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Ezetimibe alone or in combination with simvastatin increases small dense low-density lipoproteins in healthy men: a randomized trial

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Aims The predominance of small dense low-density lipoproteins (sdLDLs) has been associated with increased cardiovascular risk. The effect of ezetimibe on LDL subfraction distribution has not been fully elucidated. This study assessed by gradient gel electrophoresis the effects of ezetimibe alone, simvastatin alone, and their combination on sdLDL subfraction distribution.

Methods and results A single-centre, randomized, parallel three-group open-label study was performed in 72 healthy men with a baseline LDL-cholesterol (LDL-C) concentration of 111 ± 30 mg/dL (2.9 ± 0.8 mmol/L). They were treated with ezetimibe (10 mg/day, $n = 24$), simvastatin (40 mg/day, $n = 24$), or their combination ($n = 24$) for 14 days. Blood was drawn before and after the treatment period. Generalized estimating equations were used to assess the influence of drug therapy on LDL subfraction distribution, controlling for within-subject patterns (clustering). We adjusted for age, body mass index, and baseline concentrations of LDL-C and triglycerides. Ezetimibe alone changed LDL subfraction distribution towards a more atherogenic profile by significantly increasing sdLDL subfractions (LDL-IVA +14.2%, $P = 0.0216$ and LDL-IVB +16.7%, $P = 0.039$; fully adjusted Wald χ^2 test). In contrast, simvastatin alone significantly decreased the LDL-IVB subfraction (−16.7%, $P = 0.002$). This effect was offset when simvastatin was combined with ezetimibe (LDL-IVB +14.3%, $P = 0.44$). All three treatments decreased the large, more buoyant LDL-I subfraction, the effects of ezetimibe being the most pronounced (ezetimibe −13.9%, $P < 0.0001$; combination therapy −7.3%, $P = 0.0743$; simvastatin −4.6%, $P < 0.0001$).

Conclusion In healthy men, treatment with ezetimibe alone is associated with the development of a pro-atherogenic LDL subfraction profile. Potentially atheroprotective effects of simvastatin are offset by ezetimibe.
This study is registered with ClinicalTrials.gov, identifier no. NCT00317993.

Keywords Ezetimibe • LDL size • LDL subfractions • Simvastatin • Small dense LDL

Introduction

Ezetimibe represents the first of a new class of lipid-lowering agents, the cholesterol absorption inhibitors. It is able to reduce low-density lipoprotein cholesterol (LDL-C) by 15–25% when given as monotherapy or added on an ongoing statin treatment.[1,2] Owing to the complementary mechanism of action of ezetimibe and statins (inhibition of cholesterol absorption and synthesis, respectively) and to their additive effects on LDL-C lowering, their combination is widely used to achieve reductions in LDL-C of up to 60%.[3,4]

However, a substantial body of evidence suggests that the 'quality', and not only the 'quantity', of LDL exerts a direct influence on cardiovascular risk (reviewed in Superko and Gadesam[5] and

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Rizzo and Berneis[6]). Low-density lipoprotein consists of a set of discrete subfractions with distinct molecular properties, among them size and density. In normal subjects, seven major LDL subfractions can be identified [I (large), IIA and IIB (medium), IIIA and IIIB (small), and IVA and IVB (very small)]. Low-density lipoprotein-I is the largest and least dense and LDL-IVB is the smallest, most dense particle.[7] The predominance of small dense LDL (sdLDL) and of small LDL particle size (diameter <258 Å) has been associated with increased cardiovascular risk.[5,8–11] In this context, the LDL-IVB subfraction has been found to be the single best lipoprotein predictor for atherosclerotic disease progression.[12]

Statins have been shown to have either no or only a moderate effect on LDL subclass distribution or particle size.[13] Few studies have so far assessed the effects of ezetimibe on LDL particle size and/or subfraction distribution (as reviewed in Rizzo *et al.*[14]), with conflicting results. Furthermore, most of these trials included subjects with concomitant metabolic disorders such as obesity, hypercholesterolaemia, diabetes, and the metabolic syndrome, and with a variety of co-medications with unknown effects on lipoproteins.

