ORIGINAL RESEARCH

Effect of Switching From Statin Monotherapy to Ezetimibe/Simvastatin Combination Therapy Compared With Other Intensified Lipid-Lowering Strategies on Lipoprotein Subclasses in Diabetic Patients With Symptomatic Cardiovascular Disease

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***Background*—**Patients with diabetes mellitus and cardiovascular disease may not achieve adequate low-density lipoprotein cholesterol (LDL-C) lowering on statin monotherapy, attributed partly to atherogenic dyslipidemia. More intensive LDL-C–lowering therapy can be considered for these patients. A previous randomized, controlled study demonstrated greater LDL-C lowering in diabetic patients with symptomatic cardiovascular disease who switched from simvastatin 20 mg (S20) or atorvastatin 10 mg (A10) to combination ezetimibe/simvastatin 10/20 mg (ES10/20) therapy, compared with statin dose-doubling (to S40 or A20) or switching to rosuvastatin 10 mg (R10). The effect of these regimens on novel biomarkers of atherogenic dyslipidemia (low- and high-density lipoprotein particle number and lipoprotein-associated phospholipase A2 [Lp-PLA_2]) was assessed.

***Methods and Results*—**Treatment effects on low- and high-density lipoprotein particle number (by NMR) and Lp-PLA_2 (by ELISA) were evaluated using plasma samples available from 358 subjects in the study. Switching to ES10/20 reduced low-density lipoprotein-particle number numerically more than did statin dose-doubling and was comparable with R10 (−133.3, −94.4, and −56.3 nmol/L, respectively; $P>0.05$). Increases in high-density lipoprotein particle number were significantly greater with switches to ES10/20 versus statin dose-doubling (1.5 and −0.5 μmol/L; $P<0.05$) and comparable with R10 (0.7 μmol/L; $P>0.05$). Percentages of patients attaining low-density lipoprotein particle number levels <990 nmol/L were 62.4% for ES10/20, 54.1% for statin dose-doubling, and 57.0% for R10. Switching to ES10/20 reduced Lp-PLA_2 activity significantly more than did statin dose-doubling (−28.0 versus −3.8 nmol/min per mL, $P<0.05$) and was comparable with R10 (−28.0 versus −18.6 nmol/min per mL; $P>0.05$); effects on Lp-PLA_2 concentration were modest.

***Conclusions*—**In diabetic patients with dyslipidemia, switching from statins to combination ES10/20 therapy generally improved lipoprotein subclass profile and Lp-PLA_2 activity more than did statin dose-doubling and was comparable with R10, consistent with its lipid effects.

***Clinical Trial Registration*—**URL: http://www.clinicaltrials.gov. Unique identifier: NCT00862251. **(*J Am Heart Assoc*. 2015;4: e001675 doi:10.1161/JAHA.114.001675)**

Key Words: diabetes mellitus • ezetimibe • lipoprotein particle number • lipoprotein subclasses • lipoprotein-associated phospholipase A_2 • NMR spectroscopy • rosuvastatin • simvastatin

Patients with diabetes mellitus have dyslipidemia and metabolic complications that contribute to an increased risk of cardiovascular disease (CVD).[1–6] Statin therapy is the recommended first-line therapeutic approach for lipid management of high-risk patients; however, many patients with diabetic dyslipidemia do not achieve adequate low-density lipoprotein (LDL) cholesterol (LDL-C) lowering on statin monotherapy.[7–11] This residual risk may be attributed in part to atherogenic dyslipidemia, characterized by high levels of triglycerides, low levels of high-density lipoprotein (HDL) cholesterol (HDL-C), and high LDL-particle (LDL-P) numbers in these patients.[1] In addition to LDL-C lowering, non–HDL-C and apolipoprotein B (apoB) have been recommended as treatment targets for assessment of individuals with diabetic dyslipidemia.[1,7,12–15]

Several international guidelines endorse statin uptitration, switching to a more-potent statin therapy, and/or combination

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therapy for high-risk individuals in need of additional cholesterol lowering.[7,12–15] US guidelines recommend using the maximum tolerated statin dose with consideration given to the addition of a nonstatin cholesterol-lowering drug if the clinical benefit outweighs the safety risk in these patients.[16] Trials that evaluate the lipid-lowering efficacy of different modes of therapy may provide helpful information for physicians when considering therapeutic options for high-risk patients on statin therapy in need of additional LDL-C lowering. A number of studies have reported that combination ezetimibe/simvastatin (ES) therapy reduces LDL-C and improves other lipids more than do statin monotherapy and doubling the statin dose or switching to a more-potent statin, across a range of commonly prescribed doses in high-risk patients including those with type 2 diabetes.[17–20] The recently completed IMPROVE-IT trial (IMProved Reduction of Outcomes: Vytorin Efficacy International Trial) assessed the incremental cardiovascular benefit of LDL-C lowering with ezetimibe 10 mg added to simvastatin (mainly 40 mg) compared with simvastatin monotherapy in patients presenting with acute coronary syndromes.[21–23] The study investigators reported that the trial met its primary and secondary composite efficacy end points.

Lipid and inflammatory markers, including LDL particle number (LDL-P), HDL particle number (HDL-P), and lipoprotein-associated phospholipase A_2 (Lp-PLA_2), may be more predictive of the atherogenic dyslipidemia present in diabetic patients with CVD.[24–26] In patients with discordant LDL-C and LDL-P levels, LDL-attributable atherogenic risk was shown to be more strongly correlated with LDL-P than LDL-C levels.[27,28] Similarly, HDL-P is proposed to be better correlated with CVD risk than HDL-C.[29–32] Increased levels of Lp-PLA_2 mass and activity have been shown to be associated with CVD risk and, compared with other inflammatory markers, provide an advantage as they are less subject to variability.[25,26]

An assessment of the efficacy of combination ES therapy versus more-intensive statin therapy on lipoprotein subclasses may aid in better understanding the lipid-altering effects of these therapies in these patients. In a previously reported multicenter, double-blind, randomized clinical trial, CVD patients with diabetes on stable, low-dose statin therapy (simvastatin 20 mg/day [S20] or atorvastatin (10 mg/day [A10]) who switched to ES 10/20 mg/day (ES10/20) had significantly greater LDL-C lowering versus doubling the statin dose (S40 or A20) and numerically greater changes than switching to rosuvastatin 10 mg/day (R10) during 6 weeks of treatment.[33] This analysis assessed the effect of these therapies on changes in LDL-P and HDL-P concentrations with the use of NMR spectroscopy,[34] as well as changes in Lp-PLA_2 concentration and activity,[35–37] by using a subset of available samples from participants in that study.

Materials and Methods

Subjects

This was a post-hoc analysis of plasma samples obtained from a 12-week randomized, double-blind, active-controlled study conducted between June 2009 and March 2011 in 86 centers in Europe, South America, and the United States (www.clinicaltrials.gov NCT00862251).[33] The protocol had been previously approved by the institutional review board at each participating clinical center, and all patients provided informed consent. In brief, participants were adults between the age of 18 and 80 with type 1 or 2 diabetes mellitus (HbA_{1c} $\leq$8.5%) and symptomatic CVD, who were naïve to statin and/or ezetimibe or were taking a stable dose of approved lipid-lowering therapy and, if needed, stable antidiabetic medication for 3 months before the screening visit, as well as willing to maintain an approved cholesterol- and glucose-lowering diet for the duration of the study. Only individuals with LDL-C $\geq$1.81 mmol/L (70 mg/dL) and $\leq$4.14 mmol/L (160 mg/dL) after 6 weeks' stabilization with either S20 or A10 were eligible for randomization. Subjects with elevated levels of triglycerides (>4.52 mmol/L, e.g., 400 mg/dL), alanine transaminase and aspartate transaminase (>2$\times$ upper limit of normal), creatinine kinase (>3$\times$ upper limit of normal) were excluded from randomization. Patients who were newly diagnosed (within 3 months before visit 1) or had any change in antidiabetic pharmacotherapy (e.g., changes in dosage [with the exception of $\pm$10 units of insulin] or had the addition of new medication) within 3 months before visit 1, as well as patients experiencing a recent history of repeated hypoglycemia or unstable glycemic control, were also excluded. Eligible participants were stratified according to the statin taken during the run-in period (S20 or A10) and randomly assigned to treatment with ES 10/20 (n=133), double the current statin dose (S40 or A20; n=74), or R10 (n=151), in a 2:1:2 ratio. Details of the randomization strategy have been published previously.[33] The current study includes a subset of the entire cohort (358 of a total of 406, or 88.2%) in whom adequate plasma volume was available at both baseline and study-end time points.

