks of high dose omega 3's with their benefits in reducing triglycerides and sudden cardiac death⁵.

Historically, CHD risk management focused primarily on LDL-C targets; recently, greater emphasis has been placed on non-HDL and apo B as more robust predictors of CHD death. Although the Emerging Risk Factors Collaboration (ERFC) reported that apo B and non-HDL-C are equivalent in their prediction of CHD⁹, others have maintained that apo B is a superior marker of cardiovascular risk¹⁰. In a study of U.S. adults, risk of CHD death doubled with each standard deviation increase in apo B⁶. Alaupovic and colleagues have further proposed that apolipoprotein-defined lipid subclasses may better predict CHD risk than traditional lipoprotein and apolipoprotein measurements^{7, 8}. A 2011 meta-analysis concluded that incorporating apo B assessment into routine clinical care could substantially improve diagnosis and treatment strategies, potentially preventing 500,000 more cardiovascular events over a 10-year period than a non-HDL-C strategy¹¹. With respect to other apos, high dose omega-3 fatty acid supplementation reduces apo C-III levels¹²—an important risk factor that links lipids to inflammation and vascular endothelial dysfunction^{13, 14}. However, the effects of eicosapentaenoic acid (EPA) and DHA on individual apolipoprotein-defined TG-rich and atherogenic apo B-containing lipoprotein subclasses are not well characterized.

Lp-PLA₂ is a lipoprotein-associated, macrophage-secreted enzyme that perpetuates plaque inflammation; elevated levels of serum Lp-PLA₂ are indicative of rupture-prone plaque(s). In population studies, Lp-PLA₂ independently predicts risk of myocardial infarction and stroke, even after adjusting for other risk factors^{15, 16}. Lp-PLA₂ is elevated in metabolic syndrome¹⁷, and lipid-lowering drugs have been shown to reduce Lp-PLA₂ mass and

activity¹⁸. Reduction of Lp-PLA₂ may be a mechanism by which omega-3 fatty acids stabilize rupture-prone atherosclerotic plaques¹⁹.

Dose-response studies examining the effects of omega-3 fatty acids on these lipoprotein associated risk factors are lacking, and questions remain about whether the commonly recommended doses of 0.85 g/d or 3.4 g/d benefit these CHD risk factors in individuals with elevated TG concentrations beyond the TG and VLDL-C lowering effects of 3.4 g/d. Thus, the purpose of the present study was to compare the effects of 0.85 g/d EPA + DHA and 3.4 g/d EPA + DHA on Lp-PLA₂ mass and activity, apos, apo A-I and apo B containing lipoprotein subclasses, and the distribution of apo C-III between these two major lipoprotein subclasses.

Methods

Subjects and design

Subject characteristics and effects of EPA + DHA (0, 0.85, and 3.4 g/d) on the lipoprotein profile have been reported previously²⁰. We enrolled healthy, nonsmoking men (n = 23) and post-menopausal women (n = 3) with TG 150-500 mg/dL⁴. One male subject was excluded due to chylomicronemia following a 12-hour fast. Participants were excluded from the study if they had a chronic disease, took nutritional supplements (other than calcium), took any medications for lipids or hypertension, or ate 2 or more servings/week of foods rich in omega-3 fatty acids. The protocol employed a randomized, double-blinded, placebo-controlled, 3-period crossover design. During each treatment period (8 weeks with a 6 week washout between periods), participants consumed either 0 g/d (placebo, corn oil), 0.85 g/d (low dose), or 3.4 g/d (high dose) of EPA + DHA in the form of prescription omega-3 fatty acid ethyl ester capsules containing EPA and DHA in a ratio of 1.2:1 (LovazaTM, GlaxoSmithKline, Philadelphia, PA). During all supplementation periods, treatment was provided as 4 identical capsules per day. The low dose was provided as one capsule of LovazaTM and 3 indistinguishable placebo capsules. Uniform dosing was achieved without compromising blinding by instructing participants to take a single capsule from one bottle and 3 capsules from a second bottle each day during all three supplementation periods. All capsules were provided by GlaxoSmithKline (LovazaTM and identical corn-oil placebo). Participants were instructed to maintain their low intake (< 2 servings/week) of oily fish and foods high in alpha-linolenic acid (ALA) during the study. The study protocol was approved by the Institutional Review Board of the Pennsylvania State University, and the trial was registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (identifier: NCT00504309). All participants provided written informed consent.

