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Effect of ezetimibe/simvastatin compared with atorvastatin on lipoprotein subclasses in patients with type 2 diabetes and hypercholesterolaemia

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Aim: To evaluate the effects of the usual starting and next higher doses of ezetimibe/simvastatin and atorvastatin on the cholesterol content of lipoprotein subclasses in patients with type 2 diabetes and hypercholesterolaemia.
Methods: This *post hoc* analysis compared the effects of treatment with ezetimibe/simvastatin 10/20 mg vs. atorvastatin 10 and 20 mg/day and ezetimibe/simvastatin 10/40 mg/day vs. atorvastatin 40 mg/day on the cholesterol content of lipoprotein subclasses in the modified intent-to-treat (mITT) population (n = 1013) and in subgroups of patients with triglyceride (TG) levels <200 mg/dl (n = 600) and ≥200 mg/dl (2.6 mmol/l) (n = 413).
Results: Ezetimibe/simvastatin significantly reduced low-density lipoprotein cholesterol (LDL-C) subclasses LDL_1-C, LDL_2-C and LDL_3-C; real LDL-C (LDL-C^r); intermediate-density lipoprotein cholesterol (IDL-C), IDL_1-C, IDL_2-C; very low-density lipoprotein cholesterol (VLDL-C), $VLDL_3$-C; and remnant-like lipoprotein cholesterol (RLP-C) from baseline more than atorvastatin at all dose comparisons ($p < 0.01$) in the mITT population. Significant improvements were also observed in high-density lipoprotein cholesterol (HDL-C) subclass HDL_3-C at the ezetimibe/simvastatin 10/20 mg vs. atorvastatin 20 mg and highest dose comparisons ($p < 0.001$) and in $VLDL_{1+2}$-C at the lowest and highest dose comparisons ($p < 0.001$). Changes in LDL_4-C and LDL-C subclass patterns (A, B and I) were comparable for both treatments. Generally, similar results were observed for patients with TG levels <200 and ≥200 mg/dl (2.3 mmol). For both treatments, notable differences between TG subgroups were that patients with elevated TGs had smaller reductions in LDL_2-C, slightly smaller decreases in all IDL subclasses and greater decreases in all VLDL-C subclasses than those with lower TG levels. Frequency of pattern B was also reduced more in patients with higher TGs for both treatments.
Conclusions: Ezetimibe/simvastatin reduced the cholesterol content of most lipoprotein subclasses from baseline with generally similar efficacy in patients with low and high TGs. Despite the different mechanism of action of ezetimibe, the response to ezetimibe/simvastatin and atorvastatin treatment related to these lipoprotein subclasses was generally consistent with the overall effects of these therapies on the major lipid/lipoprotein classes. The clinical significance of these results awaits further study.

Keywords: atorvastatin, ezetimibe/simvastatin, hypertriglyceridaemia, lipoprotein subclasses, type 2 diabetes
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Introduction

Patients with type 2 diabetes mellitus are at increased risk for cardiovascular disease [1,2]. This elevated risk has been attributed in part to lipid abnormalities (atherogenic or diabetic dyslipidaemia) commonly associated with type 2 diabetes and metabolic syndrome including high levels of triglycerides (TGs) and TG-rich lipoproteins [e.g. very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and remnant-like lipoprotein (RLP)], low levels of high-density lipoprotein (HDL) and a preponderance of small dense low-density lipoprotein (LDL) particles [3].

Guidelines from the American Diabetes Association (ADA) and National Cholesterol Education Program (NCEP) Adult Treatment Program (ATP) III continue to identify elevated LDL cholesterol (LDL-C) as the primary target for lipid-modifying therapy [1,3]. Statin treatment is recommended as an adjunct to lifestyle modification in patients with type 2 diabetes to reduce LDL-C. In addition, combination drug therapy may be needed to achieve more aggressive LDL-C lowering and to improve the lipid abnormalities in patients with dyslipidaemia [3].

Standard lipid measurements may underestimate cardiovascular risk in type 2 diabetes patients because of the complexity of the lipoprotein profile. Evaluation of lipoprotein subclass profiles may provide additional information in guiding the assessment of cardiovascular risk in type 2 diabetes and lipid-modifying therapy. The major lipoprotein classes are composed of heterogeneous mixtures of lipoprotein subspecies that differ in particle size, density and lipid content. On the basis of density, LDL lipoproteins can be distributed into four subclasses ranging from large buoyant LDL_1 and LDL_2 to small dense LDL_3 and LDL_4. Studies have indicated that the presence of higher levels of smaller, more dense LDL subclasses (referred to as LDL pattern B) may confer greater cardiovascular risk in patients with dyslipidaemia than larger more buoyant subclasses (pattern A) for any given level of LDL-C [4–7]. TG-rich lipoproteins can also be distributed by density into subclasses, and high levels of all IDL and VLDL subfractions are associated with an increased atherogenic potential [5,8]. In contrast, HDL lipoprotein levels are inversely associated with cardiovascular risk, and both HDL_2 and HDL_3 subclasses may have cardioprotective effects [9,10].

The present analysis compares the effects of the usual, recommended starting doses and the next higher doses of ezetimibe/simvastatin and atorvastatin on the cholesterol content of lipoprotein subclasses in patients with type 2 diabetes and hypercholesterolaemia who participated in the previously reported *Vyt*orin vs. *A*torvastatin in Patients With Type 2 Diabetes Me*l*litus and Hypercholesterolaemia (VYTAL) study [11]. These comparisons were also made in patient subgroups with TG levels <200 and ≥200 mg/dl (2.3 mmol/l).

