Published in final edited form as:
Metabolism. 2006 January ; 55(1): 26–34. doi:10.1016/j.metabol.2005.06.022.

Combining β-adrenergic and peroxisome proliferator—activated receptor γ stimulation improves lipoprotein composition in healthy moderately obese subjects

Thomas A. Hughes[a,*], **Frankie Stentz**[a], **Thomas Gettys**[b], and **Steven R. Smith**[b]

a*Health Science Center, Department of Medicine, University of Tennessee, Memphis, TN 38163, USA*

b*Molecular Endocrinology Laboratory and Adipose Tissue Signaling Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA*

Abstract

Current pharmacological regimens for hypertriglyceridemia and low high-density lipoprotein (HDL) are limited to the peroxisome proliferator—activated receptor (PPAR) α activating fibrates, niacin, and statins. This pilot study examined the impact of simultaneous stimulation of cyclic adenosine monophosphate with a β-adrenergic agonist and PPARγ with pioglitazone (PIO) on lipoprotein composition in moderately obese, healthy subjects. Subjects were treated with PIO (45 mg) to stimulate PPARγ or a combination of ephedrine (25 mg TID), a β-agonist, with caffeine (200 mg TID), a phosphodiesterase inhibitor (ephedrine plus caffeine), or both for 16 weeks. Lipoproteins were separated by gradient ultracentrifugation into very low-density lipoprotein (VLDL), intermediate-density lipoprotein, low-density lipoprotein (LDL), and 3 HDL (L, M, and D) subfractions. Apolipoproteins were measured by high-performance liquid chromatography. PIO alone reduced the core triglyceride (TG) content relative to cholesterol ester (CE) in VLDL (−40%), IDL (−25%), and HDL-M (−38%). Ephedrine plus caffeine alone reduced LDL CE (−13%), phospholipids (−9%), and apolipoprotein (apo) B (−13%); increased HDL-M LpA-I (HDL containing apoA-I without apoA-II, 28%), CE/TG (23%), and CE/apoA-I (8%) while reducing apoA-II (−10%); and increased HDL-L LpA-I (29%). Combination therapy reduced total plasma TG (−28%), LDL cholesterol (LDL-C, −10%), apoB(−16%), apoB/apoA-I ratio(−21%), while increasing HDL cholesterol (HDL-C, 21%), total plasma apoA-I (12%), LpA-I (43%), and apoC-I (26%). It also reduced VLDL total mass (−34%) and apoC-III (−39%), LDL CE (−13%), apoB (−13%), and total mass (−11%). Combination therapy increased HDL-L CE/TG (32%), apoC-I (30%), apoA-I (56%), and LpA-I (70%), as well as HDL-M CE (35%), phospholipids (24%), total mass (19%), apoC-I (25%), apoA-I (18%), and LpA-I (56%). In conclusion, simultaneous β-adrenergic and PPARγ activation produced beneficial effects on VLDL, LDL, HDL-L, and HDL-M. Perhaps the most important impact of combination therapy was dramatic increases in LpA-I and apoC-I in HDL-L and HDL-M, which were much greater than the sum of the monotherapies. Because LpA-I appears to be the most efficient mediator of reverse-cholesterol transport and a major negative risk factor for cardiovascular disease, this combination therapy may provide very effective treatment of atherosclerosis.

*Corresponding author. Tel.: +1 901 4485744, +1 901 482 9286 (mobile); fax: +1 901 448 5332. E-mail address: thughes303@aol.com (T.A. Hughes).

1. Introduction

The sympathetic nervous system, via its intracellular messenger, cyclic adenosine monophosphate (cAMP), and the peroxisome proliferator—activated receptor γ (PPARγ) system cooperate in controlling substrate metabolism and body weight. The data reported here is one component of a study to investigate the impact of simultaneous stimulation of these systems on body weight, body composition, fat-derived cytokines, and changes in adipocyte gene activation. This report will focus on the lipoprotein compositional changes induced by combined activation.

Both of these systems are known to affect a number of important lipoprotein control points, either directly or indirectly, including the ABCA1 transporter, lipoprotein lipase (LPL), hepatic lipase, lipogenesis, and the low-density lipoprotein (LDL) receptor. The ABCA1 transporter activity, which is responsible for cholesterol efflux from most tissues, including vascular foam cells, has been shown to be stimulated by both cAMP [1,2] and PPARγ [3,4]. Similarly, LPL is also stimulated by both cAMP [5,6] and PPARγ [7,8]. Laplante et al [8] demonstrated that PPARγ activated LPL only in subcutaneous fat, not visceral fat. Deshaies et al [9], on the other hand, showed that β-adrenergic stimulation increased LPL in brown adipose tissue and muscle, but inhibited its activity in white adipose tissue. Conversely, hepatic lipase is inhibited by adrenergic stimulation [10], whereas there are no data on the effect of PPARγ activation. Hepatic lipase removes whole high-density lipoprotein (HDL) particles from the plasma; thus, inhibiting its activity increases HDL plasma concentrations.

Pioglitazone (PIO), a thiazolidinedione (TZD) with PPARγ agonist activity, has been shown to lower triglycerides (TGs) in clinical studies [11]. However, there does not appear to be an effect of PPARγ on very low-density lipoprotein (VLDL) TG secretion [12] despite the reduction in circulating free fatty acids induced by TZDs [13]. In addition, unlike PPARα [14], PPARγ does not appear to inhibit the synthesis of apolipoprotein (apo) C-III, an inhibitor of LPL. Therefore, it appears likely that the primary mechanism by which PIO lowers TGs is by increasing LPL activity in adipose tissue. cAMP [15], on the other hand, is a powerful inhibitor of hepatic fatty acid synthesis and VLDL secretion. This inhibition probably prevents an increase in plasma TGs that would be expected from the increase in adipocyte lipolysis and plasma free fatty acids induced by β-adrenergic stimulation [16]. Similarly, increased β-receptor sensitivity [17] has been shown to be correlated with reduced VLDL TG and apoB levels. Conversely, β-blockers are well known to increase TGs [18,19].

TZDs have a mixed effect on LDL concentrations. PIO tends to have a neutral or slightly positive effect, whereas rosiglitazone increases LDL [11]. Because LDL receptor synthesis is stimulated by insulin [20,21], it is not surprising that insulin sensitizers increase LDL plasma levels when they reduce portal insulin concentrations. However, troglitazone, another TZD, has been shown to directly stimulate LDL receptor messenger RNA synthesis [20], and this effect is additive to insulin. Therefore, the ultimate impact of any TZD will be a balance between the reduction in plasma insulin concentrations and any direct effects on LDL receptor synthesis or other aspects of apoB metabolism. cAMP also stimulates LDL receptor messenger RNA synthesis [22,23], whereas adrenergic stimulation has been shown to reduce LDL levels and increase HDL [24].