Laboratory Analyses

Collected plasma specimens were stored at -80°C at a central biospecimen archiving center and shipped to the Biomarker Core Laboratory (Atlanta, GA) on dry ice. Matching baseline and study-end samples from the same participant were sorted in dry ice and stored at -80°C until ready for analysis. Specimens were thawed in batches of 240 samples or 120 participants for analysis on a weekly basis. Lp-PLA_2 concentrations (PLAC) were determined with the use of ELISA

(diaDexus), and Lp-PLA$_2$ activities (ACAM) were determined through an enzymatic method with the Beckman AU480 using reagents from diaDexus. On completion (within 48 hours), each batch was shipped on ice to Liposcience for NMR analysis. Plasma samples were subjected to only a single freeze–thaw cycle, and all analyses were completed within 7 days.

Statistical Methods

Baseline was defined as the end of the 6-week run-in period with low-dose statin, either S20 or A10. Net differences were calculated between baseline and after 6 weeks of treatment with the intensified lipid-lowering therapies (S40, A40, E/S10/20, or R10) for each of the measured parameters. Paired *t* test was used to assess the changes from baseline within each treatment group, and 2-sample *t* test was used to assess the significance of the net differences between treatment groups. One-way ANOVA was used to assess the effect of the 3 more-intensive cholesterol-reduction regimens. For the present analysis, all subjects receiving double the run-in statin dose were considered as a single group, independent of whether the statin used was S20 or A10. The percent attainment of on-treatment target levels of LDL-C (<2.33 mmol/L, <90 mg/dL), LDL-P (<990 nmol/L), and non–HDL-C (<2.85 mmol/L, <110 mg/dL), defined as percentage of individuals below these levels, were assessed. These target levels for LDL-C, non–HDL-C, and LDL-P corresponded to population equivalent levels (≤20th and <5th percentiles) as previously reported in the MESA (Multi-Ethnic Study of Atherosclerosis) study cohort.[25,38] No statistical analysis was performed with respect to these percentages.

Simple linear regression analysis was used to explore the relationship among the percent changes for the various novel markers. Only relationships with $P<0.01$ were considered significant.

Results

The baseline characteristics of the 358 study participants assessed in this analysis are presented in Table 1. Baseline values for lipids and biomarkers, determined after 6 weeks of treatment on the starting dose of statin (S20 or A10), were generally consistent among the 3 treatment groups and were not statistically different.

Effect on Lipids and Apolipoproteins

Compared with baseline, significant reductions were obtained in all 3 groups for total cholesterol, LDL-C, non–HDL-C, and apoB (Table 2 and Figure 1). In comparing treatment groups (Table 2), significantly greater reductions for total cholesterol, LDL-C, non–HDL-C, and apoB were achieved when the participants were switched to either ES 10/20 or R10 than when the initial statin dose was doubled. Net changes with ES 10/20 were not statistically different from changes obtained with R10. Both R10 and ES10/20 increased apoA-I levels from baseline significantly more than did doubling the statin dose ($P<0.05$). Changes from baseline in triglycerides, HDL-C, and high-sensitivity C-reactive protein were not significant for any of the 3 treatment groups, and there were no significant between-treatment differences.

Table 1. Baseline Characteristics

Mean (SD)	2× Statin (n=74)	R10 (n=151)	ES10/20 (n=133)
Age, y	64.1 (8.1)	62.9 (8.6)	64.2 (7.9)
CHOL, mmol/L	4.65 (0.67)	4.61 (0.68)	4.70 (0.84)
TG, mmol/L	1.64 (0.62)	1.72 (0.72)	1.68 (0.70)
HDL-C, mmol/L	1.35 (0.31)	1.29 (0.31)	1.32 (0.37)
LDL-C, mmol/L	2.55 (0.58)	2.52 (0.52)	2.61 (0.60)
Non–HDL-C, mmol/L	3.30 (0.68)	3.32 (0.69)	3.38 (0.73)
hsCRP, mg/L	4.58 (6.19)	4.20 (6.53)	3.29 (3.34)
apoA-I, g/L	1.53 (0.23)	1.49 (0.24)	1.49. (0.31)
apoB, g/L	1.00 (0.18)	1.00 (0.19)	1.02 (0.21)
PLAC, ng/mL	334.5 (121.6)	322.5 (93.8)	321.5 (110.8)
ACAM, n mol/min/mL	196.7 (87.3)	198.7 (84.9)	209.2 (101.3)
TRL-P, nmol/L	58.7 (26.5)	64.9 (33.6)	60.6 (31.7)
LDL-P, nmol/L	1096.0 (269.7)	1063.2 (216.8)	1121.2 (295.4)
HDL-P, μmol/L	31.8 (5.0)	31.3 (4.7)	31.2 (6.1)

Patients on initial simvastatin 20 mg and atorvastatin 40 mg doses. International to conventional unit conversion factors: to convert cholesterol to mg/dL, divide by 0.0259; triglycerides to mg/dL, divide by 0.0113; apolipoprotein to mg/dL, divide by 0.01. 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; ACAM, lipoprotein-associated phospholipase A$_2$ activities; Apo, apolipoprotein; CHOL, total cholesterol; ES10/20, switch to ezetimibe/simvastatin 10/20 mg; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle; PLAC, lipoprotein-associated phospholipase A$_2$ concentration; R10, switch to rosuvastatin 10 mg; TG, triglycerides; TRL-P, triglyceride-rich lipoprotein particles.

Effect of More Intensive Therapy on LDL-P Concentrations

LDL-P concentrations were significantly reduced with all 3 treatments, ($P<0.05$ for 2× statin, $P<0.001$ for R10 and ES10/20) (Table 3 and Figure 2A). The net changes in LDL-P observed in subjects who were switched to either ES10/20 or R10 were comparable (−133.3 and −94.4 nmol/L, respectively) and were significantly greater than the changes obtained when the statin dose was doubled (−56.3 nmol/L) ($P<0.05$).

Table 2. Changes From Baseline in Lipid, Apolipoprotein, and hsCRP Levels

Mean (SD)	CHOL (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	non–HDL-C (mmol/L)	hsCRP (mg/dL)	apoA-I (g/L)	apoB (g/L)
Changes from baseline within treatment group								
2× statin	−0.22 (0.79)‡	−0.05 (0.70)*	−0.02 (0.22)*	−0.18 (0.67)†	−0.21 (0.75)†	0.6 (6.8)	−0.07 (0.23)†	−0.07 (0.18)‡
R10	−0.50 (0.64)§	−0.04 (0.54)*	0.02 (0.18)*	−0.51 (0.54)§	−0.50 (0.73)§	0.9 (14.9)*	0.02 (0.20)*	−0.14 (0.20)§
ES10/20	−0.57 (0.92)§	−0.04 (0.63)*	0.02 (0.26)*	−0.57 (0.78)§	−0.59 (0.81)§	0.3 (4.5)*	0.01 (0.25)*	−0.15 (0.22)§
P value for differences between treatment groups‖								
ES 10/20 vs 2× statin	0.05	NS	NS	0.05	0.05	0.05	0.05	0.05
ES 10/20 vs R10	NS	NS	NS	NS	NS	NS	NS	NS
R10 vs 2× statin	0.05	NS	NS	0.05	0.05	NS	0.05	0.05
ANOVA¶	0.01	NS	NS	0.001	0.001	NS	0.05	0.01

International to conventional unit conversion factors: to convert cholesterol to mg/dL, divide by 0.0259; triglycerides to mg/dL, divide by 0.0113; apolipoprotein to mg/dL, divide by 0.01. 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; apo, apolipoprotein; CHOL, total cholesterol; ES10/20, switch to ezetimibe/simvastatin 10/20 mg; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; R10, switch to rosuvastatin 10 mg; TG, triglycerides.

*$P>0.05$; †$P<0.05$; ‡$P<0.01$; §$P<0.001$ by paired *t*-test.

‖Pairwise comparison between any 2 intervention groups.

¶Comparison of differences among all 3 groups.

NS, not significant.

As illustrated in Figure 2B through 2D, the percent changes in LDL-P were strongly associated with changes in the concentrations of small LDL particles (sLDL-P) ($P<0.0001$). Patients receiving ES10/20 or R10 tended to have greater reductions in the concentrations of sLDL-P (34.7 and 23.3 nmol/L, respectively) compared with those who were taking double the dose of the initial statin (1.0 nmol/L); however, the between-group differences were not significant (data not shown).

Effect of More Intensive Therapy on HDL-P Concentrations

Statistically significant increases in HDL-P from baseline were observed with switching to ES10/20 ($P<0.001$) and R10 ($P<0.05$), whereas doubling the statin dose had no effect (Table 3 and Figure 3A). Net changes in HDL-P were significantly greater when switching to ES10/20 compared with doubling the statin dose ($P<0.05$) but not significantly different than switching to R10 ($P>0.05$). Net changes in HDL-P with R10 were not statistically different from changes obtained with doubling the statin dose. As illustrated in Figure 3B through 3D, the overall changes in HDL-P were associated with changes in large HDL-P number for all 3 treatment groups ($P<0.0001$).