Methods

This *post hoc* analysis used data from a previously reported multicenter, randomized, double-blind, 6-week trial that assessed the efficacy and safety of the usual starting doses of ezetimibe/simvastatin (10/20 mg/day) and atorvastatin (10 and 20 mg/day) and the next dose levels of these agents (ezetimibe/simvastatin 10/40 mg and atorvastatin 40 mg) in 1229 type 2 diabetes patients with hypercholesterolaemia [11]. Eligible patients, 18–80 years of age with haemoglobin A1C (HbA1C) levels ≤8.5%, were enrolled at 147 centres in the USA.

Patients discontinued their current lipid-modifying therapy during a 3- to 5-week washout period and then maintained their ADA-compatible diets during a 4-week placebo run-in phase. Patients who were not at the NCEP ATP III LDL-C goal of <100 mg/dl (2.6 mmol/l) and had TG levels ≤400 mg/dl (4.5 mmol/l) during the third week of the run-in period were then randomly assigned to one of five treatment arms: ezetimibe/simvastatin 10/20 or 10/40 mg or atorvastatin 10, 20 or 40 mg, each once daily for 6 weeks.

For the present analysis, the Vertical Auto Profile II method (Atherotech, Birmingham, AL, USA) was used to quantify the amount of cholesterol associated with the following lipoprotein subclasses: $LDL_{1–4}$, real LDL-C ($LDL\text{-}C^r$) [real index of cholesterol associated with true LDL particle and excludes IDL and lipoprotein (a)], total IDL, $IDL_{1 \text{ and } 2}$, total VLDL, $VLDL_{1+2}$, $VLDL_3$, HDL_2 and HDL_3 and RLP in plasma samples collected at baseline and at week 6 following a ≥12-h fast. The cholesterol content of these lipoprotein subclasses was also compared in subgroups of patients with TGs <200 or ≥200 mg/dl (2.3 mmol/l). LDL subclass pattern was assessed by segmented gradient gel electrophoresis (S_3GGE) (Berkeley HeartLab, Inc., Burlingame, CA, USA).

The lipoprotein subclass analysis was performed in the modified intent-to-treat (mITT) population, which included all randomized patients with a valid baseline and at least one valid postbaseline measurement. Per cent change from baseline in the lipoprotein subclasses was not expected to satisfy the assumption of a parametric analysis of covariance (ANCOVA). Therefore, the primary analysis was based on the per cent change from baseline in all lipoprotein subclasses (except LDL pattern) using a non-parametric ANCOVA model that included terms for

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treatment (ezetimibe/simvastatin 10/20 or 10/40 mg and atorvastatin 10, 20 or 40 mg), pretreatment LDL-C stratum [≥100 to <130 mg/dl (2.6–3.4 mmol/l), ≥130 to <160 mg/dl (3.4–4.1 mmol/l), ≥160 to <190 mg/dl (4.1–4.9 mmol/l) and ≥190 mg/dl (4.9 mmol/l)] and baseline value of the dependent variable. Differences between ezetimibe/simvastatin 10/20 mg and atorvastatin 10 mg, ezetimibe/simvastatin 10/20 mg and atorvastatin 20 mg and ezetimibe/simvastatin 10/40 mg and atorvastatin 40 mg were compared by ANCOVA using ranks based on normal scores. For the treatment group comparisons within the TG subgroups, no formal statistical testing was performed. Differences in medians and their corresponding 95% confidence intervals were evaluated to identify possible trends or to support previous findings.

Differences among treatment groups in post-treatment LDL-C^r were compared using the Cochran–Mantel–Haenszel chi-squared statistic. To control inflation of the type I error rate, multiplicity adjustment using the false discovery rate procedure was applied to these lipoprotein subclass variables [12].

Results

Baseline Characteristics

Of the 1229 patients randomized in the original study, 1013 patients in the mITT population had baseline and postbaseline measurements for lipoprotein subclasses [11]. Baseline characteristics as previously reported were similarly distributed among treatment groups. Mean baseline levels were 145 mg/dl (3.8 mmol/l) for LDL-C and 45 mg/dl (1.2 mmol/l) for HDL cholesterol (HDL-C), and the median baseline level for TGs was 178 mg/dl (2.0 mmol/l). Median baseline values for the various LDL, IDL, VLDL and HDL subclasses and for RLP at baseline were comparable across treatment groups in the mITT population (table 1). Among LDL lipoprotein subclasses, baseline levels of LDL_3-C were highest and LDL_4-C levels were lowest. Within IDL and VLDL lipoprotein subclasses, levels of IDL cholesterol (IDL_2-C) were greater than those of IDL_1-C and VLDL cholesterol ($VLDL_3$-C) was slightly higher than $VLDL_{1+2}$-C. Baseline HDL_3-C levels were greater than those of HDL_2-C.