The recent ENHANCE trial found that although the addition of ezetimibe (10 mg/day) to simvastatin (80 mg/day) in patients with heterozygous familial hypercholesterolaemia caused an additional 16.5% reduction in LDL-C, it did not significantly affect the primary endpoint, i.e. the mean change in intima–media thickness (IMT), compared with simvastatin monotherapy.[15] Subsequently, the value of ezetimibe in the arena of cardiovascular prevention was questioned, and although various theories have been proposed to explain these surprising results, the actual reasons remain unclear.[16,17]

The purpose of the present study was to test the hypothesis that ezetimibe may alter LDL subfraction distribution and particle size towards a pro-atherogenic profile in healthy subjects.

Methods

Study design

Low-density lipoprotein particle size and subfractions were analysed from frozen samples of a single-centre, randomized, parallel three-group open-label study that investigated the effects of ezetimibe and simvastatin, alone or in combination, on lipid metabolism. The primary results of this randomized trial have been reported previously.[4,18] A total of 72 subjects were randomized to receive ezetimibe (10 mg/day), simvastatin (40 mg/day), or ezetimibe (10 mg/day) plus simvastatin (40 mg/day) for 2 weeks ($n = 24$ for each group). Ezetimibe and simvastatin were taken once a day in the evening. Blood was drawn before the initiation of treatment and at the end of the treatment period, and the samples were analysed in a blinded manner.

Subjects

Inclusion criteria were age between 18 and 60 years, body mass index (BMI) between 18.5 and 30 kg/m^2, fasting LDL-C concentrations <190 mg/dL, triglyceride concentrations <250 mg/dL, and normal blood pressure ($<140/90$ mmHg). Subjects who had received lipid-lowering drugs within 12 weeks prior to study entry, those with a history of excessive alcohol intake, liver disease, renal dysfunction (glomerular filtration rate <60 mL/min), coronary heart disease, diabetes mellitus or other endocrine disorders, eating disorders, a history of recent substantial ($>10\%$) weight change, a history of obesity (BMI > 35 kg/m^2), or taking medications known to affect lipoprotein metabolism were excluded from the study.

The protocol was approved by the Ethics Committee of the University of Cologne, and all subjects gave written informed consent. The study conformed to the Declaration of Helsinki. All subjects completed the study. Body weight did not change in any treatment group. The subjects did not use any extra medications, had no illnesses, and did not deviate from the study protocol. No serious side effects were reported.

Biochemical analyses

Blood was drawn by venipuncture in the morning after a 12 h fast to obtain serum for analysis of lipids. Total cholesterol, LDL-C, and high-density lipoprotein cholesterol (HDL-C) as well as triglycerides were determined by enzymatic methods (CHOD-PAP and GPO-PAP; Roche Diagnostics, Mannheim, Germany) on the day of blood collection in the laboratories of the Cologne University Medical Center (inter-assay coefficient of variation for total cholesterol, LDL-C, HDL-C, and triglycerides was 1.09, 2.79, 0.81, and 1.72%, respectively). Serum was obtained by centrifugation at 1600 g for 30 min at 4°C within 15 min after venipuncture and aliquots were stored immediately at -80°C for future analysis.

Non-denaturing polyacrylamide gradient gel electrophoresis (GGE) of serum was performed in the laboratory of K.B. at the University Hospital Zurich, Switzerland, in a blinded manner. Samples were shipped from Germany in dry ice and immediately analysed by GGE without re-freezing. Previous studies have shown that freezing and thawing has no effect on the measurement of LDL subfractions.[19] Gradient gel electrophoresis was performed at 10–14°C in 2–16% polyacrylamide gradient gels. Gels were subjected to electrophoresis for 24 h at 125 V in tris borate buffer (pH 8.3) as described previously.[7,20] Gels were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55°C. Gels were placed on a light source and photographed using a Luminescent Image Analyzer, LAS-3000 of Fujifilm. Migration distance for each absorbance peak was determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA, USA), thyroglobulin, and apoferritin (HMW Std, Pharmacia, Piscataway, NJ, USA) having molecular diameters of 380, 170, and 122 Å, respectively, and lipoprotein calibrators of previously determined particle size. The coefficient of variation in repeated measurements was 1.3%. Low-density lipoprotein subfraction distribution (LDL-I, -IIA, -IIB, -IIIA, -IIIB, -IVA, and -IVB) as percentage of total LDL was calculated as described previously.[7]