Effect of More Intensive Therapy on Triglyceride-Rich Lipoprotein Particle Concentrations

Reductions from baseline in the total concentrations of triglyceride-rich lipoproteins were moderate for all 3 treatment groups, with only R10 being statistically significant ($P<0.05$) (Table 3). This was predominantly attributed to reduction in the concentration of small very low-density lipoprotein particle concentration (from 35.2 to 31.6 nmol/L, $P<0.01$). There were no statistically significant differences between treatment groups.

Effect on Lp-PLA$_2$

Switching to either ES10/20 or R10 resulted in significant reductions from baseline in PLAC ($P<0.001$), whereas small, nonsignificant reductions were observed with doubling the statin dose ($P>0.05$) (Table 3). Also, compared with baseline, significant reductions in Lp-PLA$_2$ activity were obtained when the subjects were switched to either ES 10/20 or R10 ($P<0.05$) but not when the initial statin dose was doubled ($P>0.05$). When Lp-PLA$_2$ specific activity calculated as the activity per unit mass of Lp-PLA$_2$ was examined, significant reductions were observed only when the participants were switched from low-dose statin to ES 10/20 ($P<0.001$) but not to any other regimen (Figure 4).

Relationship Between ApoB and LDL-P Concentrations

Figure 5 presents the relationship between on-treatment levels of LDL-P and apoB using all available samples including data while on low-dose statin (S20 or A10) and on all 3 more-intensive cholesterol-reduction therapies

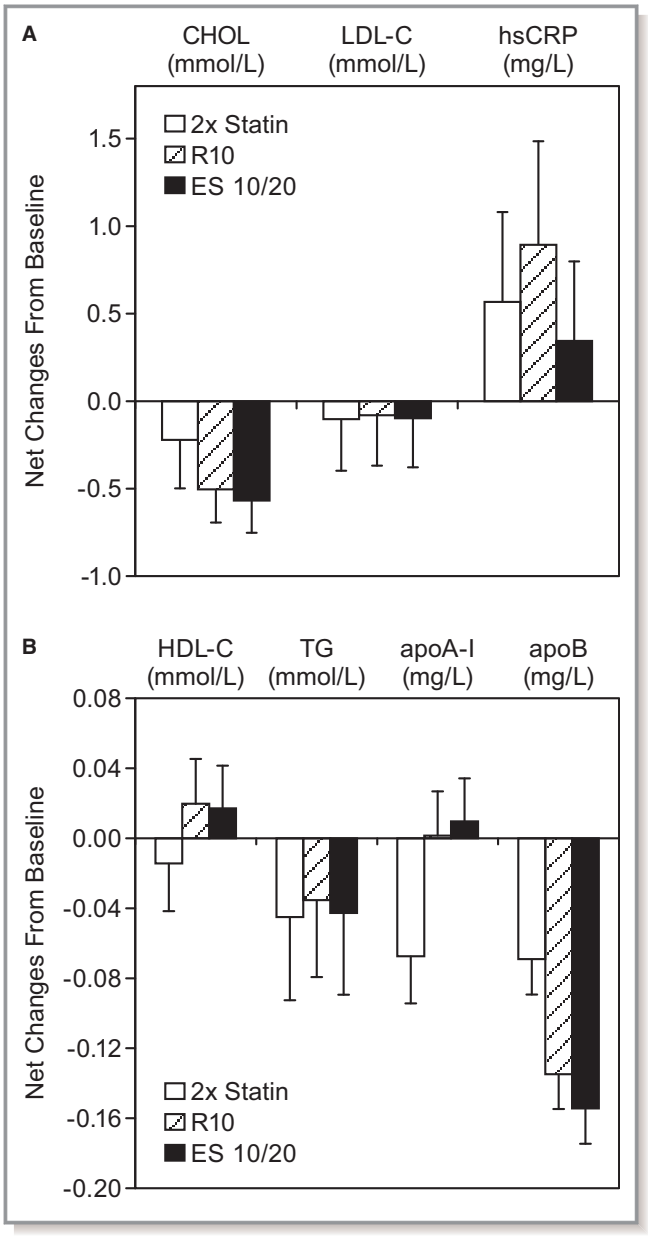

Figure 1. Net changes from baseline in (A) CHOL, LDL-C, and hsCRP and in (B) HDL-C, TG, apoA-I, and apoB for the more-intensive therapies. Apo indicates apolipoprotein; ES, ezetimibe/simvastatin; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; R, rosuvastatin; CHOL, total cholesterol; TG, triglycerides. See Table 1 for actual values and standard deviations.

(2× statin, ES 10/20, and R10). Combining all data with apoB ranging from 50 mg/dL to 200 mg/dL, LDL-P concentration (nmol/L) could be estimated from total plasma apoB concentration (mg/dL) by using the equation:

LDL-P=9.373×(apoB)+157.13 (R=0.706, P<0.0001).

Effect of Different Intensive Therapies on the Relationship Among LDL-C, ApoB, and LDL-P

Figure 6 illustrates the relationships between the percent changes in LDL-P, LDL-C, and apoB for all 3 more-aggressive cholesterol reduction regimens. As expected, there was a strong correlation between percent changes in LDL-C and in total plasma apoB (Figure 6A, R=0.894, P<0.0001). Data from all 3 more-intensive therapies are tightly clustered around the regression line. In contrast, the relationships between changes in LDL-P and LDL-C (Figure 6B, R=0.583, P<0.0001) and between changes in LDL-P and apoB were weaker (Figure 6C, R=0.608, P<0.0001), although statistically significant. In 2.3% of the patients randomized to ES 10/20, there was an unexpected increase in LDL-P despite reductions in both LDL-C and apoB. The percent of patients exhibiting this discordant response was higher with 2× statin dose (5.4%) and highest for patients randomized to R10 (8.6%).

Efficacy of More Intensive Therapy With Respect to Target Levels

The percentage of patients who achieved on-treatment target levels for LDL-C (<2.33 mmol/L, <90 mg/dL), non–HDL-C (<2.85 mmol/L, <110 mg/dL), and LDL-P (<990 nmol/L) corresponding to the 20th population equivalent of the MESA cohort are shown in Figure 7A. With the starting dose of statin (either simvastatin 40 mg/day or atorvastatin 20 mg/day), ~41.1% of the patients achieved target levels for LDL-C, 37.7% were at target levels for LDL-P, and 26.8% for non–HDL-C. After 6 weeks of treatment with all 3 intensive lipid-lowering therapies, the percentages of patients who achieved target levels of LDL-C and LDL-P increased substantially, with the largest improvements in LDL-C. Percent achievement of LDL-C target levels was 51.4% for doubling the statin dose, 72.2% for switching to R10, and 75.2% for switching to ES10/20. The percentages of patient attainment for LDL-P were 54.1%, 57.0%, and 62.4% for doubling the statin dose and switching to R10 and ES10/20, respectively. Percentages of patient attainment for non–HDL-C were 62.2%, 57.0%, and 64.7%, respectively, for statin dose-doubling and switching to R10 and to ES10/20.

For the more-stringent targets, equivalent to the 5th percentile based on the MESA cohort[25,38] (LDL-C <1.8 mmol/L, 70 mg/dL, and non–HDL-C <2.3 mmol/L, 90 mg/dL), the improvements in LDL-C and non–HDL-C were consistently greater for E/S 10/20 and R10 than those for doubling the statin dose (Figure 7B). With low-dose starting statin, either simvastatin (20 mg/day) or atorvastatin (10 mg/day), the percentage of patients achieving this LDL-C target was less than 0.6%. With more-intensive therapy, the percentage of individuals reaching the LDL-C target level improved with

Table 3. Changes in Lp-PLA$_2$ and Lipoprotein Particle Concentrations

Mean (SD)	PLAC (ng/mL)	ACAM (nmol/min per mL)	TRL-P (nmol/L)	LDL-P (nmol/L)	HDL-P (μmol/L)
Changes from baseline within treatment group					
2× statin	−6.7 (71.9)*	−3.8 (51.3)*	−1.9 (28.2)*	−56.3 (256.0)†	−0.5 (4.4)*
R10	−16.7 (58.5)§	−18.6 (3.87)†	−5.0 (25.5)†	−94.4 (242.0)§	0.7 (1.4)†
ES10/20	−23.7 (70.2)§	−28.0 (3.98)†	−2.4 (29.7)*	−133.3 (317.5)§	1.5 (3.8)§
P value for differences between treatment groups‖					
ES 10/20 vs 2× statin	NS	<0.05	NS	<0.05	<0.05
ES 10/20 vs R10	NS	NS	NS	NS	NS
R10 vs 2× statin	NS	<0.05	NS	<0.05	NS
ANOVA¶	NS	0.002	NS	NS	0.005