Within the subgroups of patients with TGs <200 and ≥200 mg/dl (2.3 mmol/L), baseline levels of lipoproteins were also comparable across treatment arms (table 1). However, the baseline levels of several lipoprotein subclasses differed among these two subgroups. Higher levels of LDL_3-C (~1.4-fold), LDL_4-C (~4.0-fold), IDL-C (~1.4-fold), IDL_1-C (~1.8-fold) and the VLDL subclasses

Table 1 Baseline median values of lipoprotein subclasses in the mITT population and TG subgroups

Lipoprotein subclass (mg/dl)*	All patients (mITT)					TGs <200 mg/dl (2.3 mmol/l)					TGs ≥200 mg/dl (2.3 mmol/l)				
	A 10 (n = 205)	E/S 10/20 (n = 206)	A 20 (n = 205)	E/S 10/40 (n = 193)	A 40 (n = 204)	A 10 (n = 116)	E/S 10/20 (n = 122)	A 20 (n = 127)	E/S 10/40 (n = 116)	A 40 (n = 119)	A 10 (n = 89)	E/S 10/20 (n = 84)	A 20 (n = 78)	E/S 10/40 (n = 77)	A 40 (n = 85)
LDL_1-C	23.1	23.5	24.0	24.0	22.6	21.9	23.4	23.6	24.0	22.3	24.8	23.5	24.1	24.0	22.8
LDL_2-C	28.0	30.8	32.3	31.5	29.9	35.8	40.0	38.6	38.8	38.7	17.8	16.4	19.1	19.2	15.8
LDL_3-C	53.6	51.8	53.9	52.5	55.3	49.1	49.3	47.7	46.1	48.2	59.7	58.0	59.9	64.3	61.0
LDL_4-C	8.1	7.3	7.2	7.2	8.3	4.0	4.4	4.4	3.8	4.2	16.6	15.8	14.3	15.5	18.6
LDL-C‡†	117.0	119.5	120.0	120.0	119.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
IDL-C	21.0	21.0	20.0	21.0	19.0	18.0	18.5	18.0	18.0	17.0	26.0	24.0	24.5	25.0	22.0
IDL_1-C	7.1	6.5	6.9	7.1	6.7	5.4	5.4	5.3	5.5	4.8	10.1	9.0	9.4	9.3	8.8
IDL_2-C	13.9	14.3	13.7	14.1	12.5	12.7	13.5	12.8	13.2	11.9	15.8	15.1	14.5	15.4	13.1
VLDL-C	29.0	29.0	29.0	30.0	29.0	25.0	24.0	25.0	25.0	24.0	40.0	38.5	38.0	39.0	36.0
$VLDL_{1+2}$-C	13.1	12.7	12.7	13.2	12.5	10.3	10.7	10.8	10.8	10.2	18.3	18.0	17.5	17.9	17.0
$VLDL_3$-C	16.0	16.5	16.0	17.0	16.0	14.0	14.0	14.0	14.0	14.0	22.0	21.0	20.0	20.0	20.0
HDL_2-C	9.0	9.0	10.0	10.0	10.0	10.0	10.0	11.0	12.0	11.0	8.0	8.0	8.0	9.0	9.0
HDL_3-C	34.0	34.0	35.0	35.0	35.0	35.0	36.0	36.0	36.0	37.0	32.0	32.0	33.0	34.0	33.0
RLP-C‡	37.0	36.0	37.0	39.0	35.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

A, atorvastatin 10, 20 and 40 mg; E/S, ezetimibe/simvastatin 10/20 and 10/40 mg; HDL-C, HDL cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, LDL cholesterol; mITT, modified intent-to-treat; nd, not determined; RLP-C, remnant-like lipoprotein cholesterol; TGs, triglycerides.

*SI conversion factors: to convert cholesterol to millimoles per litre, multiply by 0.0259.

†Real index of cholesterol associated with true LDL particle, excludes IDL and lipoprotein (a).

‡Remnant-like lipoprotein (chylomicrons, IDL and $VLDL_3$).

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(~1.5- to 1.7-fold) were observed in patients with TG levels ≥200 vs. <200 mg/dl (2.3 mmol/L), while levels of LDL_2-C (~2.0-fold) were higher in patients with TGs <200 vs. ≥200 mg/dl (2.3 mmol/L). Levels of LDL_1-C, IDL_2-C, HDL_2-C and HDL_3-C were more similar in patients, irrespective of TG levels.

Changes from Baseline in Lipoprotein Subclasses

LDL Subclasses

Treatment with ezetimibe/simvastatin significantly reduced the cholesterol in LDL subfractions (LDL_1-C, LDL_2-C, LDL_3-C and LDL-C^r) compared with atorvastatin at all dose comparisons in the mITT population ($p < 0.01$) (figure 1A). Levels of LDL_4-C were decreased from baseline by a smaller percentage than those of the other LDL subclasses for both treatments, resulting in non-significant treatment comparisons.

Regardless of baseline TG levels above or below 200 mg/dl (2.3 mmol/l), ezetimibe/simvastatin reduced LDL_1-C, LDL_2-C and LDL_3-C more than atorvastatin at all dose comparisons (figure 1B, C), consistent with the effects observed for these subclasses in the mITT population. For both treatments, reductions from baseline in LDL_1-C and LDL_3-C were generally similar among patients with TGs <200 or ≥200 mg/dl (2.3 mmol/l), whereas reductions in LDL_2-C were more pronounced in patients with TG levels <200 mg/dl (2.3 mmol/l) compared with levels of ≥200 mg/dl (2.3 mmol/l). Both treatments lowered LDL_4-C from baseline in the elevated TG subgroup and increased this subfraction in the lower TG subgroup, although as noted above, the baseline level of LDL_4-C was substantially higher in patients with TGs ≥200 mg/dl (2.3 mmol/l) and very low for those with TGs <200 mg/dl (2.3 mmol/l).