Based on these multiple potential effects of adrenergic and PPARγ stimulation on lipid metabolism, this study is designed to determine whether simultaneous stimulation of these 2 systems will produce an additive or synergistic benefit. Subjects were treated with PIO to stimulate PPARγ and a combination of ephedrine, a β-agonist that will increase the production of cAMP, with caffeine, a phosphodiesterase inhibitor, which inhibits cAMP catabolism.

Detailed lipoprotein compositional analysis was performed before and after 16 weeks of therapy.

2. Methods

2.1. Study design

Fourteen men and 43 women completed a 16-week randomized, placebo-controlled, parallel-arm, proof-of-concept clinical trial implemented in Baton Rouge, LA, at the Pennington Biomedical Research Center. The subjects were healthy men and women between 18 and 50 years with a body mass index (BMI) of 30 to 37 kg/m^2. They had no significant diabetes, hypertension, renal, cardiac, liver, lung, neurological, or thyroid disease. They also had no history of thrombophlebitis or other vascular or blood-clotting disorders. They were not taking TZDs, β-blockers, orlistat, sibutramine, ephedrine, phenylpropanolamine (Dexatrim), corticosteroids, statins, fibrates, cholesterol-binding drugs, or herbal supplements containing ephedrine and/or caffeine, using alcohol, or other illicit drugs. The subjects were nonsmokers.

Subjects were randomized to 4 groups: placebo/placebo, ephedrine plus caffeine (E + C)/placebo, PIO/placebo, or PIO + (E + C). Pharmaceutical ephedrine HCl (25 mg) ("Breath Easy," Contract Pharmacal, Hauppauge, NY) and caffeine (200 mg) (Contract Pharmacal; IND No. 65075) were administered as follows: 1 of each pill per day for 7 days at breakfast, then increased to 1 each at breakfast and lunch for the next 7 days, then increased to 1 each at breakfast, lunch, and dinner for the remainder of the protocol. Subjects that could not tolerate 3 doses of E + C per day were allowed to continue in the protocol on 2 doses per day. Subjects who could not tolerate 2 doses per day were dropped from the protocol. Medication doses were taken at least 4 hours apart. This titration was designed to down-regulate β_1- and β_2-adrenergic receptors and thus reduce side effects while leaving β_3 receptors unaffected. PIO was initiated at 15 mg/d and increased by 15 mg each week until the maximum dose of 45 mg was achieved. All subjects were given instruction on diet and behavior modification as well as instructions on healthy activity levels (eg, walking). All subjects provided written informed consent. The protocol was conducted under the approvals of the Food and Drug Administration (IND No. 65075) and the Pennington Institutional Review Board (Protocol No. 22021).

2.2. Lipoprotein separation by ultracentrifugation

Lipoproteins were isolated and analyzed as previously described [25] using a gradient ultracentrifugation — high-performance liquid chromatography (HPLC) technique with revisions made that shortened the ultracentrifugation time [26]. Blood was drawn after a 12-hour fast into tubes containing EDTA and immediately placed on ice. Plasma was separated as soon as possible from the blood cells by centrifugation at 4°C. Nine milliliters of plasma was centrifuged and collected as previously described [26]. The fractions were pooled into VLDL, intermediate-density lipoprotein (IDL), LDL, and 3 HDL subfractions designated as L, M, and D (lowest to highest density), respectively. Lipoprotein a [Lp(a)] was also pooled if it as present as a peak between LDL and HDL-L.

The major protein in each of the HDL subfractions is apoA-I, and the subfractions are subdivided based on their apoA-II/A-I ratio. HDL-M has the highest apoA-II/apoA-I ratio and a medium buoyant density (d = 1.11-1.16 mg/mL), whereas both HDL-L (least dense) and HDL-D (most dense) have substantially lower apoA-II/apoA-I ratios.

2.3. Apolipoprotein analysis by HPLC

One milliliter of VLDL and each HDL pool and 2 mL of IDL were delipidated with human insulin added as an internal standard. The proteins were injected onto an HPLC column and analyzed as previously described [25]. The coefficients of variation for the apolipoprotein

concentrations were 0.4 (apoA-I), 3.9 (apoA-II), 3.6 (apoC-III), 2.3 (apoC-II), and 5.4 (apoC-I). LpA-I/A-II particles (HDL particles containing both apoA-I and apoA-II) in HDL-L and HDL-M have a molar A-II/A-I ratio of 3:4, whereas HDL-D has a ratio of 1:2. From these known ratios, the number of LpA-I (HDL containing apoA-I without apoA-II) particles can be estimated from the measured apoA-II/ apoA-I ratios in each of these subfractions. The concentrations of each are given as millimoles per liter. To convert to milligrams per deciliter, multiply by 2.83 (A-I), 1.74 (A-II), 0.95 (C-III), 0.88 (C-II), and 0.66 (C-I).

2.4. Enzymatic and chemical assays

Total cholesterol, TGs, free cholesterol (FC; Roche, Indianapolis, IN), phospholipids (PLs), and free fatty acids (Wako, Richmond, VA) were assayed using standard enzymatic assays. This laboratory participates in the laboratory quality control testing program sponsored by the Centers for Disease Control for the total cholesterol and TG assays. The Centers for Disease Control does not provide unknowns for the FC or PL assays.

The apoB (mmol/L; convert to mg/dL × 54.9) content of LDL and Lp(a) was determined [25,27] by Lowry assay with bovine serum albumin used as a standard. The apoB concentrations in VLDL and IDL were determined by precipitating the apoB with 50% isopropanol + water[25,28,29]. The pellet was dried under vacuum and resolubilized in sodium dodecyl sulfate and NaOH at 37°C. The recovery of protein is linear up to 10 μg per tube.

LDL density was estimated by identifying the peak position of LDL after ultracentrifugation. There are 6 potential 1-mL fractions where the LDL peak position can be seen; therefore, the peak position is designated at positions 1 (least dense) through 6 (most dense) in increments of 0.5 mL.