International to conventional unit conversion factors: to convert cholesterol to mg/dL, divide by 0.0259; triglycerides to mg/dL, divide by 0.0113; apolipoprotein to mg/dL, divide by 0.01.
2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; ACAM, lipoprotein-associated phospholipase A$_2$ activities; ES10/20, switch to ezetimibe/simvastatin 10/20 mg; HDL-P, high-density lipoprotein particle; LDL-P, low-density lipoprotein particle; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; PLAC, lipoprotein-associated phospholipase A2 concentration; R10, switch to rosuvastatin 10 mg; TRL-P, triglyceride-rich lipoprotein particles.
*P>0.05; †P<0.05; ‡P<0.01; §P<0.001 by paired t- test.
‖Pairwise comparison between any 2 intervention groups.
¶Comparison of differences among all 3 groups.
NS, not statistically significant at the 0.05 α level (P>0.05).

doubling the statin dose, R10 and ES 10/20 (24.3%, 42.4%, and 52.6%, respectively). Similarly, the percentage of patients reaching the target level for non–HDL-C increased substantially from 1.7% at the initial starting dose to 17.6%, 29.8%, and 41.4% with 2×statin, R10, and ES10/20, respectively. The percentage of individuals achieving the target level for LDL-P (770 nmol/L) also improved from 11.2% at the starting dose to 18.9%, 25.8%, and 27.1%, with 2× statin, R10, and ES 10/20, respectively.

Discussion

This analysis showed that switching from S10 or A20 therapy to more-intensive lipid-lowering therapies had favorable effects on several biomarkers considered to be important measures of atherogenic dyslipidemia in diabetic patients with symptomatic CVD. Switching to combination ES10/20 provided greater reductions from baseline in LDL-P as well as attainment of LDL-P levels <990 nmol/L compared with doubling the statin dose and reductions that were comparable with R10. Switching to the combination ES10/20 therapy also resulted in modest increases in HDL-P that were significantly greater than doubling the starting dose and similar to R10. Combination ES10/20 therapy improved Lp-PLA$_2$ activity significantly more than statin dose-doubling and comparably with R10, and moderately decreased Lp-PLA$_2$ concentration. Overall, the treatment effects on these atherogenicity markers were consistent with the lipid changes demonstrated in the full cohort of the original study.

The lipid changes observed in this subgroup analysis were generally similar to those reported in the full cohort of the original trial, in which switching to ES combination therapy improved LDL-C lowering and achievement of LDL-C levels <70 and <100 mg/dL compared with doubling the baseline statin dose and switching to R10.[33] Other studies have also reported that switching from low-dose statin monotherapy to ES combination therapy significantly improved LDL-C lowering compared with doubling the statin dose[39–41] and switching to R10.[42,43] Taken together, these results indicate that more-intensive lipid-lowering therapy may contribute to improved lipid management in these high-risk patients.

Given that measurement of on-treatment levels of LDL-P may be more predictive of residual CVD risk than LDL-C when the 2 measures are discordant, it has been suggested that intensification of lipid-lowering therapy is reasonable to consider when elevated LDL-P is present in patients receiving statin therapy.[25,44] To assess the response to lipid-lowering therapy, LDL-P targets have been proposed, based on population equivalent levels for LDL-C targets (<20th percentile for high- and very high-risk patients). It should be noted that while treatment targets for LDL-C, non–HDL-C, and apoB have been recommended in various national dyslipidemia guidelines, LDL-P targets have not been endorsed.[1,7,12,15] Achievement of LDL-P targets that are equivalent to LDL-C and non–HDL-C targets based on population percentiles have been shown to require intensive therapy.[44] Our study showed that ≤41% of patients at baseline had levels of LDL-C <2.33 mmol/L (90 mg/dL), non–HDL-C <2.85 mmol/L (110 mg/dL), and LDL-P <990 nmol/L after 6 weeks of pretreatment with S20 or A10. After switching to ES10/20 or R10, >72% of patients achieved the LDL-C level and >57% achieved the LDL-P and non–HDL-C targets, highlighting the importance of more-intensive therapy in

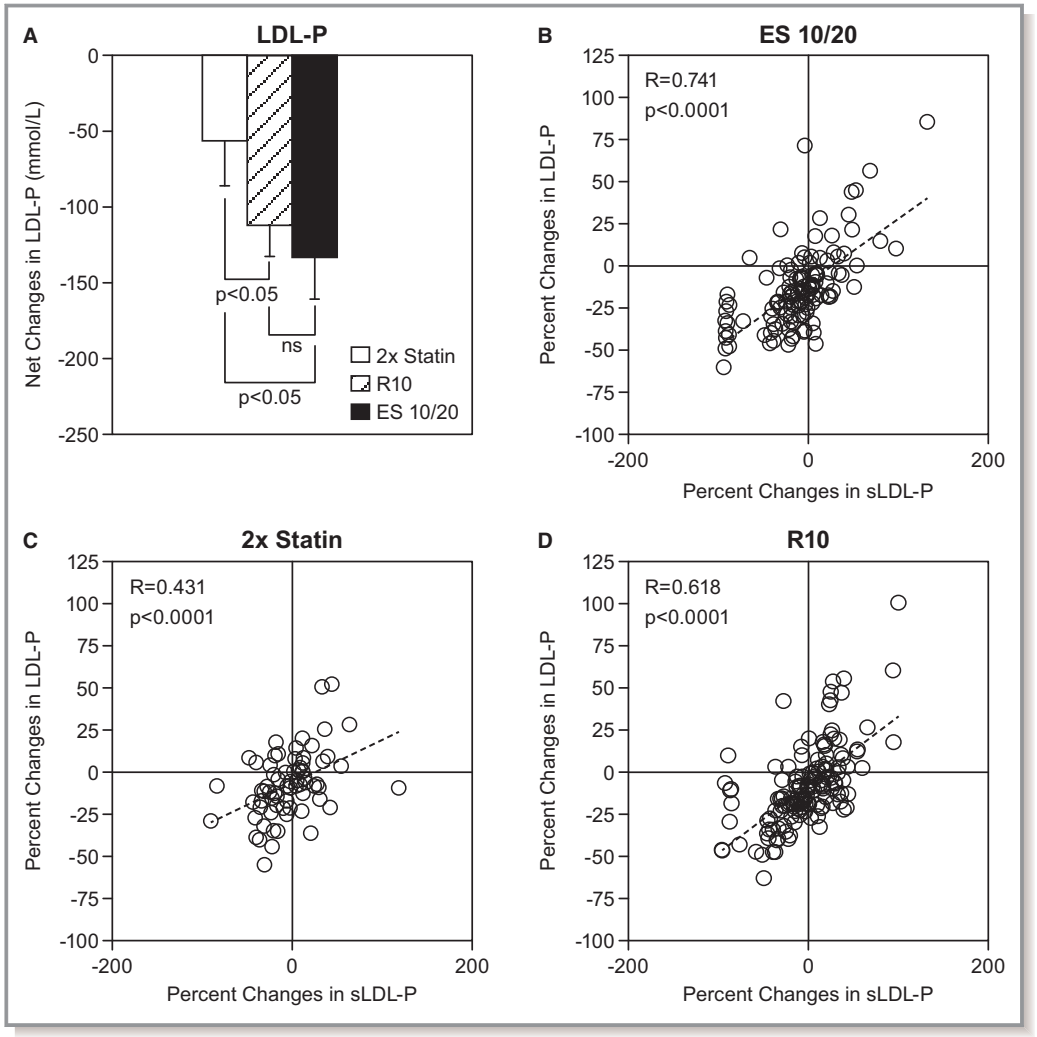

Figure 2. Effect of more-intensive therapy on LDL-P. A, Reductions in LDL-P from baseline were significant with switching to 2× statin ($P<0.05$), ES 10/20 ($P<0.001$), and R10 ($P<0.001$). There was a significant association between the percent changes in total LDL-P and the percent changes in the concentration of small LDL particles with ES 10/20 (B), 2× statin (C), and with R10 (D). 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; ES10/20, switch to ezetimibe/simvastatin 10/20 mg; LDL-P, low-density lipoprotein particle number; R10, switch to rosuvastatin 10 mg.

reducing the atherogenic lipoprotein profile in these patients. The observation that more patients achieved the LDL-C than LDL-P and non–HDL-C target levels may be attributed to a high LDL-P number that is not reflected by LDL-C measurement, consistent with findings in patients where these 2 measures are discordant.[44] To the best of our knowledge, this study is the first to evaluate the effect of these therapies on LDL-P targets.