Statistical analysis

Descriptive data are presented as mean values (SD) unless otherwise stated. We performed multivariate analyses using generalized estimating equations to assess the influence of therapy on LDL subclass distribution, controlling for within-subject patterns (clustering). We adjusted for age, BMI, and baseline concentrations of LDL-C and triglycerides. Statistical analyses were conducted using Stata version 9 (StataCorp, College Station, TX, USA). We used Stata's xtgee command to model panel data. All reported *P*-values were calculated two-sided. Statistical significance was assumed at *P*-values <0.05.

Results

Baseline subject characteristics are shown in *Table 1* and were not different among the three treatment groups. The flow of participants

Table I Demographic data and biochemical baseline characteristics of the study participants (total *n* = 72)

Parameter	Total cohort (*n* = 72)	Ezetimibe only (*n* = 24)	Ezetimibe plus simvastatin (*n* = 24)	Simvastatin only (*n* = 24)
Age (years)	32 (9)	29 (7)	34 (11)	32 (9)
Height (cm)	181 (7)	181 (7)	181 (7)	182 (6)
Weight (kg)	85 (12)	82 (11)	84 (12)	87 (12)
BMI (kg/m^2)	25.7 (3.2)	25.0 (3.3)	25.8 (3.1)	26.4 (3.2)
Fasting plasma glucose (mg/dL)	88 (8)	87 (6)	89 (2)	86 (7)
Smoking status				
Current smoker [*n* (%)]	21 (29)	7 (29)	6 (25)	8 (33)
Ex-smoker [*n* (%)]	9 (12.5)	4 (17)	3 (13)	2 (8)
Never smoker [*n* (%)]	42 (58.3)	13 (54)	15 (63)	14 (58)
Serum lipoproteins				
Total cholesterol (mg/dL)	189 (35)	180 (28)	194 (41)	194 (34)
LDL cholesterol (mg/dL)	111 (30)	105 (23)	116 (35)	113 (30)
HDL cholesterol (mg/dL)	64 (15)	64 (13)	61 (14)	65 (18)
Triglycerides (mg/dL)	95 (43)	78 (32)	106 (48)	101 (45)
LDL particle size (Å)	276 (9)	279 (7)	273 (9)	277 (11)
LDL subclasses				
LDL-I (%)	37.2 (6.7)	39.6 (5.5)	35.4 (7.3)	36.8 (6.7)
LDL-IIA (%)	17.4 (3.6)	16.3 (2.9)	19.1 (4.6)	16.8 (2.3)
LDL-IIB (%)	16.0 (4.3)	14.2 (1.8)	18.1 (5.3)	15.7 (4.1)
LDL-IIIA (%)	10.7 (2.6)	10.3 (1.6)	10.6 (2.4)	11.3 (3.5)
LDL-IIIB (%)	4.7 (1.0)	5.1 (1.0)	4.3 (1.0)	4.9 (0.9)
LDL-IVA (%)	6.8 (1.6)	7.2 (1.4)	6.2 (1.8)	7.0 (1.6)
LDL-IVB (%)	7.3 (2.1)	7.4 (1.7)	6.5 (2.2)	8.0 (2.2)

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein. Data are presented as mean (SD) or counts (percentages). There were no significant differences between the three treatment groups.

through the trial is shown in *Figure 1*. All subjects completed the study and their adherence was excellent, as based on pill counts [mean (SD) adherence, 99.1 (3.7)%]. As shown in *Table 2*, total cholesterol and LDL-C levels significantly decreased in all treatment groups ($P < 0.001$ for all), whereas triglycerides decreased only in the groups receiving simvastatin. High-density lipoprotein-cholesterol concentrations remained unchanged in all groups.