2.5. Statistical analysis

Data are presented as the means ± SEM unless otherwise stated. Differences between groups in lipoprotein compositional changes were determined by analysis of variance using Student t tests and the NPAR1WAY with the TTEST procedure in SAS (SAS Institute, Cary, NC). If the variances of the 2 groups proved to be unequal, then the Satterthwaite approximation for reducing the degrees of freedom was used. Differences within groups between pre- and posttreatment lipoprotein composition values were determined by paired differences, also using the repeated measures analysis of variance with the GLM procedure in SAS. The main effects of group and time were cross-classified, and the random effect of subject was nested within group. Only preplanned contrasts were made. An α of .05 was considered statistically significant.

3. Results

3.1. Subject characteristics

The population characteristics of the 57 participants are presented in Table 1. The 4 study groups, ranging from 13 to 17 subjects per group, were middle-aged individuals with moderate obesity who were well matched for age, weight, and BMI. All groups were predominately female, and most groups were relatively balanced by race except for the control group that was predominately white. One subject from each of the PIO and E + C groups were only able to tolerate 2 medication doses per day instead of 3, whereas 3 subjects in the combined group were treated only twice a day. There were no differences in the baseline standard lipid measurements between the treated groups and the control group except for total TGs. The control group had significantly higher TGs because 5 of the 14 subjects had TGs greater than 2.00 mmol/L, whereas the only other subject with a TG of greater than 2.00 mmol/L was in the PIO group. Subjects with hypertriglyceridemia tend to be biologically labile, and this may

have lead to some of the significant changes noted below in this group. Only subjects with both lipoprotein samples were included in this analysis. There were complete data on almost all variables (101 measured and 231 calculated) on all subjects. Forty-three of these variables had 1 to 3 nonuseable values of the maximum of 114 (before and after therapy on 57 subjects). The changes in the anthropomorphic parameters will be reported separately.

3.2. Total plasma lipoproteins

There were no medication effects on total cholesterol, but combination therapy significantly lowered TGs (−28%, $P = .001$), whereas E + C had a marginal impact (−15%, $P = .056$) (Fig. 1). The combination therapy also lowered − LDL cholesterol (LDL-C, −10%, $P = .009$) and total plasma apoB (−16%, $P = .003$), whereas E + C again had a marginal effect (cholesterol, −12%, $P = .069$; apoB, −7%, $P = .056$) (Fig. 2). There were no treatment effects on−the total plasma apoC levels except for an increase in apoC-I (26%, $P = .025$) in the combination group. However, there was a marginal increase in apoC-I in the placebo group (18%, $P = .062$).

Combination therapy increased total plasma HDL cholesterol (HDL-C, 21%, $P = .039$) (Fig. 1) and apoA-I (12%, $P = .034$) (Fig. 2). However, the placebo group also had a marginal change in HDL-C (17%, $P = .065$). Although both PIO and E + C tended to reduce total plasma apoA-I and apoA-II, with E + C producing the only significant reduction (apoA-II, 13%, P .024), combination therapy increased both apolipoproteins with the change in apoA-I being significant (12%, $P = .034$, $P = .012$ vs placebo). There were also increases in total plasma LpA-I in the combination (43%, $P = .016$) and E + C groups (24%, $P = .018$). Combination therapy also increased the total plasma HDL PL (20%, $P = .003$). There were no changes in total plasma HDL TG, apoC-III, or apoC-II, but there were increases in total plasma HDL apoC-I in the combination (26%, $P = .005$) and PIO (13%, $P = .034$) groups.

All groups had significant reductions in the LDL/HDL ratio, including the placebo group, but only the combination patients had a reduction in the apoB/apoA-I ratio (21%, $P = .001$, $P = .029$ vs placebo) (Fig. 2).

3.3. Apoβ-containing subfractions

Only the combination group had a reduction in VLDL TG (−39%, $P = .0004$, $P = .08$ vs placebo), FC (−29%, $P = .002$, $P = .009$ vs placebo), PL (−34%, $P = .0004$, $P = .034$ vs placebo), and total mass (−34%, $P = .0008$), whereas there was no change in cholesterol esters (CEs) (Fig. 3). There was no significant change in VLDL apoB in this group, but there were reductions in apoC-III (−39%, $P = .014$), apoC-II (−44%, $P = .0008$), and apoC-I (−33%, $P = .039$). The CE/TG ratios were increased in both the combination (31%, $P = .01$ l, $P = .003$ vs placebo) and PIO (40%, $P = .002$, $P = .002$ vs placebo) groups, whereas the FC/PL ratios were increased in the combination (14%, $P = .05$) and E + C (17%, $P = .005$) groups. There were no changes in the lipid/apoB ratios.

The only medication effect on IDL component concentrations or composition was an increase in the CE/TG ratio in the combination (41%, $P = .003$) and PIO (25%, P .027) groups.

LDL CE (−13%, $P = .015$), apoB (−13%, $P = .009$), and total mass (−11%, $P = .014$) were reduced in both the combination group and the E + C group (CE, −14%, $P = .002$; apoB, −9%, $P = .07$; mass, −11%, $P = .003$), whereas there were trends toward a reduction in CE (−8%, $P = .07$) and total mass (−7%, $P = .07$) in the PIO group (Fig. 4). The E + C group also had a reduction in the LDL PL (−9%, $P = .01$). There were no changes in TG, FC, or lipid ratios, but the combination group did have increases in the PL/apoB (8%, $P = .022$) and FC/apoB (11, $P = .035$) ratios. LDL peak position shifted to a more favorable, less dense position in the combination (−10%, $P = .008$) and E + C (−7%, $P = .048$) groups.

3.4. HDL subfractions

There were no significant medication effects on HDL-L lipid concentrations, but the combination group had a significant increase in the CE/TG ratio (32%, $P = .043$) and a decrease in the FC/PL ratio (−18%, $P = .0062$, $P = .07$ vs placebo) (Fig. 5). There was also a trend toward a decrease in the FC/PL in the PIO group (−15%, $P = .053$). Combination therapy significantly increased HDL-L apoA-I (56%, $P = .001$, $P = .03$ vs placebo), but left apoA-II unchanged, leading to an increase in LpA-I (70%, $P = .006$). There was also an increase in the LpA-I (29%, $P = .033$) in the E + C group due to a small increase in apoA-I with a reduction in apoA-II. Combination therapy also increased HDL-L apoC-III (53%, $P = .001$), apoC-II (44%, $P = .001$), and apoC-I (30%, $P = .003$) while producing a trend toward an increase in total mass (31%, $P = .07$). However, there was a similar trend in HDL-L mass in the controls.