Several studies have demonstrated that the prevalence of dense sLDL-P may be an independent risk factor for cardiovascular disease.[45–48] Data from this study would suggest that the changes in LDL-P observed when patients were switched to either ES10/20 or R10 were primarily driven by changes in the concentrations of sLDL-P as assessed with NMR spectroscopy. For patients who were receiving double the initial statin dose, the relationship between the changes in the number of sLDL-P and the overall changes in LDL-P number was weaker. Thus, aggressive LDL-reduction therapy with combination ES 10/20 and R10 may have a preferential impact on dense sLDL-P compared with either simvastatin (40 mg/day) or atorvastatin (20 mg/day).

Because apoB is the primary protein component of LDL-P, measurement of apoB concentration is considered to provide a direct estimate of LDL-P, and thus it would be anticipated that changes between apoB and LDL-P would be highly associated.[49,50] In our study, changes in LDL-P and apoB were only moderately correlated, and response to

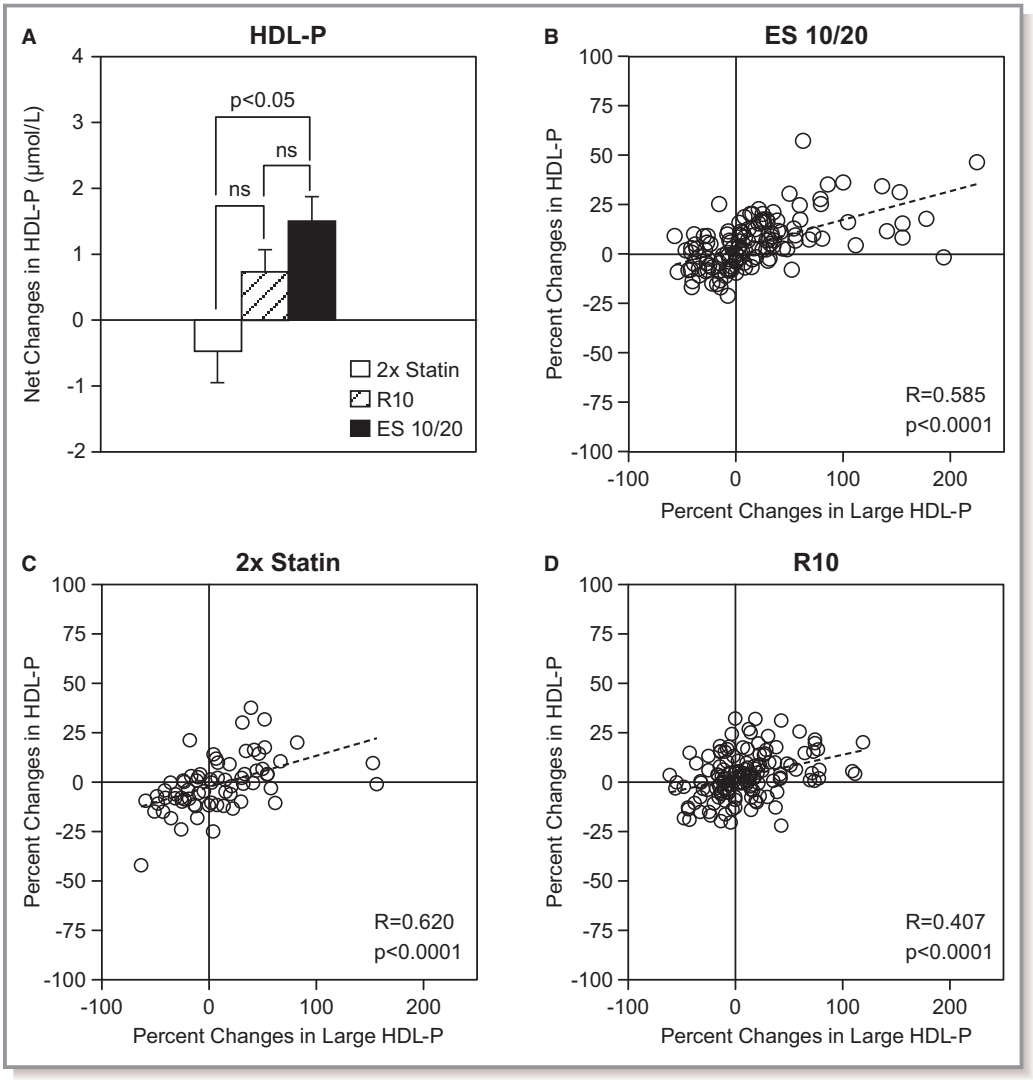

Figure 3. Effect of more-intensive therapy on HDL-P. A, Net changes (SD) from baseline in HDL-P concentrations (µmol/L) were statistically significant only for ES 10/20 ($P<0.001$) and nonsignificant ($P>0.05$) for doubling the statin dose (2× statin) and R10. Significant associations were observed between HDL-P and large HDL-P with ES 10/20 in (B), 2× statin in (C), and R10 in (D). 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; ES10/20, switch to ezetimibe/simvastatin 10/20 mg; HDL-P, high-density lipoprotein particle number; R10, switch to rosuvastatin 10 mg; SD, standard deviation.

treatment was found to be discordant in some patients, particularly those who had increases in LDL-P despite apoB reductions. Differences in baseline levels of triglycerides, HDL-C, LDL-C, apoB, and body mass index in these patients may have contributed to this discrepancy. A prior study showed that while apoB and LDL-P levels were concordant in a majority of individuals, discordance was observed in patients who had low apoB and high LDL-P levels but not in those with higher apoB levels, indicating that apoB and LDL-P measurement of atherogenic lipoproteins may not be comparable in all settings.[49] Additionally, differences in assessment of apoB with immunoassay [LDL, very low-density lipoprotein, intermediate-density lipoprotein, and lipoprotein(a)] and of LDL-P with NMR [LDL subfractions, intermediate-density lipoprotein, and lipoprotein(a)] may partly account for these discordant observations. In line with this, a review of 25 clinical trials showed that although apoB and LDL-P were comparably associated with clinical outcomes in most studies, some discordance was noted, which was attributed to inherent methodological differences in measurement of these markers.[51] Further studies are needed to better understand the relationship between apoB and LDL-P, as well as assessment of these markers in various populations.

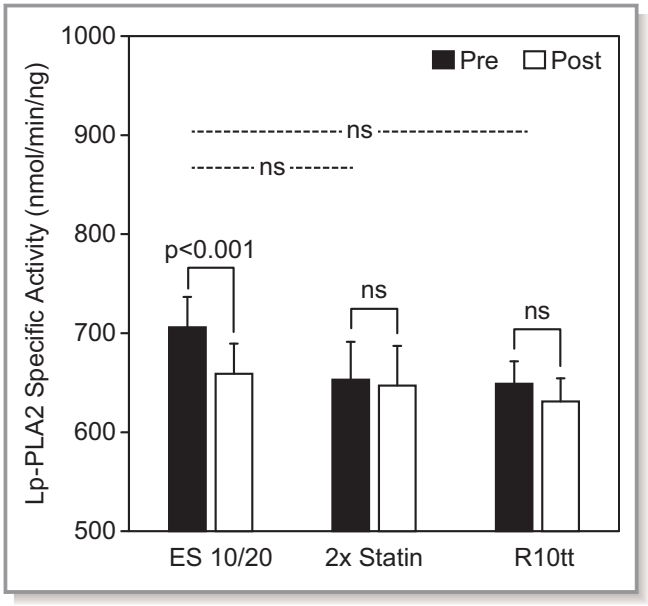

Figure 4. Changes in Lp-PLA$_2$ specific activity with the 3 more-intensive cholesterol-reduction regimens. E/S indicates ezetimibe/simvastatin; Lp-PLA$_2$ lipoprotein-associated phospholipase A2; R, rosuvastatin.

Studies have also suggested that HDL-P is a better marker of residual CVD risk than are HDL-C and apoA-I levels and HDL-P size, including patients treated to very low LDL-C levels

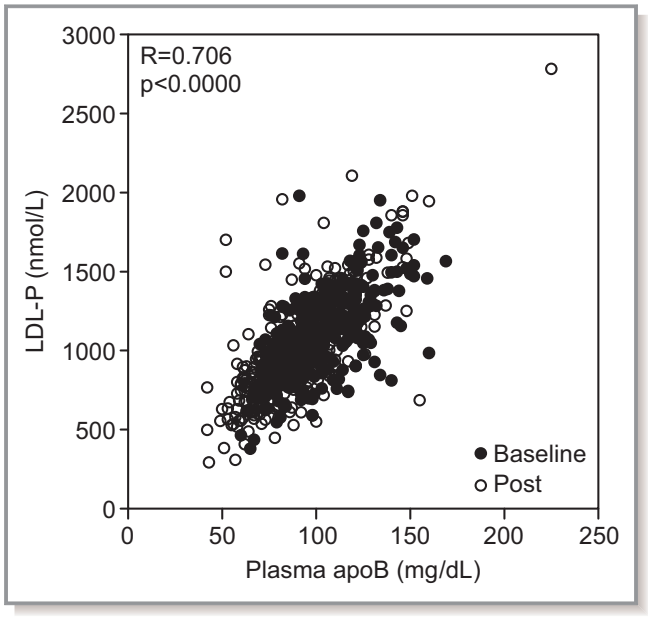

Figure 5. Relationship between on-treatment levels of total plasma apoB and LDL-P concentration based on all samples for participants stabilized on low-dose statin (S20 or A10) as well as more-intensive cholesterol reduction (2× statin, ES 10/20, and R10). 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; A, atorvastatin; Apo, apolipoprotein; E/S, ezetimibe/simvastatin; LDL-P, low-density lipoprotein particle number; R, rosuvastatin; S, simvastatin.