Significant changes in LDL subfraction distribution were observed in all treatment groups after adjusting for age, BMI and baseline concentrations of LDL-C and triglycerides (Wald χ^2 $P < 0.05$). The results are depicted in *Figure 2*. Adjusted comparisons within individual subclasses showed that ezetimibe treatment significantly increased LDL-IIB (+11.2%), LDL-IIIA (+19.5%), LDL-IIIB (+11.9%), LDL-IVA (+14.2%), and LDL-IVB (+16.7%) (*Table 2* and *Figure 2A*). Combination treatment (ezetimibe plus simvastatin) significantly increased LDL-IIIB (+27%) and LDL-IVA (+28.5%) (*Table 2* and *Figure 2B*). Treatment with simvastatin alone significantly increased LDL-IIB (+11.3%), LDL-IIIA (+15.4%), LDL-IIIB (+17.3%), and LDL-IVA (+2.5%), but significantly decreased LDL-IVB, the most atherogenic LDL subfraction (−16.7%, $P = 0.002$) (*Figure 2C*). This effect was offset when ezetimibe was added to simvastatin (*Table 2*, and *Figures 2B* and *3*).

All treatments decreased the larger, more buoyant LDL-I (*Table 2* and *Figures 2A–C*). The decrease was most pronounced in the ezetimibe alone group (−13.9%), followed by the combination group (−7.3%) and smallest in the simvastatin alone group (−4.6%; *Figure 3*). The reported within-group changes did not reach statistical significance in between-group comparisons due to the overall small sample size.

In multivariate analyses there was a significant influence of baseline LDL-C concentrations on changes in LDL subfraction distribution in the ezetimibe alone group. The increase in atherogenic sdLDL was more pronounced when baseline LDL-C was higher and vice versa (data not shown).

None of the treatments had an effect on overall LDL particle size (*Table 2*).

Discussion

One essential finding of our study is that treatment with ezetimibe alone or in combination with a statin increases sdLDL proportions, thus resulting in a more pro-atherogenic LDL subfraction profile. Small dense LDL has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III.[21] Moreover, a consensus statement endorsed by the American Diabetes Association and the American College of Cardiology advocated measuring LDL particle concentration in subjects at high risk for cardiometabolic disorders and pointed