In HDL-M, there were increases in CE (35%, $P < .0001$, $P = .0004$ vs placebo) and PL (24%, $P = .001$, $P = .01$ vs placebo) in the combination group with trends toward increases in the PIO group (CE, 10%, $P = .053$; PL, 8%, $P = .09$) (Fig. 5). All therapies increased the CE/TG (PIO, 38%, $P = .007$; E + C, 23%, $P = .032$; combination, 43%, $P = .003$, $P = .045$ vs placebo) and CE/apoA-I ratios (PIO, 11%, $P = .015$; E + C, 8%, $P = .037$; combination, 14%, $P = .002$, $P = .06$ vs placebo). PIO increased the PL/apoA-I ratio (6%, $P = .045$), whereas there was a trend in the combination group (6%, $P = .09$). ApoA-I (18%, $P = .001$, $P = .014$ vs placebo) increased in the combination group, whereas the E + C group had a reduction in apoA-II (−10%, $P = .034$), leading to increases in LpA-I in both groups (56%, $P = .018$; 28%, $P = .011$, respectively). The only change in the apoC's was an increase in apoC-I in the combination group (25%, $P = .039$). The combination therapy was the only treatment that produced an increase in total HDL-M mass (19%, $P = .007$, $P = .01$ vs placebo).

The only treatment effect on HDL-D was an increase in the cholesterol to apoA-I ratio in the PIO group (22%, $P = .014$). However, a similar trend was seen in the placebo group (14%, $P = .09$).

4. Discussion

Dyslipidemia, consisting of elevated TGs and a low HDL, is the sine qua non of the metabolic syndrome. The underlying pathophysiology of these abnormalities lies in the presentation of excess lipid to the liver with subsequent increases in VLDL production, reduced LPL activity, and increased clearance of HDL. Increased adipose tissue lipolysis contributes to the increased lipid flux into the liver. β-Adrenergic signaling increases fat oxidation, LPL activity, cholesterol efflux via ABCA1, and LDL receptors while inhibiting fatty acid synthesis, VLDL secretion, and hepatic lipase. Activation of the PPARγ nuclear hormone receptor increases lipid uptake and storage within adipocytes and may act directly on the muscle to increase fat oxidation. PPARγ also increases LPL activity and may increase LDL receptors. Consistent with these complementary effects on lipid metabolism and storage, we found that combination therapy significantly reduced VLDL and LDL while increasing HDL-L and HDL-M total mass and LpA-I.

The primary impact of PIO alone was on the core lipid composition. CE/TG ratios increased in VLDL (40%), IDL (25%), and HDL-M (38%). In VLDL and IDL, this was caused by simply reducing TG, whereas in HDL-M, there was an increase in CE as well as a reduction in TG. Typically, individuals with the metabolic syndrome have TG-enriched lipoprotein particles based on this core ratio; therefore, this increase in CE/TG indicates that there was a normalization of the particle compositions. However, we did not see the increase in total plasma HDL-C reported with PIO in diabetic subjects [11]. PIO did increase the HDL particle size as reflected in the marginal increase in HDL-M PLs (8%), leading to an increase in PL/apoA-I (6%) along with the increase in CE/apoA-I (11%). We also found trends toward reductions in

LDL CE and total mass as opposed to the increase in LDL-C reported in diabetic subjects [11]. However, similar to Goldberg et al [11], we found that there was no impact of PIO on LDL apoB and, thus, particle number. These compositional changes have not been previously reported with PIO therapy, but all would be considered beneficial in cardiovascular risk.

Despite the subtle changes in lipoprotein composition, PIO did not produce a significant change in either total TGs or HDL-C in our population as was previously reported [11]. However, previous studies were done in diabetic patients who had significant reductions in plasma glucose concentrations with therapy. Because plasma glucose becomes a major substrate for TG synthesis in the hyperglycemic state, this reduction in glucose may have been the primary mechanism by which PIO lowered the plasma TGs. Our subjects were not diabetic; therefore, they did not have this reduction in glucose or TGs. Without a change in TGs, the impact of PIO on HDL-C would also be reduced.

The cAMP-generating combination of E + C primarily altered LDL and HDL-M while marginally lowering total plasma TGs (−15%). Similar to a previous study with the β-agonist albuteral [24], E + C reduced LDL CE (−14%), PL (−9%), and apoB (−9%) while shifting the LDL peak density to a less dense position. These changes in LDL could have been caused by an increase in LDL receptor synthesis [22,23] or an inhibition of hepatic lipase [10] (which is required for conversion of IDL to LDL) or both. E + C increased the CE/TG ratio (23%) and CE/apoA-I (8%) in HDL-M (similar to PIO) while reducing apoA-II (−10%). This reduction in apoA-II without a change in apoA-I led to an increase in LpA-I (28%). There was a similar increase in LpA-I (29%) in HDL-L. Despite a minimal change in total plasma HDL-C, E + C did reduce total plasma apoA-II (−13%) while increasing total plasma LpA-I (24%). Again, all of these changes would be considered beneficial in CVD risk, but have not been previously described. In contrast to the large impact on HDL, the only effect of E + C on VLDL or IDL was an increase in the FC/PL ratio (17%) on the surface of VLDL.

Only the combination of cAMP generation and PPARγ stimulation produced a significant reduction in total plasma TGs (−28%). This is consistent with complementary actions of each therapy on overall fat oxidation and sequestration of lipid within adipocytes, respectively. In addition, combination therapy also produced the only significant effect on total plasma HDL-C (21%). However, the placebo group also had a marginal increase in HDL-C (17%), so the significance of this observation is uncertain. As previously mentioned, the control group did have a number of subjects with hypertriglyceridemia, which will make their lipoprotein composition more labile, perhaps explaining some of these changes. Both monotherapies also tended to lower LDL total cholesterol and total plasma apoB, but again, only combination therapy (−10% and −16%) produced significant reductions. Both PIO and E + C tended to lower total plasma apoA-I and apoA-II, whereas combination therapy reversed this effect by increasing apoA-I significantly (12%) and apoA-II marginally (6%). Because the molar increase in apoA-I was greater than the increase in apoA-II, there was a significant increase in total plasma LpA-I. Neither PIO nor E + C had any impact on the apoB/apoA-I ratio, whereas combination therapy produced a very significant reduction (−21%) in this important ratio.