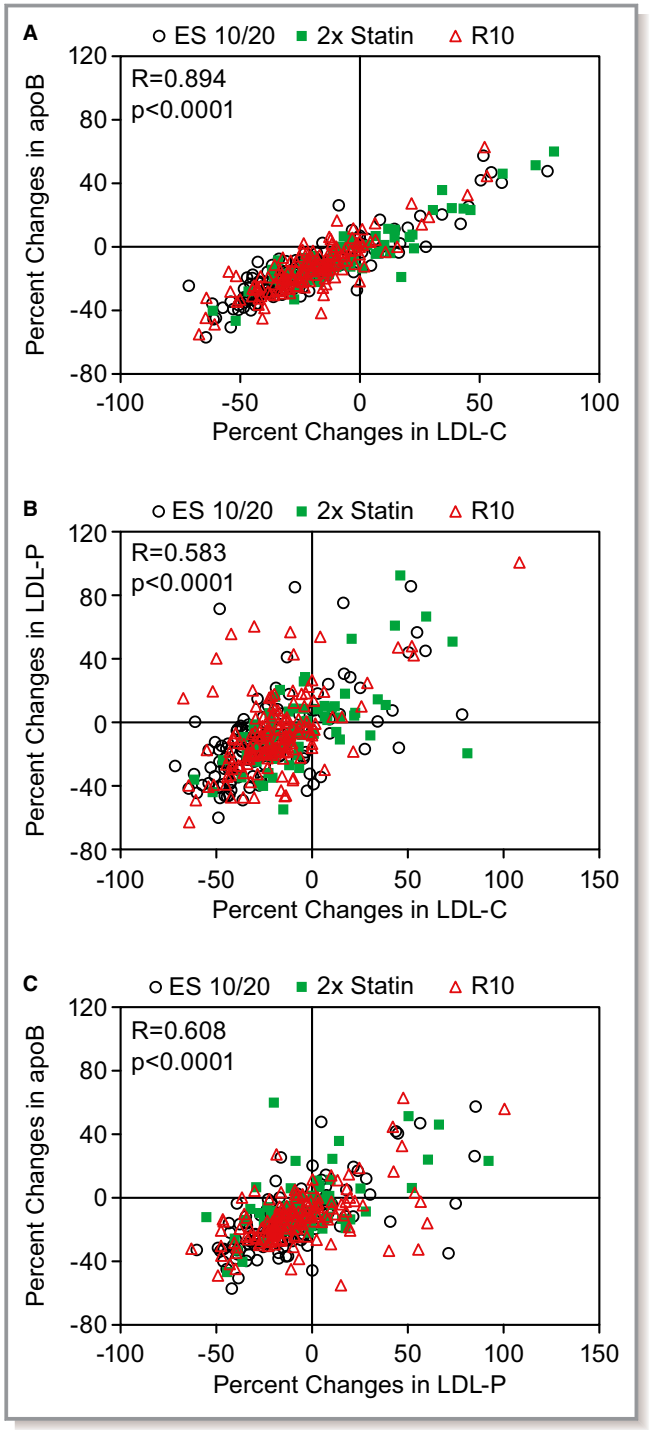

Figure 6. Effect of more-intensive cholesterol reducing regimens (ES 10/20, 2× statin, and R10) on the percent changes for various measures of LDL. (A) LDL-C versus total plasma apoB, (B) LDL-C versus LDL-P, and (C) total plasma apoB versus LDL-P. 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; E/S, ezetimibe/simvastatin; LDL-P, low-density lipoprotein particle number; R, rosuvastatin.

with potent statin therapy.[52] In our study, in patients treated with S10 or A20 therapy, switching from statin therapy to ES10/20 had modest, nonsignificant effects on apoA-I and

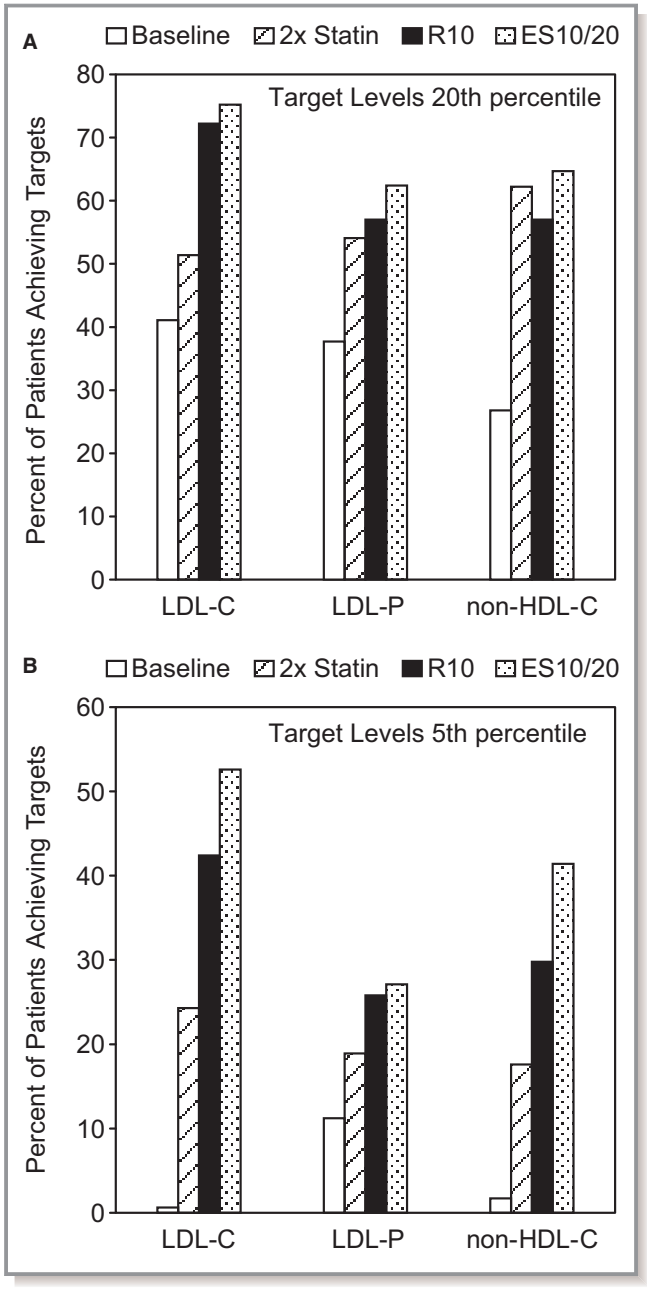

Figure 7. Percent of individuals who reached (A) the 20th percentile target levels for LDL-C, LDL-P, and non–HDL-C or (B) the 5th percentile target levels based on the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. Baseline corresponds to either simvastatin 20 mg or atorvastatin 10 mg. 2× statin indicates doubling the statin dose to simvastatin 40 mg or atorvastatin 20 mg; E/S, ezetimibe/simvastatin; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle number; non–HDL-C, non–high-density lipoprotein cholesterol R, rosuvastatin.