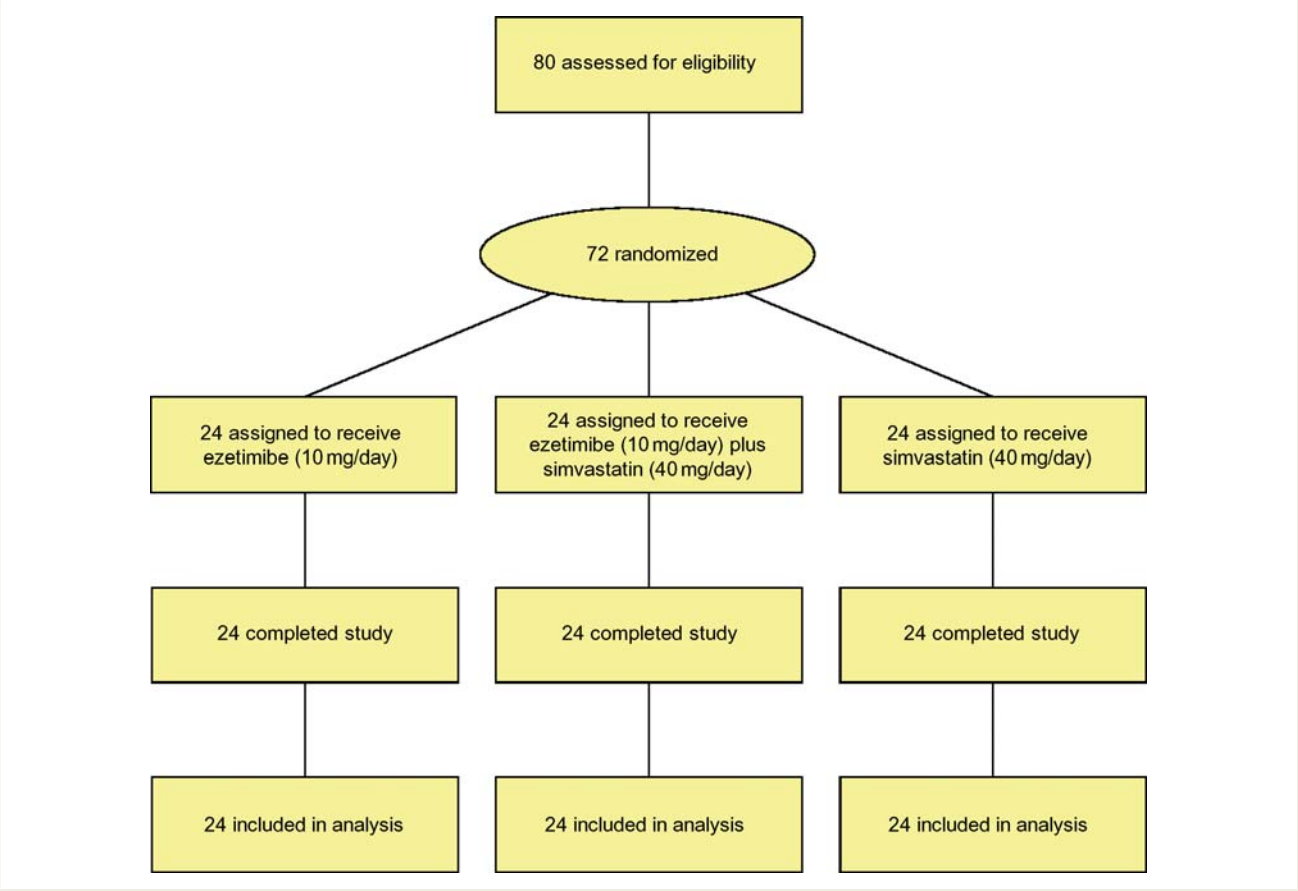

Figure 1 CONSORT flow diagram: flow of participants through the trial.

out the pro-atherosclerotic effects of sdLDL.[22] The mechanisms through which sdLDL may promote atherosclerosis include increased endothelial permeability, impaired clearance from the circulation, easier oxidation and glycation, and increased ability to bind to proteoglycans in the vessel wall.[23,24]

Although it cannot be fully excluded that the increased risk associated with smaller LDL phenotype may also be a consequence of the broader pathophysiology of which sdLDL are a part (e.g. high triglycerides, low HDL-C, increased LDL particle number, obesity, insulin resistance, diabetes, and metabolic syndrome),[23,25–27] some studies have shown that sdLDLs are a strong and independent predictor of coronary artery disease (CAD).[28] Other studies have investigated whether the therapeutic modification of LDL subfractions reduces cardiovascular risk. Such investigations used angiographic changes as outcome variables and have reported benefit in patients with a predominance of sdLDL who received treatment such as statins and bile acid-binding resins that tend to reduce the amount of such particles.[8,29,30]

In fact, various lipid-lowering drugs are able to favourably alter sdLDL, and fibrates and nicotinic acid seem to be the most effective in this respect (reviewed in Gazi *et al.*[31] and Rizzo *et al.*[32]). As we were also able to show in the present study, simvastatin has been found to have either no or only a marginal net effect on LDL subfraction distribution.[13] This is true for the majority of the statins.[33] Interestingly, rosuvastatin, the latest statin to be introduced in the market, seems to be more efficient in modulating plasma lipids and LDL subfractions (reviewed in Rizzo *et al.*[13,34]). In contrast, the effects of the cholesterol absorption inhibitor ezetimibe on LDL size and subfraction distribution have been contradictory. Ezetimibe monotherapy was found to be associated with a small but significant decrease in sdLDL concentrations and increase in LDL particle size in patients with primary dyslipidaemia,[35] mixed hyperlipidaemia,[36] and in obese and overweight patients with hypercholesterolaemia.[37] On the other hand, Ose *et al.*[38] in a 12-week trial found no effects of ezetimibe monotherapy on sdLDL concentrations and LDL particle size in patients with hypercholesterolaemia. Moreover, in patients with mixed hyperlipidaemia Tribble *et al.*[39] found that ezetimibe caused reductions in both the large and sdLDL subfractions and had no effects on LDL particle size. Furthermore, Geiss *et al.*[40] found no effect of ezetimibe on LDL subfraction distribution in patients concomitantly treated with LDL apheresis and statins. Three recent studies support our findings. Winkler *et al.*[41] showed that in patients with the metabolic syndrome, the combination of simvastatin with ezetimibe reduces LDL radius of the sdLDL subfractions even further. Moreover, Stojakovic *et al.*[42] found that in patients at high risk for coronary events, the addition of ezetimibe to fluvastatin does not result in any further reduction of dense LDL