Blood sampling procedures

Serum and plasma samples were obtained from fasting subjects (12 hours with nothing but water, 48 hours without alcohol, and 2 hours without vigorous exercise) at the end of each treatment period.

Apolipoprotein-Defined Lipoprotein Subclasses

Plasma concentrations of apos A-I, B and C-III were determined by the immunoturbidimetric procedure of Riepponen *et al.*²¹ using corresponding monospecific polyclonal antisera produced in this laboratory. Apos A-II²², C-III²³, and E²⁴ were measured by electroimmunoassay for monitoring the isolated apo B-containing lipoprotein subclasses. Apo C-III bound to apo A-I-containing Lps (apo C-III-HS) and apo B-containing Lps (apo C-III-HP) were measured by electroimmunoassay in heparin soluble (HS) and heparin precipitate (HP) fractions²⁵. Quantitative determination of LpA-I and LpA-I:A-II subclasses were performed according to the procedure by März *et al.*²⁶. As previously described in detail²⁷, the quantitative determination of individual apo B-containing Lp subclasses LpB, LpB:E + LpB:C:E, LpB:C and LpA-II:B:C:D:E were expressed in terms of their apo B contents. The inter-assay CV's for immunoprecipitation with antisera to apo A-II or antisera to apo A-II + apo E were 2-4%, and the corresponding CV for immunoprecipitation with an antiserum to apo C-III was 6-7%.

Lipoprotein associated phospholipase A2 mass and activity

Lp-PLA₂ concentration levels (enzyme mass) were measured using an enzyme-linked immunoassay (PLAC[®] test, diaDexus, Inc., South San Francisco, CA), and Lp-PLA₂ activity was measured using a colorimetric activity method (diaDexus CAM Kit, South San Francisco, CA) in plasma aliquots that were stored at -80°C until batch analysis at the end of the study.

Statistical analysis

Statistical analyses were performed using SAS (Statistical Analysis System, Version 9.2, Cary, NC). Normality was tested, and a log transformation was applied to the following variables: TG, apo C-III, apo C-III-HP, and apo C-III ratio. We report least squares means $\pm$ SEM. The mixed models procedure in SAS was used to test effects of treatment. Subject was treated as a random effect. Treatment by period interactions were universally nonsignificant and removed from final models of treatment effects. When period effects were significant, they were retained in the models. For between treatment comparisons, *post hoc* tests with Tukey-Kramer adjusted p-values < 0.05 were considered significant. All p-values for between treatment comparisons are Tukey adjusted.

Results

Baseline characteristics are presented in Table 1. Additional baseline characteristics have been reported previously²⁰. Participants were predominantly male (n = 3 post-menopausal females), white and non-Hispanic (n = 1 Southeast Asian), middle-aged (mean age = 44 years), overweight (mean BMI = 29 kg/m²), and normotensive (mean blood pressure = 123/82 mm Hg)²⁰. Baseline Lp-PLA₂ values were markedly elevated (mean = 317 ng/mL) relative to the reported 50th percentile value of 235 ng/mL²⁸. Only one participant had an Lp-PLA₂ blood level of less than 200 ng/mL at study entry, which has been suggested as the threshold above which CVD risk is increased²⁹.