HDL-C that were comparable with those of R10, while doubling the statin dose had no effect. On the other hand, increases in HDL-P with switching to ES10/20 were significantly greater than those with doubling the statin dose and slightly larger than R10. Similarly, in a previously reported study in rosuvastatin- and atorvastatin-treated metabolic syndrome patients, the HDL-P response was greater than those for HDL-C or apoA-I.[31] Such treatment effects could reflect an increase in cholesterol-depleted HDL-P.[31] In a previous analysis of combination ES10/20 therapy in hyperlipidemic patients, we reported that ES10/20+niacin and niacin increased HDL-C more than HDL-P, whereas ES10/20 increased HDL-P more than HDL-C,[53] which is consistent with findings in other studies that have evaluated niacin and statin effects on HDL-P.[31,53,54]

With respect to HDL subclasses, the current data suggest that with either the combination therapy ES 10/20 or doubling of the initial dose of simvastatin or atorvastatin, the changes in overall HDL-P number were closely associated with the changes in the concentration of large HDL-P. The changes in HDL-P observed when the patients were switched to rosuvastatin (10 mg/day) were less dependent on changes in the concentration of large HDL-P. It remains unclear whether changes in the concentrations of any specific HDL subpopulations are more important in risk reduction than overall changes in HDL-P number.[55–57]

In our study, reductions in Lp-PLA_2 were greatest with switching to ES10/20 in terms of plasma concentration and enzyme activity, followed by R10, while reductions with doubling the statin dose were smallest. These results are in line with previous studies that showed reductions in Lp-PLA_2 mass and activity with statins and ES were proportionate to the extent of LDL-C lowering efficacy[25,58,59] and that combination ezetimibe therapy with rosuvastatin and simvastatin provided additional reductions in Lp-PLA_2 mass and activity in patients already receiving statin monotherapy.[58] Ezetimibe, rosuvastatin, and fenofibrate monotherapies have been shown to primarily reduce Lp-PLA_2 activity and mass associated with LDL lipoprotein subfractions via receptor-mediated uptake.[60] On the other hand, it has also been suggested that statin and nonstatin lipid-lowering therapies reduce Lp-PLA_2 through a receptor-independent clearance mechanism and that Lp-PLA_2 changes are weakly correlated with LDL-C changes, indicating that Lp-PLA_2 reduction is only partly explained by LDL-C lowering.[61] In our analysis, reductions in Lp-PLA_2 activity and concentration were consistent with the degree of cholesterol lowering and LDL-P reduction observed for these agents. Similarly, only ES 10/20 treatment resulted in a statistically significant reduction in Lp-PLA_2 specific activity (ie, enzyme activity per unit mass).

A limitation of our study was that the samples used were those available from the original trial and thus were not randomly selected. Nonetheless, the baseline characteristics were generally similar for the treatment groups indicating no selection bias, and the effect of treatment on lipids was comparable to that observed in the original trial. It should be

noted that our analysis was exploratory in nature and thus the results should be considered hypothesis generating. However, the treatment effects observed on the biomarkers in this study are generally consistent with lipid changes observed for these treatments in this and other studies. The treatment effects observed in this study were limited to a 6-week duration, and thus, longer-term effects on these biomarkers are not known. Additionally, the diabetic patients in this study were a mix of both types 1 and 2, and the relevance of these results to each type was not ascertained.

In summary, in diabetic patients with symptomatic CVD on statin monotherapy, switching to more-intensive therapy provided additional improvements in the lipid profile, as assessed by a more-advanced risk panel including lipoprotein subclass analysis with NMR spectroscopy and Lp-PLA_2. These effects were generally greater with combination ES10/20 and R10 therapies than statin dose-doubling, and ES10/20 and R10 were more comparable, consistent with the lipid-altering effects demonstrated for these therapies in these patients.

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Disclosures

Dr Le is a consultant with Liposcience. Dr Wilson is a consultant with Merck and Liposcience. Dr Tershakovec, Dr Tomassini, and Dr Neff are employees and/or former employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ and may hold stock/stock options in the company.

References

1. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care*. 2008;31:811–822.
2. Dunn FL. Hyperlipidemia in diabetes mellitus. *Diabetes Metab Rev*. 1990;6:47–61.
3. Feher MD, Caslake M, Foxton J, Cox A, Packard CJ. Atherogenic lipoprotein phenotype in type 2 diabetes: reversal with micronised fenofibrate. *Diabetes Metab Res Rev*. 1999;15:395–399.
4. Gregg EW, Gu Q, Cheng YJ, Narayan KM, Cowie CC. Mortality trends in men and women with diabetes, 1971 to 2000. *Ann Intern Med*. 2007;147:149–155.
5. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998;339:229–234.
6. Krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care*. 2004;27:1496–1504.
7. Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman M, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D. ESC/EAS Guidelines for the management of dyslipidaemias The task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis*. 2011;217:3–46.
8. Foody JM, Sajjan SG, Hu XH, Ramey DR, Neff DR, Tershakovec AM, Tomassini JE, Wentworth C, Tunceli K. Loss of early gains in low-density lipoprotein cholesterol goal attainment among high-risk patients. *J Clin Lipidol*. 2010;4:126–132.
9. Rublee DA, Burke JP. LDL-C goal attainment in patients who remain on atorvastatin or switch to equivalent or non-equivalent doses of simvastatin: a retrospective matched cohort study in clinical practice. *Postgrad Med*. 2010;122:16–24.
10. Santos RD, Waters DD, Tarasenko L, Messig M, Jukema JW, Chiang CW, Ferrieres J, Foody JM. A comparison of non-HDL and LDL cholesterol goal attainment in a large, multinational patient population: the Lipid Treatment Assessment Project 2. *Atherosclerosis*. 2012;224:150–153.
11. Waters DD, Brotons C, Chiang CW, Ferrieres J, Foody J, Jukema JW, Santos RD, Verdejo J, Messig M, McPherson R, Seung KB, Tarasenko L. Lipid treatment assessment project 2: a multinational survey to evaluate the proportion of patients achieving low-density lipoprotein cholesterol goals. *Circulation*. 2009;120:28–34.
12. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R, Francis GA, Poirier P, Lau DC, Grover S, Genest J Jr, Carpentier AC, Dufour R, Gupta M, Ward R, Leiter LA, Lonn E, Ng DS, Pearson GJ, Yates GM, Stone JA, Ur E. 2012 Update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol*. 2013;29:151–167.
13. Grundy SM. Low-density lipoprotein, non-high-density lipoprotein, and apolipoprotein B as targets of lipid-lowering therapy. *Circulation*. 2002;106:2526–2529.
14. International Atherosclerosis Society. An International Atherosclerosis Society Position Paper: global recommendations for the management of dyslipidemia-full report. *J.Clin.Lipidol*. 2014;8:29–60.
15. Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, Shepherd MD, Seibel JA, Kreisberg R, Goldberg R. American Association of Clinical Endocrinologists' guidelines for management of dyslipidemia and prevention of atherosclerosis. *Endocr Pract*. 2012;18(suppl 1):1–78.
16. Stone NJ, Robinson J, Lichtenstein AH, Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr, Watson K, Wilson PW. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013;29(25 Suppl 2):S1–S45.
17. Bays HE, Neff D, Tomassini JE, Tershakovec AM. Ezetimibe: cholesterol lowering and beyond. *Expert Rev Cardiovasc Ther*. 2008;6:447–470.
18. Catapano AL, Toth PP, Tomassini JE, Tershakovec AM. The efficacy and safety of ezetimibe co-administered with statin therapy in various patient groups. *Clin Lipidol*. 2013;8:13–41.
19. Morrone D, Weintraub WS, Toth PP, Hanson ME, Lowe RS, Lin J, Shah AK, Tershakovec AM. Lipid-altering efficacy of ezetimibe plus statin and statin monotherapy and identification of factors associated with treatment response: a pooled analysis of over 21,000 subjects from 27 clinical trials. *Atherosclerosis*. 2012;223:251–261.
20. Toth PP, Catapano A, Tomassini JE, Tershakovec AM. Update on the efficacy and safety of combination ezetimibe plus statin therapy. *Clin Lipidol*. 2010;5:655–684.
21. Blazing MA, Giugliano RP, Cannon CP, Musliner TA, Tershakovec AM, White JA, Reist C, McCagg A, Braunwald E, Califf RM. Evaluating cardiovascular event reduction with ezetimibe as an adjunct to simvastatin in 18,144 patients after acute coronary syndromes: final baseline characteristics of the IMPROVE-IT study population. *Am Heart J*. 2014;168:205–212.
22. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; on behalf of the IMPROVE-IT investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397.
23. Blazing MA, Guigliano R, Cannon CP, Musliner TA, Tershakovec AM, White JA, Reist C, McCagg A, Braunwald E, Califf RM. IMProved reduction of outcomes: vytorin efficacy international trial on-treatment analysis. Available at: http://

my.americanheart.org/idc/groups/ahamah-public/@wcm/@sop/@scon/documents/downloadable/ucm_469652.pdf. Accessed October 13, 2015.