Combination therapy significantly reduced VLDL mass (−34%), which was greater than the sum of the mono-therapies (PIO, −10%; E + C, −18%). This mass reduction was accompanied by a decrease in all VLDL lipids (except CE) and apoCs. There was also a 20% reduction in VLDL apoB (and thus particle number), but this change did not reach statistical significance. Both PIO and combination therapy produced a similar improvement in the VLDL core lipid by increasing the CE/TG ratio (40%, 31%), indicating that E + C had little impact on this parameter. Similarly, PIO had little impact on the FC/PL ratio because E + C and combination therapy increased this parameter similarly (17% and 14%, respectively). These data indicate that both monotherapies have beneficial effects on VLDL, but the combination may have a

synergistic impact. There was probably a reduction in both particle number and size with combination therapy; however, because these changes did not reach statistical significance, additional studies will be required to confirm these trends.

Only combination therapy reduced VLDL apoC-III, which would be expected to increase LPL activity and improve clearance of the VLDL particles into the liver (thus providing a potential mechanism for the VLDL mass reduction). However, this reduction in apoC-III is probably not a direct effect on apoC-III synthesis because the total plasma apoC-III was not reduced. It appears that the apoC-III was simply transferred to HDL, probably because of an increase in LPL activity, as previously described for both of these therapies [5-8]. Consistent with this interpretation, the apoC-III/apoB ratio in VLDL, which represents the number of apoC-III molecules per particle, was not reduced by therapy.

As noted above, E + C produced the greatest impact on LDL lipids and total mass. However, LDL apoB was reduced significantly (13%) only by combination therapy. All 3 therapeutic groups also had small (7%-11%) shifts toward a lower-density LDL, possibly related to the reductions in VLDL mass. There were no changes in the core or surface lipid compositions of LDL. Although the effects of these therapies on LDL were small, they would generally be considered beneficial.

These interventions had no effect on HDL-L lipids except for an increase in the CE/TG ratio (similar to VLDL and IDL) in the combination group (32%) and a trend in the PIO (29%) group. However, the combination group did have an increase in apoA-I (56%) and LpA-I (70%). The E + C group also had a small increase in LpA-I (29%). These increases in LpA-I are very important because LpA-I appears to be a more efficient promoter of reverse-cholesterol transport than LpA-I/A-II [30-32] and thus better at preventing cardiovascular disease.

Combination therapy had a dramatic effect on HDL-M lipid content: increasing CE (35%), PL (24%), and total mass (19%). There were trends in the PIO group in the same lipids (CE 10%, PL 8%). Again, all therapeutic groups had increases in the CE/TG ratios. Similar to HDL-L, the combination group had a significant increase in apoA-I (18%) and LpA-I (56%). E + C also increased LpA-I (28%) while reducing LpA-I/A-II (as reflected by the significant reduction in apoA-II). The CE/apoA-I ratios increased significantly (8%-14%) in all 3 treatment groups, indicating an increase in particle size. Again, increases in LpA-I and HDL size are indicators of improved reverse-cholesterol transport.

Combination therapy increased apoC-I in total plasma (26%), HDL-L (30%), and HDL-M (25%). ApoC-I has been shown to inhibit CE transfer protein [33,34] that permits the transfer of CE and TG among the lipoproteins. Therefore, this increase in apoC-I may have inhibited the transfer of CE out of HDL-M and, thus, led to the increase in CE seen in this subfraction. Conversely, CE transfer protein inhibition would have inhibited the transfer of TGs into HDL, accounting for the reduction in the CE/TG ratio. ApoC-I also inhibits hepatic lipase [35], which would increase HDL by preventing removal of HDL particles by the liver.

In summary, the combination of PIO and E + C produced substantial beneficial changes in VLDL and HDL-M while inducing smaller improvements in LDL and HDL-L. Perhaps, the most clinically important effect was the dramatic increases in LpA-I and apoC-I in both HDL-L and HDL-M, suggesting an impressive increase in reverse-cholesterol transport. These data indicate that simultaneous cAMP generation and PPARγ activation produces important beneficial effects on lipoprotein metabolism.

Acknowledgment

This study was funded by a grant from Takeda Pharmaceuticals North America, Lincolnshire, IL.

We would like to thank Ann Ammons and Suzanne Hughes at the University of Tennessee for their excellent technical assistance and the patients who participated in this study because without their invaluable assistance, this study could not have been completed.

References

1. Oram JF, Lawn RM, Garvin MR, Wade DP. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J Biol Chem 2000;275:34508–11. [PubMed: 10918070]
2. Haidar B, Denis M, Krimbou L, Marcil M, Genest J. cAMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts. J Lipid Res 2002;43:2087–94. [PubMed: 12454270]
3. Hsueh WA, Law RE. PPAR gamma and atherosclerosis—effects on cell growth and movement. Arterioscler Thromb Vasc Biol 2001; 21:1891–5. [PubMed: 11742860]
4. Hodgkinson CP, Ye S. Microarray analysis of peroxisome proliferator—activated receptor gamma—induced changes in gene expression in macrophages. Biochem Biophys Res Commun 2003;308:505–10. [PubMed: 12914779]
5. Kuusela P, Rehnmark S, Jacobsson A, Cannon B, Nedergaard J. Adrenergic stimulation of lipoprotein lipase gene expression in rat brown adipocytes differentiated in culture: mediation via beta(3)- and alpha(1)-adrenergic receptors. Biochem J 1997;321:759–67. [PubMed: 9032464]
6. Ballart X, Siches M, PeinadoOnsurbe J, Lopez Tejero D, Llobera M, Ramirez I, et al. Isoproterenol increases active lipoprotein lipase in adipocyte medium and in rat plasma. Biochimie 2003;85:971–82. [PubMed: 14644552]
7. Kageyama H, Hirano T, Okada K, Ebara T, Kageyama A, Murakami T, et al. Lipoprotein lipase mRNA in white adipose tissue but not in skeletal muscle is increased by pioglitazone through PPAR-gamma. Biochem Biophys Res Commun 2003;305:22–7. [PubMed: 12732191]
8. Laplante M, Sell H, MacNaul KL, Richard D, Berger JP, Deshaies Y. PPAR-gamma activation mediates adipose depot-specific effects on gene expression and lipoprotein lipase activity—mechanisms for modulation of postprandial lipemia and differential adipose accretion. Diabetes 2003;52:291–9. [PubMed: 12540599]
9. Deshaies Y, Geloen A, Paulin A, Marette A, Bukowiecki LJ. Tissue-specific alterations in lipoprotein lipase activity in the rat after chronic infusion of isoproterenol. Horm Metab Res 1993;25:13–6. [PubMed: 8428704]
10. Neve BP, Verhoeven AJM, Kalkman I, Jansen H. Maturation and secretion of rat hepatic lipase is inhibited by alpha(1B)-adrenergic stimulation through changes in Ca2+ homeostasis: thapsigargin and EGTA both mimic the effect of adrenaline. Biochem J 1998;330: 701–6. [PubMed: 9480878]
11. GoldbergRBKendallDMDeegMAA comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care2005 [in press].
12. Lefebvre AM, PeinadoOnsurbe J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, et al. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler Thromb Vasc Biol 1997;17:1756–64. [PubMed: 9327774]
13. Tan MH. How pioglitazone affects glucose and lipid metabolism. Exp Clin Endocrinol Diabetes 2000;108:S224–33.
14. Fredenrich A. Role of apolipoprotein CIII in triglyceride-rich lipoprotein metabolism. Diabetes Metab 1998;24:490–5. [PubMed: 9932215]
15. Bjornsson OG, Sparks JD, Sparks CE, Gibbons GF. Regulation of Vldl secretion in primary culture of rat hepatocytes—involvement of cAMP and cAMP-dependent protein kinases. Eur J Clin Invest 1994; 24:137–48. [PubMed: 8206083]
16. Blaak EE. Fatty acid metabolism in obesity and type 2 diabetes mellitus. Proc Nutr Soc 2003;62:753–60. [PubMed: 14692611]