Significant treatment effects were observed for several endpoints (Table 2, Figure 1, and Figure 2). The 3.4 g/d dose significantly reduced apo B by 6% (Figure 1, $p = 0.01$), apo C-III by 14% (Figure 2, $p = 0.05$), and VLDL-C by 29% compared to placebo ($p = 0.002$). Following the 0.85 g/d and 3.4 g/d doses, plasma concentrations were significantly different for heparin-precipitated apo C-III, LpB, apo B/apo A-I ratio, and LpA-I (Table 2, $p < 0.05$). The 0.85 g/d dose significantly decreased the apo C-III ratio relative to placebo ($p = 0.03$). There was a trend for a 6% reduction in Lp-PLA₂ mass following the high dose, but this was not significant after applying a penalty for multiple treatment comparisons (Figure 3, $p = 0.1$). In a preplanned, un-penalized comparison with baseline values, the 3.4 g/d high dose resulted in a 16 ng/mL reduction in Lp-PLA₂ ($p = 0.03$). There were no significant treatment effects for the other endpoints assessed (Table 2).

Discussion

Our results demonstrate beneficial effects of 3.4 g/d EPA + DHA supplementation on apo B and apo C-III, in addition to corroborating its effectiveness for reducing plasma VLDL-C. The 0.85 g/d dose of EPA + DHA had no effect on apo B, apo C-III, or VLDL-C levels. These findings agree with our original report²⁰ that the 3.4 g/d dose reduced TG by 27%, with no effects observed after supplementation with 0.85 g/d. However, neither the current analysis nor our earlier report²⁰ found significant effects of either dose on the measured markers of inflammation (interleukin-1 β , interleukin-6, tumor necrosis factor- α , high-sensitivity C-reactive protein, and Lp-PLA₂) and endothelial function (flow-mediated dilation and EndoPAT scores).

The beneficial effects of omega-3 supplementation on apos have not been demonstrated consistently and may be unique to individuals with elevated TG. Twelve weeks of supplementation with 4 g/d of EPA has been shown to decrease apo B-containing lipoprotein subclasses in individuals with very high TG.³⁰ The 8.5% reduction in apo B reported by Bays *et al.* is similar to the 6% reduction we observed. Our finding that the concentration of apo C-III was also reduced by the high dose but not the low dose of omega-3 fatty acids is similarly in agreement with earlier studies³¹⁻³⁴. As anticipated, we found no effects of either dose on apo A-I³¹⁻³⁶. There were no significant differences between placebo and 0.85 g/d in any outcome measurement, except for the apo C-III ratio. This can likely be attributed to chance as no effect on the apo C-III ratio was observed for the 3.4 g/d dose. There were significant differences between the low and high dose for heparin-precipitated apo C-III, LpB, apo B/apo A-I ratio, and LpA-I. However, neither differed significantly from the placebo, which makes findings for these outcomes difficult to interpret. Significant differences between the low and high doses, rather than between the high dose and placebo, may be explained by a combination of trends toward detrimental changes during the low dose period and/or improvements during the placebo and high dose periods. Therefore, these significant *post hoc* treatment comparisons are likely to be coincidental because no dose response was observed.

The reduction in apo C-III that we observed following high dose omega-3 fatty acid supplementation could have implications for CHD risk. Increased concentrations of apo C-III have been shown to inhibit lipoprotein lipase activity³⁷ and to interfere with binding of

apo B-containing lipoproteins to hepatic lipoprotein receptors³⁸. Apo C-III also plays a role in inflammatory processes as an activator of monocytic and endothelial cells, demonstrating the link between inflammation and atherosclerosis^{13, 39, 40}. Furthermore, it has been established that apo C-III bound to apo B-containing lipoproteins is an independent risk factor for atherosclerosis and a significant contributor to the progression of atherosclerotic lesions⁴¹⁻⁴⁶. Loss of function mutations in the apo C-III gene are also associated with lower plasma TG and reduced risk of coronary heart disease⁴⁷. In the Framingham cohort, each 1 mg/dL decrease in apo C-III was associated with a 4% decrease in incident coronary heart disease during a mean of 14.4 years of follow up⁴⁷. In aggregate, these findings suggest that apo C-III plays a pathophysiologic role linking lipids, inflammation, and atherogenesis. Therefore, it could be hypothesized that modest reductions in apo C-III resulting from omega-3 fatty supplementation may attenuate coronary heart disease risk; however, long term clinical trials of 3.4 g/d EPA + DHA are needed to evaluate this question.