IDL and VLDL Subclasses

Ezetimibe/simvastatin treatment significantly reduced IDL-C, IDL_1-C and IDL_2-C compared with atorvastatin at all dose comparisons in the mITT population ($p < 0.01$) (figure 2A). Similar to the effects of ezetimibe/simvastatin in the mITT population, ezetimibe/simvastatin was observed to reduce the cholesterol in all IDL subclasses to a greater extent than atorvastatin in patients with TGs <200 and ≥200 mg/dl (2.3 mmol/l) (figure 2B, C). Reductions from baseline in IDL-C and its subclasses were slightly greater among patients with TGs <200 mg/dl (2.3 mmol/l) compared with ≥200 mg/dl (2.3 mmol/l) for both treatments.

Reductions from baseline in VLDL-C and $VLDL_3$-C were significantly greater upon treatment with ezetimibe/simvastatin compared with atorvastatin at all dose comparisons (all $p < 0.001$ except $p < 0.01$ for VLDL-C ezetimibe/simvastatin 10/20 mg vs. atorvastatin 20 mg) (figure 3A). Per cent reductions from baseline in $VLDL_{1+2}$-C were significantly higher for ezetimibe/simvastatin 10/20 mg vs. atorvastatin 10 mg and ezetimibe/simvastatin 10/40 mg vs. atorvastatin 40 mg ($p < 0.001$) and were not found to be different for ezetimibe/simvastatin 10/20 mg vs. atorvastatin 20 mg ($p = 0.085$). Both ezetimibe/simvastatin and atorvastatin treatments lowered cholesterol in VLDL to a smaller degree than was observed for LDL and IDL lipoproteins (figure 3).

Among patients with TGs <200 or ≥200 mg/dl (2.3 mmol/l), VLDL-C and $VLDL_3$-C were reduced to a greater extent by ezetimibe/simvastatin compared with atorvastatin at all dose comparisons (figure 3B, C). These results were also consistent with the significantly greater effects of ezetimibe/simvastatin compared with atorvastatin in the mITT population. Per cent declines in $VLDL_{1+2}$-C also favoured ezetimibe/simvastatin over atorvastatin at each of the dose comparisons. Within each treatment group, per cent reductions from baseline in VLDL-C, $VLDL_{1+2}$-C and $VLDL_3$-C were more marked among patients with TGs ≥200 mg/dl (2.3 mmol/l) compared with those with TGs <200 mg/dl (2.3 mmol/l).

HDL Subclasses

Ezetimibe/simvastatin increased HDL_3-C from baseline by 2.7% at the 10/20 mg dose and 2.6% at the 10/40 mg dose, while atorvastatin had no effect at the 10 or 20 mg dose and decreased HDL_3-C by 2.5% at the 40 mg dose ($p < 0.001$ for ezetimibe/simvastatin 10/20 mg vs. atorvastatin 20 mg and ezetimibe/simvastatin 10/40 mg vs. atorvastatin 40 mg and $p = 0.078$ for ezetimibe/simvastatin 10/20 mg vs. atorvastatin 10 mg) (figure 4). In patients with TGs <200 or ≥200 mg/dl (2.3 mmol/l), ezetimibe/simvastatin treatment increased HDL_3-C levels comparably to those observed in the mITT population (figure 4). In both TG subgroups, atorvastatin 10 mg had no effect on HDL_3-C levels. Atorvastatin 20 mg reduced HDL_3-C by 2.6% in patients with lower TGs and had no effect in those with higher TGs. At atorvastatin 40 mg, HDL_3-C was reduced by 3.1% in patients with TGs <200 mg/dl (2.3 mmol/l) and increased by 2.4% in those with TGs ≥200 mg/dl (2.3 mmol/l). Both treatments were similarly ineffective in raising HDL_2-C in the mITT population and in the TG subgroups (data not shown).