17. Arner P, Wahrenberg H, Lonnqvist F, Angelin B. Adipocyte beta-adrenoceptor sensitivity influences plasma lipid levels. Arterioscler Thromb 1993;13:967–72. [PubMed: 8391311]

18. Rabkin SW, Huff MW, Newman C, Sim D, Carruthers SG. Lipids and lipoproteins during antihypertensive drug therapy—comparison of doxazosin and atenolol in a randomized, double-blind trial: the Alpha Beta Canada Study. Hypertension 1994;24:241–8. [PubMed: 8039850]

19. Boquist S, Ruotolo G, Hellenius ML, DanellToverud K, Karpe F, Hamsten A. Effects of a cardioselective beta-blocker on postprandial triglyceride-rich lipoproteins, low density lipoprotein particle size and glucose-insulin homeostasis in middle-aged men with modestly increased cardiovascular risk. Atherosclerosis 1998;137:391–400. [PubMed: 9622282]

20. AlRayyes O, Floren CH. Troglitazone upregulates LDL receptor activity in HepG2 cells. Diabetes 1998;47:1193–8. [PubMed: 9703316]

21. Duvillard L, Florentin E, Lizard G, Petit JM, Galland F, Monier S, et al. Cell surface expression of LDL receptor is decreased in type 2 diabetic patients and is normalized by insulin therapy. Diabetes Care 2003;26:1540–4. [PubMed: 12716819]

22. Liu JQ, Heikkila P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. Eur J Endocrinol 2000;142:677–82. [PubMed: 10832106]

23. Liu JW, Ahlborn TE, Briggs MR, Kraemer FB. Identification of a novel sterol-independent regulatory element in the human low density lipoprotein receptor promoter. J Biol Chem 2000;275:5214–21. [PubMed: 10671569]

24. Maki KC, Skorodin MS, Jessen JH, Laghi F. Effects of oral albuterol on serum lipids and carbohydrate metabolism in healthy men. Metabolism 1996;45:712–7. [PubMed: 8637445]

25. Hughes TA, Moore MA, Neame P, Medley Mark F, Chung BS. Rapid quantitative apolipoprotein analysis by gradient ultracentrifugation and reversed-phase HPLC. J Lipid Res 1988;29:363–76. [PubMed: 3379347]

26. Hughes TA, Gaber Ahmed O, Montgomery CE. Plasma distribution of cyclosporin within lipoproteins and bin vitroQ transfer between very-low-density lipoproteins, low density lipoproteins, and high density lipoproteins. Ther Drug Monit 1991;13:289–95. [PubMed: 1780959]

27. Shen MMS, Krauss RM, Lindgren FT, Forte TM. Heterogeneity of serum low density lipoproteins in normal human subjects. J Lipid Res 1981;22:236–44. [PubMed: 7240955]

28. Holmquist L, Carlson K, Carlson LA. Comparison between the use of isopropanol and tetramethylurea for solubilization and quantitation of human serum very low density apolipoproteins. Anal Biochem 1978;88:457–60. [PubMed: 211874]

29. Egusa G, Brady DW, Grundy SM, Howard Barbara V. Isopropranol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density and low density lipoprotein apolipoprotein B. J Lipid Res 1983;24:1261–7. [PubMed: 6631250]

30. Duriez P, Fruchart JC. High-density lipoprotein subclasses and apolipoprotein A-I. Clin Chim Acta 1999;286:97–114. [PubMed: 10511287]

31. Rinninger F, Brundert M, Budzinski RM, Fruchart JC, Greten H, Castro GR. Scavenger receptor BI (SR-BI) mediates a higher selective cholesteryl ester uptake from LpA-I compared with LpA-I: A-II lipoprotein particles. Atherosclerosis 2003;166:31–40. [PubMed: 12482548]

32. deBeer MC, Castellani LW, Cai L, Stromberg AJ, deBeer FC, vanderWesthuyzen DR. ApoA-II modulates the association of HDL with class B scavenger receptors SR-BI and CD36. J Lipid Res 2004;45:706–15. [PubMed: 14729860]

33. Gautier T, Masson D, deBarros JPP, Athias A, Gambert P, Aunis D, et al. Human apolipoprotein C-I accounts for the ability of plasma nigh density lipoproteins to inhibit the cholesteryl ester transfer protein activity. J Biol Chem 2000;275:37504–9. [PubMed: 10978346]

34. Gautier T, Masson D, Jong MC, Duverneuil L, LeGuern N, Deckert V, et al. Apolipoprotein CI deficiency markedly augments plasma lipoprotein changes mediated by human cholesteryl ester transfer protein (CETP) in CETP transgenic/ApoCI-knocked out mice. J Biol Chem 2002;277:31354–63. [PubMed: 12070157]

35. CondeKnape K, Bensadoun A, Sobel JH, Cohn JS, Shachter NS. Overexpression of apoC-I in apoE-null mice: severe hypertriglyceridemia due to inhibition of hepatic lipase. J Lipid Res 2002;43: 2136–45. [PubMed: 12454276]