In dyslipidemic patients with elevated CVD risk, statins are typically indicated for initial therapy, and omega-3 fatty acids—including EPA-only preparations—may be considered as an adjuvant therapy for further improving dyslipidemia. In agreement with our monotherapy results, addition of 3.4 g/d EPA + DHA to statin therapy in individuals with TG 200-499 mg/dL decreased apo B—as well as non-HDL-C and total cholesterol—and increased HDL-C⁴⁸. EPA ethyl esters affect the lipid profile differently than DHA-containing preparations and do not raise LDL-C or HDL-C^{49, 50}. While we did not find a significant increase in LDL-C in the present study, LDL-C increased proportionally to TG reductions²⁰, and increases in LDL-C typically occur with large reductions in TG following EPA + DHA therapy⁵¹. This may be explained by the observation that EPA + DHA increases lipoprotein particle size^{12, 52, 53} while EPA alone does not⁵⁴. The lack of clinical studies evaluating major adverse cardiovascular events following high dose omega-3 fatty acid supplementation for TG reduction limits the evaluation of whether either EPA or DHA or the combination of the two is superior for risk management in individuals with elevated TG. Due to this limitation, the 2013 ACC/AHA Guidelines have not issued recommendations for omega-3 fatty acids and other non-statin therapies⁵⁵. Additional prescribing considerations (e.g., individual patient characteristics, tolerability, and cost considerations) will continue to play a critical role in selection of initial and additional therapies for risk management of the hyperlipidemic patient.

The modest reduction in apo B that we observed following high dose omega-3 fatty acid supplementation may also have implications for CHD risk management. The recently released National Lipid Association (NLA) Recommendations for Patient-Centered Management of Dyslipidemia emphasize that apo B and non-HDL-C are superior primary targets than LDL-C because they better predict ASCVD risk⁵⁶. Non-HDL-C is recommended by the NLA over apo B largely due to greater accessibility⁵⁶. However, apo B measurement is amenable to standardization, and clinical assays are becoming more reliable and cost-effective⁵⁷. We found a significant reduction in apo B without effects on non-HDL-C. Although apo B and non-HDL-C are highly correlated, apo B concentrations specify the number of circulating particles with atherogenic potential, whereas non-HDL-C refers to the total mass of cholesterol contained in atherogenic lipoproteins. Although the ERFC meta-analysis concluded that non-HDL-C and apo B are equivalent risk predictors⁹, this may not

be the case when non-HDL-C and apo B are discordant^{10, 11}. In patients with discordant non-HDL-C and apo B levels, apo B has been suggested to be a superior predictor of cardiovascular risk¹¹. Cholesterol-lowering drug therapies (i.e., statins) can also alter the relationship between atherogenic cholesterol and apo B, often lowering the cholesterol concentration more than the apo B level. Thus, in some individuals, apo B could more directly contribute to identifying residual ASCVD risk and individuals as apo B may remain elevated after treatment goals for non-HDL-C and LDL-C have been attained¹¹.

The high dose omega-3 in the present study did not result in statistically significant reductions in Lp-PLA₂ concentrations in peripheral blood after adjustments for multiple treatment comparisons. However, other research powered with greater numbers of subjects has demonstrated the effectiveness of adding high doses of omega-3 fatty acids to statin therapy for reducing this marker of vascular inflammation^{12, 58}. This effect could partly account for the finding that omega-3 fatty acid supplementation can stabilize atherosclerotic plaques¹⁹. Further, the action of Lp-PLA₂ on oxidized LDL may lead to isoprostane formation; thus, reduced Lp-PLA₂ may be a mechanism for the decrease in isoprostane levels found in other studies of omega-3 supplementation⁵⁹. Modulation of Lp-PLA₂ catalyzed hydrolysis of oxidized lipoprotein may represent a crucial link between oxidation and inflammation that can be attenuated by omega-3 supplementation. Further studies with larger sample sizes are needed, however, to clarify this. The effect of a pharmacological reduction in Lp-PLA₂ on cardiovascular outcomes has not yet been evaluated, and such results would aid in the interpretation of these findings.