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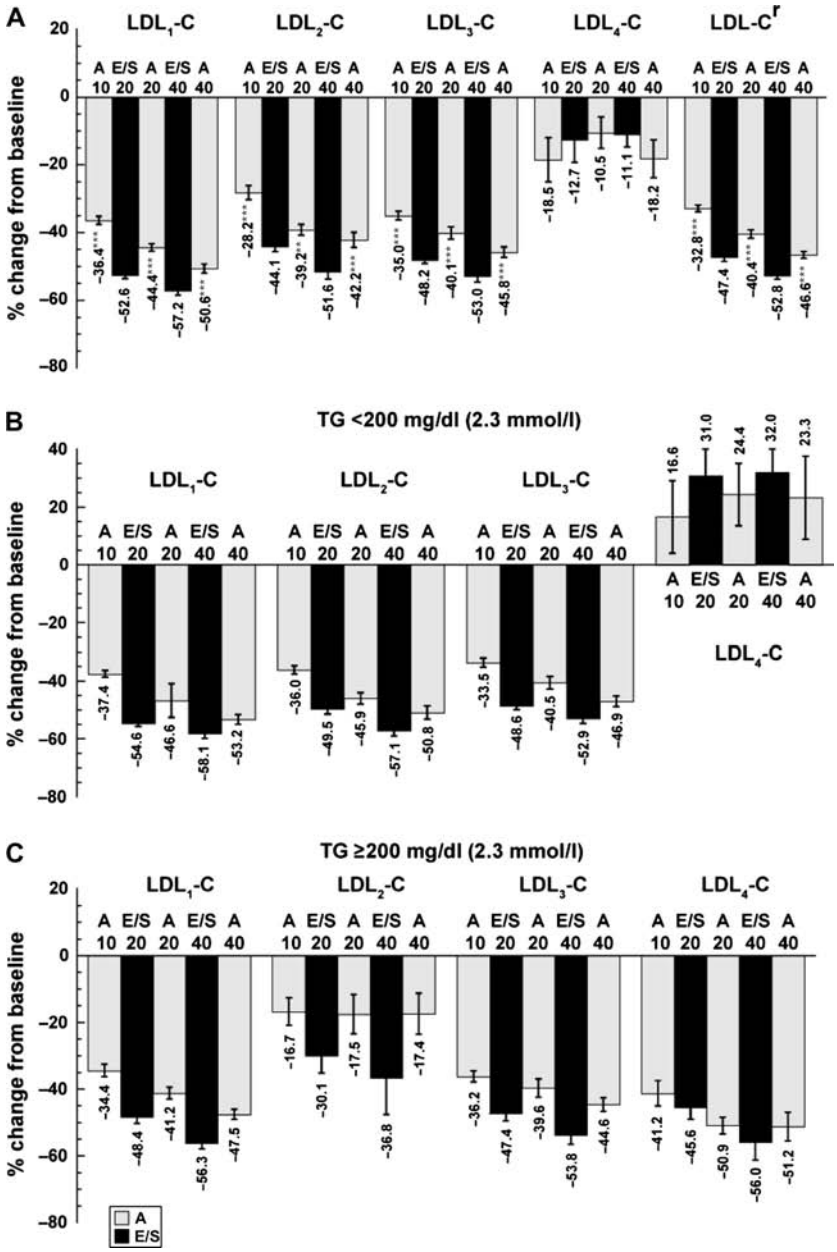

Fig. 1 Per cent change from baseline in LDL cholesterol subclasses in the mITT population (panel A), subgroup with TGs <200 mg/dl (2.3 mmol/l) (panel B) and subgroup with TGs ≥200 mg/dl (2.3 mmol/l) (panel C). **$p < 0.01$ and ***$p < 0.001$ for the indicated treatment group comparisons: E/S 10/20 mg vs. A 10 mg, E/S 10/20 mg vs. A 20 mg and E/S 10/40 mg vs. A 40 mg. A, atorvastatin; E/S, ezetimibe/simvastatin; mITT, modified intent-to-treat; TGs, triglycerides.

Remnant-like lipoprotein

Treatment with ezetimibe/simvastatin also lowered RLP cholesterol (RLP-C) from baseline significantly more than atorvastatin at all dose comparisons in the mITT population (data not shown). Treatment with ezetimibe/simvastatin 10/20 mg reduced RLP-C by 55.3% compared with 40.0% for atorvastatin 10 mg ($p < 0.001$) and 46.2% for atorvastatin 20 mg ($p < 0.001$) and by 58.3 and 50.9% for ezetimibe/simvastatin 10/40 mg, vs. atorvastatin 40 mg, respectively ($p < 0.001$).

LDL Subclass Pattern

The proportions of patients having LDL subclass pattern A, B and I phenotypes at baseline in the mITT population

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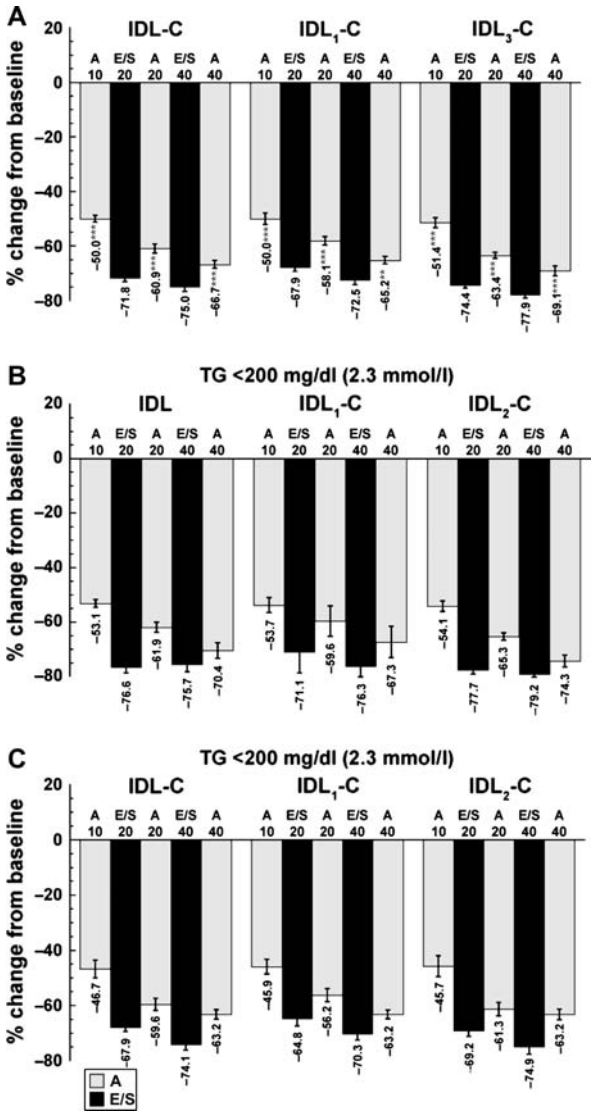