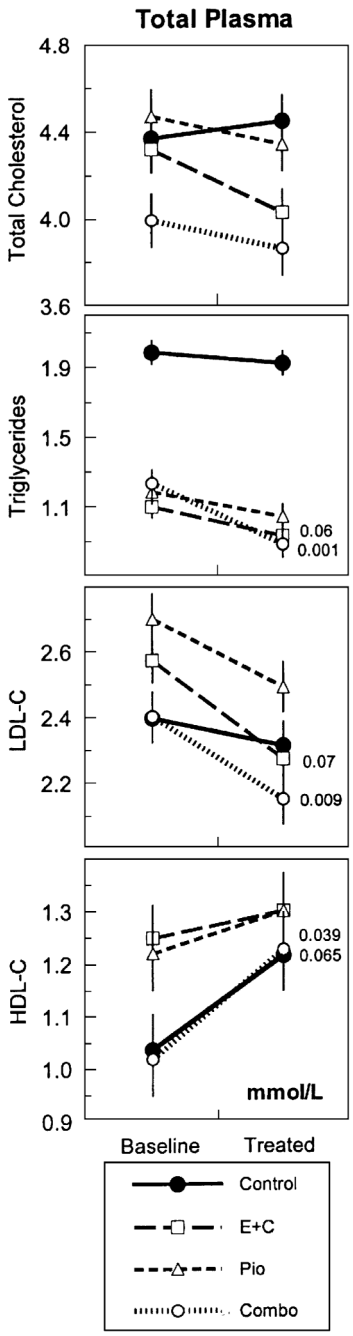

Fig. 1.

Total plasma lipid (mmol/L) concentrations before and after 16 weeks of therapy. Number of subjects: controls, 14; E + C, 17; PIO, 13; combined, 13. The numbers are the *P* values for the paired differences for the change from baseline within the group.

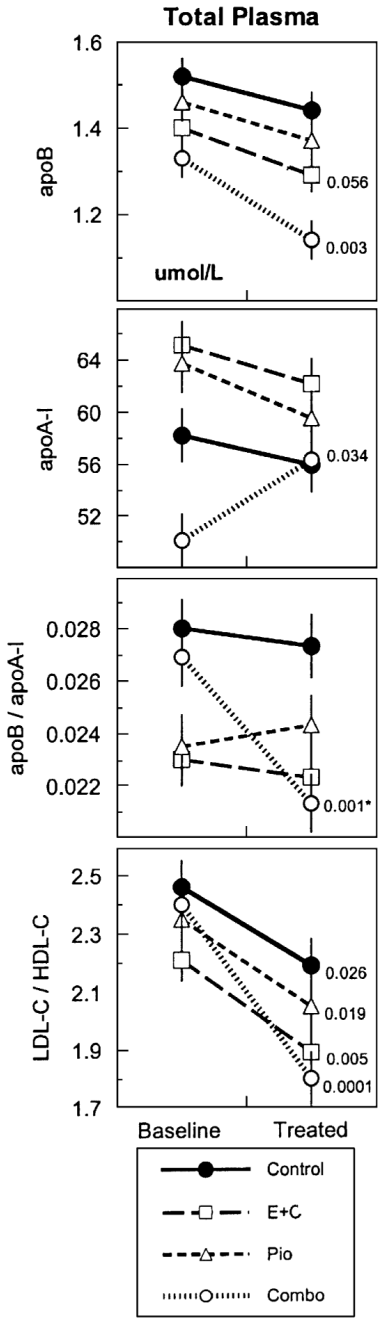

Fig. 2.

Total plasma apolipoprotein (μmol/L) concentrations and the LDL-C/HDL-C ratios before and after 16 weeks of therapy. A *P* value with an asterisk indicates that this change is significantly different than the change in the control group (means ± SEM). Details are explained in the footnote to Fig. 1.

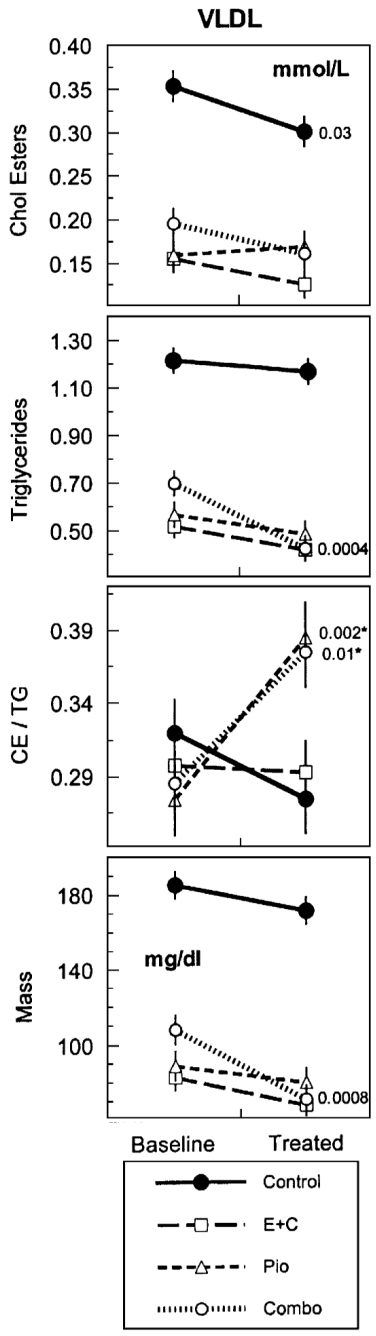

Fig. 3.

VLDL lipid (mmol/L) concentrations and total mass (mg/dL) before and after 16 weeks of therapy. A *P* value with an asterisk indicates that this change is significantly different than the change in the control group (means ± SEM). Details are explained in the footnote to Fig. 1.