Strengths and Limitations

We compared two clinically relevant doses of omega-3 fatty acids using a crossover study design to maximize statistical power. In addition, we examined effects of omega-3 fatty acid monotherapy, and participants were not using any lipid-lowering or anti-hypertensive medications. This allowed us to examine omega-3 fatty acid treatment effects without confounding factors. However, we did not measure particle size, small-dense LDL concentrations, oxidized LDL, or plasma isoprostane concentrations, which would have added to the understanding of treatment effects. The short treatment duration employed and our relatively small sample size (n= 25), which consisted predominantly of white men, are also factors that limit the interpretation of our findings.

Conclusions

This study demonstrates potential mechanisms by which omega-3 fatty acids may decrease CHD risk by reducing atherogenic apolipoproteins in individuals with elevated TG. Treatment with 3.4 g/d EPA + DHA significantly reduced apo B and apo C-III concentrations, and trended toward a modest reduction in Lp-PLA₂. However, as previously reported, these improvements were not accompanied by reductions in markers of inflammation or improvements in endothelial function²⁰. Long-term studies using higher doses of omega-3 fatty acids and powered with a greater number of participants are needed to determine whether these effects on intermediate CHD risk factors translate into improved clinical outcomes in individuals with hypertriglyceridemia.

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- People with elevated triglycerides (150-500 mg/dL) took 0, 0.85, and 3.4 g/d EPA+DHA.
- The study was a randomized crossover with 8-week treatment periods.
- Apolipoproteins B and C were reduced by 3.4 g/d EPA + DHA.
- The low dose (0.85 g/d) did not result in improvements.

Figure 1. Effects of EPA + DHA treatment on plasma apolipoprotein B concentrations
Change from baseline is presented as mean $\pm$ SEM based on the MIXED procedure (SAS, Version 9.2, Cary, NC). *There was a significant main effect of treatment, and the 3.4 g/d dose was significantly different from 0 g/d and 0.85 g/d (Tukey $p < 0.01$).

Figure 2. Effects of EPA + DHA treatment on plasma apolipoprotein C-III concentrations
Change from baseline is presented as mean $\pm$ SEM based on the MIXED procedure (SAS, Version 9.2, Cary, NC). *There was a significant main effect of treatment, and the 3.4 g/d dose was significantly different from 0 g/d and 0.85 g/d (Tukey $p < 0.05$).

Figure 3. Effects of EPA + DHA treatment on plasma apolipoprotein Lp-PLA₂ concentrations
Change from baseline is presented as mean $\pm$ SEM based on the MIXED procedure (SAS, Version 9.2, Cary, NC). No groups were significantly different. In a preplanned, unadjusted analysis, Lp-PLA₂ mass following the 3.4 g/d treatment was significantly different vs. baseline ($p = 0.03$).

Table 1Baseline values for apolipoproteins, apolipoprotein-defined lipid subclasses, and Lp-PLA₂ (n=25).¹

Parameter	Mean ± SEM	Range
<i>Apolipoproteins</i>		
Apo A-I, mg/dL	128 ± 2.0	113-152
Apo B, mg/dL	104 ± 2.3	84-128
Apo B / Apo A-I	0.81 ± 0.02	0.68-0.96
Apo C-III, mg/dL	13.9 ± 1.0	6.5-27
Apo C-III-HS, mg/dL	6.68 ± 0.58	2.1-13.5
Apo C-III-HP, mg/dL	6.18 ± 0.44	3.5-12
Apo C-III ratio	1.12 ± 0.09	0.47-2.74
<i>Apolipoprotein-defined Lipoproteins</i>		
LpB, mg/dL	64 ± 1.6	52-83
LpB:C, mg/dL	11 ± 0.64	3.6-16
LpB:C:E, mg/dL	13 ± 1.1	1.4-21.8
LpA-II:B:C:D:E, mg/dL	17 ± 1.2	2.3-27
LpA-I, mg/dL	34 ± 0.62	29-38
LpA-I:A-II, mg/dL	94 ± 2.1	73.2-121
<i>Lp-PLA₂ Mass and Activity</i>		
Mass, ng/mL	317 ± 15	198-449
Activity, nmol/min/mL	219 ± 9.48	121-348

¹Values are expressed as mean ± SEM. Values were derived from the UNIVARIATE procedure (SAS, Version 9.2, Cary, NC). Lipid and lipoprotein values have been previously reported²⁰.