Fig. 2 Per cent change from baseline in intermediate-density lipoprotein cholesterol subclasses in the mITT population (panel A), subgroup with TGs <200 mg/dl (2.3 mmol/l) (panel B) and subgroup with TGs ≥200 mg/dl (2.3 mmol/l) (panel C). $^{**}p < 0.01$ and $^{***}p < 0.001$ for the indicated treatment group comparisons: E/S 10/20 mg vs. A 10 mg, E/S 10/20 mg vs. A 20 mg and E/S 10/40 mg vs. A 40 mg. A, atorvastatin; E/S, ezetimibe/simvastatin; mITT, modified intent-to-treat; TGs, triglycerides.

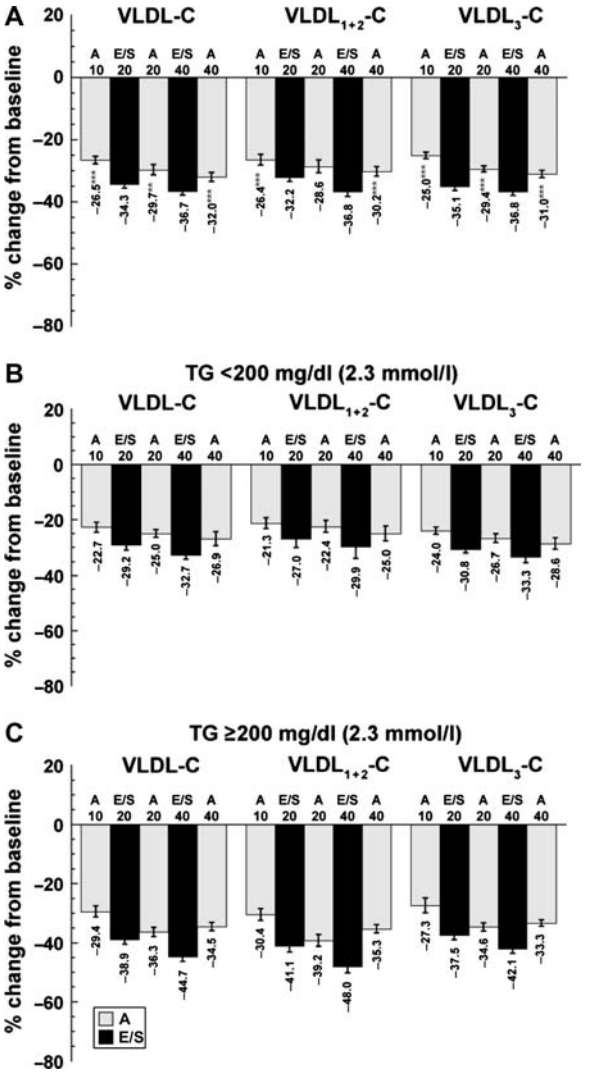

Fig. 3 Per cent change from baseline in very low-density lipoprotein cholesterol subclasses in the mITT population (panel A), subgroup with TGs <200 mg/dl (2.3 mmol/l) (panel B) and subgroup with TGs ≥200 mg/dl (2.3 mmol/l) (panel C). $^{**}p < 0.01$ and $^{***}p < 0.001$ for the indicated treatment group comparisons: E/S 10/20 mg vs. A 10 mg, E/S 10/20 mg vs. A 20 mg and E/S 10/40 mg vs. A 40 mg. A, atorvastatin; E/S, ezetimibe/simvastatin; mITT, modified intent-to-treat.

were generally similar at all doses and across pooled doses for both ezetimibe/simvastatin and atorvastatin, with a slightly higher frequency of pattern B (figure 5). In patients with TG levels <200 mg/dl (2.3 mmol/l), the frequencies of patterns A and I at baseline were higher relative to those of pattern B, and in patients with TG levels ≥200 mg/dl (2.3 mmol/l), the frequencies of patterns A and I were substantially lower compared with those of pattern B at individual doses and across doses for both treatments (figure 5). Overall, the numbers of patients with patterns A and I were greatest in patients with low TGs, and those with pattern B were greatest in patients with elevated TGs. Both treatments had minimal effect on changes in LDL subclass pattern at the end of the study in the mITT population and lower TG

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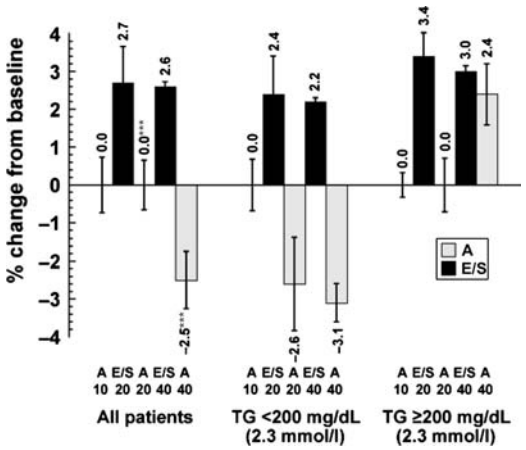

Fig. 4 Per cent change from baseline in HDL_3-C. ***$p < 0.001$ for the indicated treatment group comparisons: E/S 10/20 mg vs. A 10 mg, E/S 10/20 mg vs. A 20 mg and E/S 10/40 mg vs. A 40 mg. A, atorvastatin; E/S, ezetimibe/simvastatin; HDL-C, HDL cholesterol.

group. In patients with high TGs, small increases in the frequencies of A and I patterns and decreases in B pattern were observed following treatment with both ezetimibe/simvastatin and atorvastatin.