24. Chehade JM, Gladysz M, Mooradian AD. Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management. *Drugs*. 2013;73:327–339.
25. Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, Brown AS, Brown WV, Cromwell WC, Goldberg RB, McKenney JM, Remaley AT, Sniderman AD, Toth PP, Tsimikas S, Ziajka PE, Maki KC, Dicklin MR. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. *J Clin Lipidol*. 2011;5:338–367.
26. Mangalmurti SS, Davidson MH. The incremental value of lipids and inflammatory biomarkers in determining residual cardiovascular risk. *Curr Atheroscler Rep*. 2011;13:373–380.
27. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J Clin Lipidol*. 2011;5:105–113.
28. Cromwell WC, Otvos JD. Heterogeneity of low-density lipoprotein particle number in patients with type 2 diabetes mellitus and low-density lipoprotein cholesterol <100 mg/dl. *Am J Cardiol*. 2006;98:1599–1602.
29. deGoma EM, Rader DJ. High-density lipoprotein particle number: a better measure to quantify high-density lipoprotein? *J Am Coll Cardiol*. 2012;60:517–520.
30. Mackey RH, Greenland P, Goff DC Jr, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol*. 2012;60:508–516.
31. Rosenson RS, Otvos JD, Hsia J. Effects of rosuvastatin and atorvastatin on LDL and HDL particle concentrations in patients with metabolic syndrome: a randomized, double-blind, controlled study. *Diabetes Care*. 2009;32:1087–1091.
32. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, Tikkanen MJ, Wareham NJ, Faergeman O, Olsson AG, Pedersen TR, Khaw KT, Kastelein JJ. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. *J Am Coll Cardiol*. 2008;51:634–642.
33. Rosen JB, Jimenez JG, Pirags V, Vides H, Hanson ME, Massaad R, McPeters G, Brudi P, Triscari J. A comparison of efficacy and safety of an ezetimibe/simvastatin combination compared with other intensified lipid-lowering treatment strategies in diabetic patients with symptomatic cardiovascular disease. *Diab Vasc Dis Res*. 2013;10:277–286.
34. Otvos JD. Measurement of lipoprotein subclass profiles by NMR spectroscopy. In: Rifai N, Warnick GR, and Dominiczak MH, eds. *Handbook of Lipoprotein Testing*. Washington DC: AACC Press; 2000:609–623.
35. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation*. 2004;109:837–842.
36. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. *Am J Cardiol*. 2008;101:41F–50F.
37. Garza CA, Montori VM, McConnell JP, Somers VK, Kullo IJ, Lopez-Jimenez F. Association between lipoprotein-associated phospholipase A2 and cardiovascular disease: a systematic review. *Mayo Clin Proc*. 2007;82:159–165.
38. Malave H, Castro M, Burkle J, Voros S, Dayspring T, Honigberg R, Pourfarzib R. Evaluation of low-density lipoprotein particle number distribution in patients with type 2 diabetes mellitus with low-density lipoprotein cholesterol <50 mg/dl and non-high-density lipoprotein cholesterol <80 mg/dl. *Am J Cardiol*. 2012;110:662–665.
39. Barrios V, Amabile N, Paganelli F, Chen JW, Allen C, Johnson-Levonas AO, Massaad R, Vandormael K. Lipid-altering efficacy of switching from atorvastatin 10 mg/day to ezetimibe/simvastatin 10/20 mg/day compared with doubling the dose of atorvastatin in hypercholesterolaemic patients with atherosclerosis or coronary heart disease. *Int J Clin Pract*. 2005;59:1377–1386.
40. Constance C, Westphal S, Chung N, Lund M, McCrary SC, Johnson-Levonas AO, Massaad R, Allen C. Efficacy of ezetimibe/simvastatin 10/20 and 10/40 mg compared with atorvastatin 20 mg in patients with type 2 diabetes mellitus. *Diabetes Obes Metab*. 2007;9:575–584.
41. Hing Ling PK, Civeira F, Dan AG, Hanson ME, Massaad R, De Tilleghem CB, Milardo C, Triscari J. Ezetimibe/simvastatin 10/40 mg versus atorvastatin 40 mg in high cardiovascular risk patients with primary hypercholesterolemia: a randomized, double-blind, active-controlled, multicenter study. *Lipids Health Dis*. 2012;11:18.
42. Bays HE, Averna M, Majul C, Muller-Wieland D, De Pellegrin A, Giezek H, Lee R, Lowe RS, Brudi P, Triscari J, Farnier M. Efficacy and safety of ezetimibe added to atorvastatin versus atorvastatin uptitration or switching to rosuvastatin in patients with primary hypercholesterolemia. *Am J Cardiol*. 2013;112:1885–1895.
43. Farnier M, Averna M, Missault L, Vaverkova H, Viigimaa M, Massaad R, Vandormael K, Johnson-Levonas AO, Brudi P. Lipid-altering efficacy of ezetimibe/simvastatin 10/20 mg compared with rosuvastatin 10 mg in high-risk hypercholesterolaemic patients inadequately controlled with prior statin monotherapy: the IN-CROSS study. *Int J Clin Pract*. 2009;63:547–559.
44. Sniderman AD. Differential response of cholesterol and particle measures of atherogenic lipoproteins to LDL-lowering therapy: implications for clinical practice. *J Clin Lipidol*. 2008;2:36–42.
45. Gerber PA, Thalhammer C, Schmied C, Spring S, Amann-Vesti B, Spinas GA, Berneis K. Small, dense LDL particles predict changes in intima media thickness and insulin resistance in men with type 2 diabetes and prediabetes: a prospective cohort study. *PLoS One*. 2013;8:e72763.
46. Nishikura T, Koba S, Yokota Y, Hirano T, Tsunoda F, Shoji M, Hamazaki Y, Suzuki H, Itoh Y, Katagiri T, Kobayashi Y. Elevated small dense low-density lipoprotein cholesterol as a predictor for future cardiovascular events in patients with stable coronary artery disease. *J Atheroscler Thromb*. 2014;21:755–767.
47. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E, Ballantyne CM. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. *Arterioscler Thromb Vasc Biol*. 2014;34:1069–1077.
48. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation*. 1997;95:69–75.
49. Morris B, McLain KH, Malave HA, Underberg JA, Le N, Shapiro MD, Winegar DA, Pourfarzib R. Relationship between plasma apoliprotein B concentration and LDL particle number. *Res Rep Clin Cardiol*. 2014;5:237–242.
50. Sniderman AD, Lamarche B, Contois JH, de Graaf J. Discordance analysis and the Gordian knot of LDL and non-HDL cholesterol versus apoB. *Curr Opin Lipidol*. 2014;25:461–467.
51. Cole TG, Contois JH, Csako G, McConnell JP, Remaley AT, Devaraj S, Hoefner DM, Mallory T, Sethi AA, Warnick GR. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices. *Clin Chem*. 2013;59:752–770.
52. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC Jr, O'Leary DH, Saad MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2007;192:211–217.
53. Le NA, Jin R, Tomassini JE, Tershakovec AM, Neff DR, Wilson PW. Changes in lipoprotein particle number with ezetimibe/simvastatin coadministered with extended-release niacin in hyperlipidemic patients. *J Am Heart Assoc*. 2013;2:e000037 doi: 10.1161/JAHA.113.000037.
54. Jafri H, Alsheikh-Ali AA, Mooney P, Kimmelstiel CD, Karas RH, Kuvin JT. Extended-release niacin reduces LDL particle number without changing total LDL cholesterol in patients with stable CAD. *J Clin Lipidol*. 2009;3:45–50.
55. Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med*. 2011;17:594–603.
56. Kontush A, Chapman MJ. Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities. *Curr Opin Lipidol*. 2010;21:312–318.
57. Rosenson RS, Davidson MH, Le NA, Burkle J, Pourfarzib R. Underappreciated opportunities for high-density lipoprotein particles in risk stratification and potential targets of therapy. *Cardiovasc Drugs Ther*. 2015;29:41–50.
58. Ballantyne CM, Hoogeveen RC, Raya JL, Cain VA, Palmer MK, Karlson BW. Efficacy, safety and effect on biomarkers related to cholesterol and lipoprotein metabolism of rosuvastatin 10 or 20 mg plus ezetimibe 10 mg vs. simvastatin 40 or 80 mg plus ezetimibe 10 mg in high-risk patients: results of the GRAVITY randomized study. *Atherosclerosis*. 2014;232:86–93.
59. Moutzouri E, Liberopoulos EN, Tellis CC, Milionis HJ, Tselepis AD, Elisaf MS. Comparison of the effect of simvastatin versus simvastatin/ezetimibe versus rosuvastatin on markers of inflammation and oxidative stress in subjects with hypercholesterolemia. *Atherosclerosis*. 2013;231:8–14.
60. Saougos VG, Tambaki AP, Kalogirou M, Kostapanos M, Gazi IF, Wolfert RL, Elisaf M, Tselepis AD. Differential effect of hypolipidemic drugs on lipoprotein-associated phospholipase A2. *Arterioscler Thromb Vasc Biol*. 2007;27:2236–2243.
61. Zhang B, Fan P, Shimoji E, Itabe H, Miura S, Uehara Y, Matsunaga A, Saku K. Modulating effects of cholesterol feeding and simvastatin treatment on platelet-activating factor acetylhydrolase activity and lysophosphatidylcholine concentration. *Atherosclerosis*. 2006;186:291–301.