Table 2

Changes in apolipoproteins, apolipoprotein-defined lipid subclasses, lipoproteins, and lipoprotein associated phospholipase A₂ following treatment with 0, 0.85, and 3.4 g/d EPA + DHA (n=25).¹

Outcome	0 g/d	0.85 g/d	3.4 g/d	p-value for treatment effect
Apolipoproteins				
Apo A-I, mg/dL	4.18 ± 2.21	1.82 ± 2.21	0.82 ± 2.21	NS
Apo B, mg/dL	-0.62 ± 2.24 ^a	-0.42 ± 2.24 ^a	-6.96 ± 2.24 ^b	0.005
Apo B/apo A-I	-0.03 ± 0.02	0.00 ± 0.02 ^a	-0.06 ± 0.02 ^b	0.01
Apo C-III, mg/dL	-1.31 ± 0.88 ^a	-0.37 ± 0.89 ^a	-3.06 ± 0.88 ^b	0.002
Apo C-III-HS, mg/dL	0.28 ± 0.51	-0.21 ± 0.51	-0.49 ± 0.51	NS
Apo C-III-HP, mg/dL	-1.20 ± 0.42	-0.40 ± 0.42 ^a	-1.79 ± 0.42 ^b	0.003
Apo C-III ratio	0.43 ± 0.11 ^a	0.08 ± 0.11 ^b	0.34 ± 0.11	0.02
Apolipoprotein-defined lipoprotein subclasses				
LpB, mg/dL	-0.03 ± 1.68	2.24 ± 1.68 ^a	-2.69 ± 1.68 ^b	0.0009
LpB:C, mg/dL	-0.62 ± 0.97	-1.07 ± 0.97	-1.23 ± 0.97	NS
LpB:C:E, mg/dL	0.18 ± 1.34	-0.95 ± 1.34	-0.98 ± 1.34	NS
LpA-I, mg/dL	-2.30 ± 0.66	-1.76 ± 0.65 ^a	-3.39 ± 0.65 ^b	0.02
LpA-I:A-II, mg/dL	5.51 ± 2.51	4.83 ± 2.50	5.10 ± 2.47	NS
LpA-II:B:C:D:E, mg/dL	-0.11 ± 1.50	-0.54 ± 1.50	-2.18 ± 1.50	NS
Lipids and Lipoproteins				
Total C, mg/dL	3.08 ± 3.50	7.24 ± 3.50	3.02 ± 3.50	NS
HDL-C, mg/dL	2.00 ± 0.91	2.16 ± 0.91	2.60 ± 0.91	NS
LDL-C, mg/dL	1.58 ± 3.37	7.30 ± 3.37	10.06 ± 3.37	NS
VLDL-C, mg/dL	2.32 ± 3.07 ^a	0.70 ± 2.96 ^a	-10.22 ± 2.96 ^b	0.001
Triglycerides, mg/dL	17.26 ± 15.0 ^a	-4.70 ± 15.0 ^a	-46.36 ± 15.0 ^b	0.002
Lipoprotein associated phospholipase A ₂				
Activity, nmol/min/mL	-4.35 ± 4.27	-5.96 ± 4.27	-6.68 ± 4.19	NS

¹ Values are expressed as mean ± SEM. Values and statistical results were derived from the MIXED procedure (SAS, Version 9.2, Cary, NC). Values with different superscripts are significantly different from each other based on Tukey adjusted p-values (< 0.05) for *post hoc* pairwise comparisons. Lipid and lipoprotein values have been previously reported²⁰.