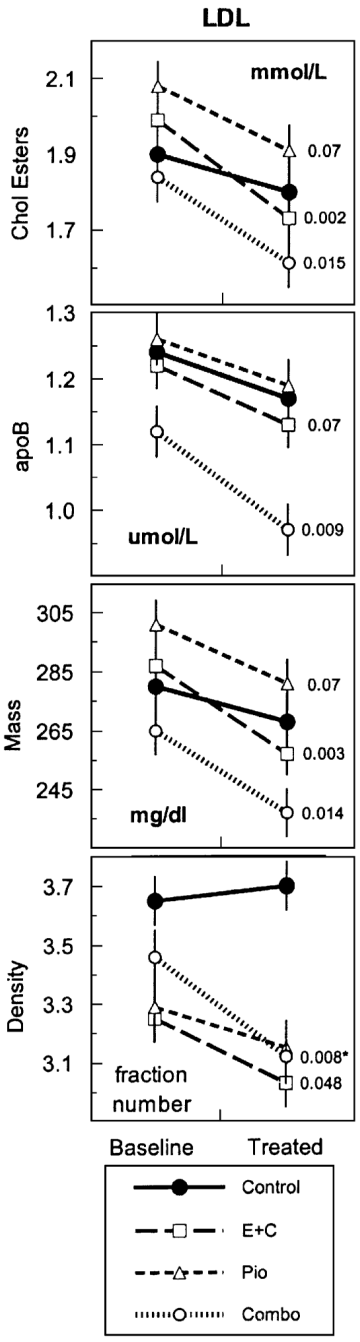

Fig. 4.

LDL CE (mmol/L), apoB (μmol/L), total mass (mg/dL) concentrations, and the peak position for LDL (fraction numbers 1-6) before and after 16 weeks of therapy. A *P* value with an asterisk indicates that this change is significantly different than the change in the control group (means ± SEM). Details are explained in the footnote to Fig. 1.

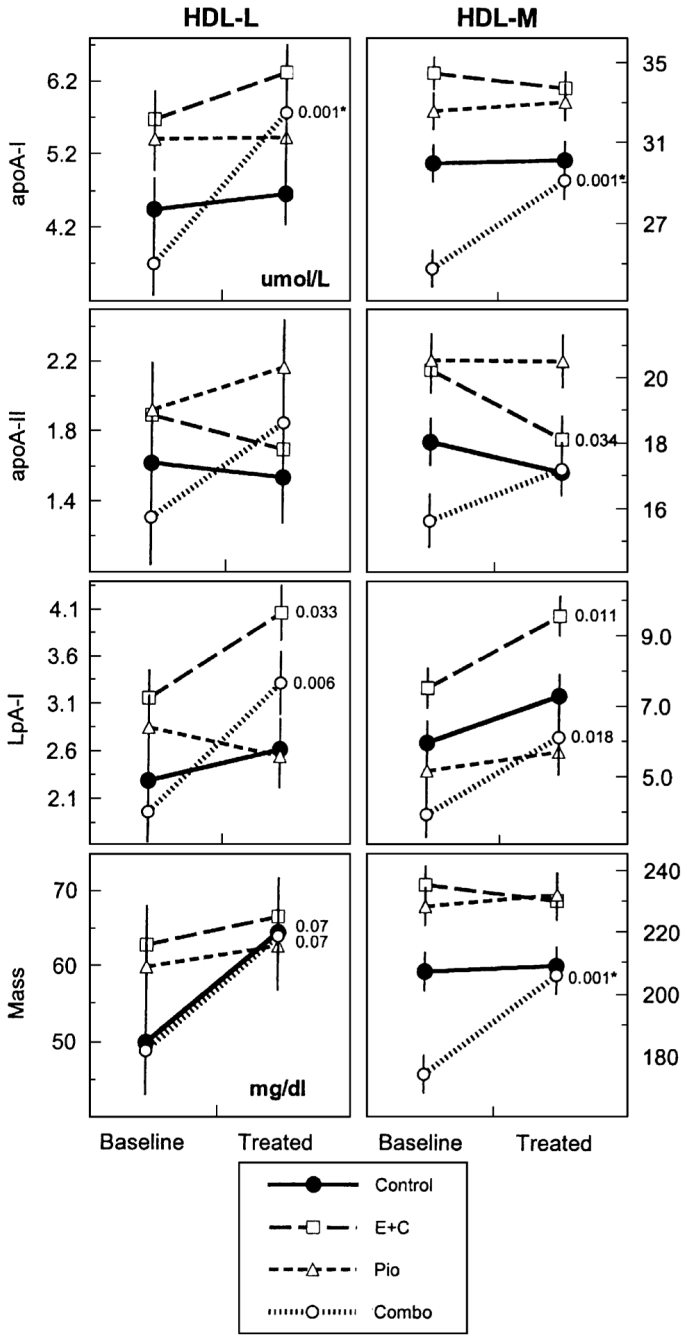

Fig. 5.

HDL apolipoprotein (μmol/L), LpA-I (μmol/L), and total mass (mg/dL) before and after 16 weeks of therapy. A *P* value with an asterisk indicates that this change is significantly different than the change in the control group (means ± SEM). Details are explained in the footnote to Fig. 1.

Table 1

Demographics and standard baseline lipids (means ± SEM)

	Control	PIO	E + C	Combination
n	14	13	17	13
Age (y)	37.3 ± 2.1	39.8 ± 2.2	40.6 ± 1.9	39.5 ± 2.2
Weight (kg)	99.4 ± 2.8	94.7 ± 3.0	93.1 ± 2.6	98.8 ± 3.0
BMI (kg/m^2)	33.9 ± 0.6	33.6 ± 0.6	33.2 ± 0.5	34.3 ± 0.6
Sex (M vs F)	4 vs 10	4 vs 9	2 vs 15	4 vs 9
Race (white vs African American)	12 vs 2	6 vs 7	8 vs 9	8 vs 5
Total cholesterol (mmol/L)	4.37 ± 0.19	4.47 ± 0.20	4.32 ± 0.17	3.99 ± 0.20
TG	1.98 ± 0.17	1.19 ± 0.18*	1.10 ± 0.16*	1.18 ± 0.19*
LDL-C	2.40 ± 0.16	2.70 ± 0.16	2.57 ± 0.14	2.40 ± 0.16
HDL-C	1.04 ± 0.08	1.22 ± 0.08	1.24 ± 0.07	1.02 ± 0.08
LDL-C/HDL-C	2.46 ± 0.19	2.35 ± 0.20	2.21 ± 0.17	2.40 ± 0.20
ApoB (μmol/L)	1.52 ± 0.07	1.46 ± 0.07	1.40 ± 0.07	1.33 ± 0.07
ApoA-I	58 ± 4	63 ± 4	65 ± 3	50 ± 4
ApoB/apoA-I	0.028 ± 0.002	0.024 ± 0.002	0.023 ± 0.002	0.027 ± 0.002

*$P < .05$ vs controls (5 subjects in control group had triglyceride >2.00 mmol/L).