Discussion

Previously, the safety and lipid-altering efficacy of ezetimibe/simvastatin were demonstrated in comparison with atorvastatin at the recommended usual and next higher doses in type 2 diabetes patients (VYTAL study) [11]. In that study, ezetimibe/simvastatin reduced levels of LDL-C, total cholesterol and non-HDL-C, increased levels of HDL-C and improved patient attainment of LDL-C levels <70 mg/dl (1.8 mmol/l) significantly more than atorvastatin at all dose comparisons ($p < 0.001$) (data not shown). In view of these results, it was of interest to further assess the effect of these treatments on lipoprotein subclasses, which may provide additional clinically relevant information. The present analysis shows that ezetimibe/simvastatin also significantly reduces the cholesterol content of most LDL lipoprotein subclasses including LDL_1, LDL_2, LDL_3 and LDL^r and TG-rich IDL, IDL_1, IDL_2, VLDL, $VLDL_3$ and RLP and did so generally to a greater extent when compared with corresponding doses of atorvastatin. In addition, ezetimibe/simvastatin significantly reduced $VLDL_{1+2}$-C and increased HDL_3-C at most dose comparisons with atorvastatin.

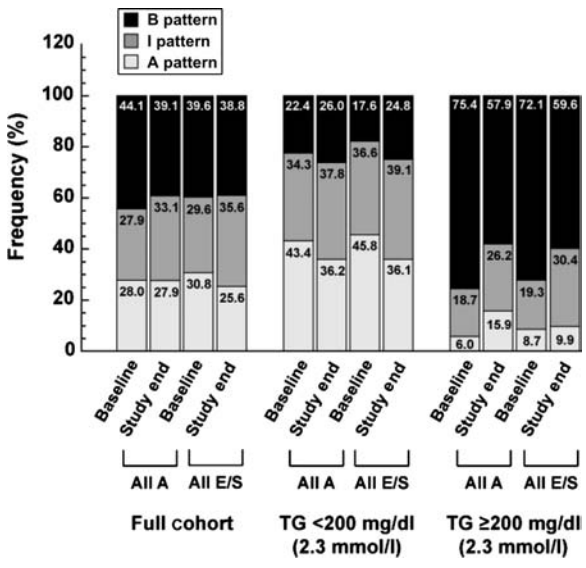

Fig. 5 Frequency of change in LDL subclass pattern at baseline and study end pooled across doses. All A, Atorvastatin 10, 20 and 40 mg; all E/S, ezetimibe/simvastatin 10/20 and 10/40 mg.

Studies have shown that patients with type 2 diabetes have altered distributions of LDL subclasses with increased levels of small dense LDL_3, reduced levels of larger more buoyant LDL_1 and LDL_2 subclasses and variable levels of LDL_4 [13–15]. In this study, baseline levels of small dense LDL_3-C were also highest among the LDL subclasses, accounting for ~45% of total LDL-C^r, while levels of LDL_1-C and LDL_2-C and smaller denser LDL_4-C comprised 20, 26 and 7%, respectively, of the measured LDL-C^r. Therefore, small dense LDL_3 and LDL_4 subclasses (pattern B) that may carry relatively greater atherogenic risk than larger more buoyant lipoproteins (pattern A) represented just over half of the total LDL-C^r at baseline, consistent with the elevated cardiovascular risk of patients with type 2 diabetes [1,2]. While both treatments substantially reduced LDL-C^r and LDL_1-C, LDL_2-C and LDL_3-C subclasses from baseline at all dose comparisons, declines in LDL_4-C were modest in the full cohort.

TG-rich lipoproteins can contribute to atherogenesis by entering the arterial intima or indirectly through lipolytic processing into other highly atherogenic particles. In particular, large VLDL can serve as a precursor for small dense LDL particles, whereas smaller VLDL and IDL may be more likely to enter the arterial intima directly to promote atherogenesis [8,13]. In type 2 diabetes patients, decreased hepatic insulin sensitivity results in enhanced VLDL secretion and reduced clearance of large VLDL, which in turn leads to increased concentrations of smaller denser LDL, IDL and other remnant lipoprotein particles [16,17]. In the present study, cholesterol levels associated with the smaller more dense IDL_2 and $VLDL_3$ subclasses were higher at baseline than

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larger more buoyant IDL_1 and $VLDL_{1+2}$, accounting for about 66 and 55% of the total IDL-C and VLDL-C concentrations, respectively. A higher cholesterol content in these lipoprotein subclasses has been previously observed in type 2 diabetes patients [18]. Treatment with ezetimibe/simvastatin was significantly more effective than corresponding doses of atorvastatin in reducing the cholesterol associated with all of the TG-rich lipoprotein subclasses assessed and that of RLP, which includes remnant chylomicrons, IDL and $VLDL_3$.

Consistent with an increased prevalence of atherogenic dyslipidaemia among type 2 diabetes patients with elevated TGs, baseline levels were ~1.4- to 2.0-fold higher for LDL_3-C, IDL-C, IDL_1-C and all VLDL subclasses and were substantially higher for LDL_4-C (approximately fourfold) than in those with lower TG levels. Irrespective of baseline TG levels, ezetimibe/simvastatin treatment was associated with greater reductions from baseline in LDL_1-C, LDL_2-C, LDL_3-C, IDL-C, IDL_1-C, IDL_2-C, VLDL-C and $VLDL_3$-C compared with corresponding atorvastatin doses, and these findings were consistent with the significant findings observed in the entire mITT cohort. Reduction in $VLDL_{1+2}$-C was also greater with ezetimibe/simvastatin treatment compared with atorvastatin in the subgroups with normal and elevated TGs.

TG levels appeared to influence the magnitude of the response to both treatments across certain lipoprotein subclasses. Compared with patients who had TGs <200 mg/dl (2.3 mmol/l), those with TGs ≥200 mg/dl (2.3 mmol/l) tended to have more modest reductions in LDL_2-C, a somewhat reduced response in all IDL-C subclasses and greater declines in all VLDL-C subclasses. Patients with elevated TGs also experienced decreases in LDL_4-C when treated with ezetimibe/simvastatin or atorvastatin compared with increases in LDL_4-C in those with normal TG levels. This effect may be related to a greater reduction of precursors, leading to the generation and/or increased processing of LDL_4 particles in the treated hypertriglyceridemic patients in whom levels of LDL_4-C at baseline were substantially higher (approximately fourfold) compared with the very low LDL_4-C levels in those with TGs <200 mg/dl (2.3 mmol/l).

In this study, the number of patients with LDL pattern B phenotype (S_3GGE) at baseline was slightly higher than that of those with pattern A or I in the mITT population, consistent with an increased prevalence of the more atherogenic LDL phenotype observed in type 2 diabetes patients [19]. The presence of pattern B phenotype was most pronounced in patients with TGs ≥200 mg/dl (2.3 mmol/l), whereas the frequencies of patterns A and I were substantially higher relative to pattern B in patients with TGs <200 mg/dl (2.3 mmol/l). In the full mITT cohort, while both ezetimibe/simvastatin and atorvastatin had minimal effect on LDL subclass pattern, both treatments effectively reduced the cholesterol content of most LDL subclasses. These results are similar to those previously reported for statins and ezetimibe with and without simvastatin, which reduced LDL subclasses but had a limited role in altering LDL size and distribution in patients with type 2 diabetes, dyslipidaemia or hypercholesterolaemia [20–22]. However, upon further review of patients with elevated TG levels, small increases in the frequency of subclass patterns A and I and decreases in pattern B were observed for both ezetimibe/simvastatin and atorvastatin treatment by the end of this 6-week study.

In contrast to other lipoprotein subclasses, HDL_2 and HDL_3 confer cardioprotective effects [13,23,24]. Diminished levels of HDL-C in type 2 diabetes patients are attributed to reductions in the HDL_2 subclass [13,19,24]. In this study, median concentrations of HDL_2-C at baseline were threefold to fourfold lower than those of HDL_3-C regardless of TG level. Treatment with ezetimibe/simvastatin slightly but significantly raised HDL_3-C from baseline but did not affect HDL_2-C in the mITT cohort. In comparison, atorvastatin had no effect and/or decreased levels of these HDL subclasses. Results in patients with elevated TGs were consistent with the overall population, although increases in HDL_3-C were slightly higher.

While this study evaluated lipoprotein subclass profiles on the basis of cholesterol content and distribution, these results may differ from those that have assessed lipoprotein size and particle concentrations [13,20]. Additional studies are needed to fully understand the relationship of differences in physicochemical properties and particle concentrations of lipoprotein subclasses to cardiovascular risk, particularly in type 2 diabetes patients who have a wide range of lipid abnormalities. These results also may not be generalizable to patients with very poorly controlled diabetes as patients enrolled in this study were required to have HbA1C levels of <8.5%. Furthermore, the consequence of these effects on cardiovascular events has not been yet investigated.

In conclusion, treatment with ezetimibe/simvastatin is significantly more effective than corresponding doses of atorvastatin monotherapy in improving the cholesterol content of most subclasses of LDL and TG-rich lipoproteins as well as HDL_3 in patients with type 2 diabetes. These improvements were generally consistent regardless of baseline TG levels, although reductions in LDL_4-C and LDL subclass pattern B and increases in HDL_3-C were observed to be greater in patients with elevated TG levels with both treatments. Although the ezetimibe

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mechanism of action in cholesterol lowering differs from that of a statin, the effect of ezetimibe/simvastatin on reducing the cholesterol content of apolipoprotein B-containing lipoprotein subclasses was generally similar to and greater than that of statin monotherapy. The changes in these lipoprotein subclasses were also generally consistent with the overall effect of ezetimibe/simvastatin on the major lipid/lipoprotein classes. Further research is necessary to determine the clinical significance of these effects and the overall benefit of lipoprotein subclass measurement beyond that of standard lipid panel testing in the assessment of cardiovascular risk.

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Author Contributions

The authors jointly developed the manuscript content and were involved in at least one of the following: conception, design, data acquisition, analysis, statistical analysis, interpretation of data, drafting the manuscript and/or revising the manuscript for important intellectual content. All authors provided final approval of the version to be published. Editorial assistance was provided by Rete Biomedical Communications Corp. (Wyckoff, NJ, USA) and Kathleen Newcomb and Martha Vollmer, Merck & Co.

Author Financial Interests

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