Table 2 Plasma lipids, low-density lipoprotein size, and low-density lipoprotein subfraction distribution before and after treatment

Parameter	Ezetimibe only				Ezetimibe plus simvastatin				Simvastatin only			
	Before therapy	After therapy	Mean per cent change	*P*-value[a]	Before therapy	After therapy	Mean per cent change	*P*-value[a]	Before therapy	After therapy	Mean per cent change	*P*-value[a]
Lipoprotein concentrations												
Total cholesterol, mg/dL	180 (28)	159 (23)	−11.2 (9.7)	0.0002	194 (41)	121 (25)	−36.9 (8.1)	<0.0001	194 (34)	145 (24)	−24.7 (7.9)	<0.0001
LDL cholesterol (mg/dL)	105 (23)	80 (16)	−22.1 (10.2)	<0.0001	116 (35)	47 (19)	−59.6 (9.7)	<0.0001	113 (30)	67 (22)	−40.7 (11.5)	<0.0001
HDL cholesterol (mg/dL)	64 (13)	65 (16)	+1.7 (11)	0.35	61 (14)	60 (14)	−1.5 (8.5)	0.23	65 (18)	65 (16)	+.7 (11.1)	0.88
Triglycerides (mg/dL)	78 (32)	88 (49)	+27 (79)	0.57	106 (48)	90 (36)	−8.9 (29.7)	0.0288	101 (45)	82 (39)	−11.8 (39.9)	0.0386
LDL particle size (Å)	279 (7)	279 (10)	+.2 (3.3)	0.22	273 (9)	276 (8)	+1.1 (2.6)	0.0975	277 (11)	276 (8)	−.4 (1.9)	0.22
LDL subclass composition												
LDL-I (%)	39.6 (5.5)	33.9 (5.9)	−13.9 (11.8)	<0.0001	35.4 (7.3)	31.8 (4.7)	−7.3 (19.1)	0.0743	36.8 (6.7)	34.5 (5.7)	−4.6 (16.7)	<0.0001
LDL-IIA (%)	16.3 (2.9)	16.1 (2.2)	−.5 (9.8)	0.89	19.1 (4.6)	18.7 (4.3)	+.6 (20.1)	0.73	16.8 (2.3)	17.3 (2.0)	+4.0 (10.1)	0.59
LDL-IIB (%)	14.2 (1.8)	15.8 (3.1)	+11.2 (15.8)	0.0003	18.1 (5.3)	17.6 (3.2)	+3.4 (27.2)	0.37	15.7 (4.1)	17.1 (3.1)	+11.3 (16.3)	0.0002
LDL-IIIA (%)	10.3 (1.6)	12.1 (1.8)	+19.5 (21.0)	<0.0001	10.6 (2.4)	11.5 (1.8)	+13.6 (28.5)	0.0609	11.3 (3.5)	12.5 (2.6)	+15.4 (24.3)	<0.0001
LDL-IIIB (%)	5.1 (1.0)	5.6 (1.0)	+11.9 (19.2)	0.0021	4.3 (1.0)	5.2 (1.2)	+27.0 (30.0)	0.0017	4.9 (.9)	5.6 (1.0)	+17.3 (24.1)	0.0011
LDL-IVA (%)	7.2 (1.4)	8.2 (2.0)	+14.2 (25.9)	0.0216	6.2 (1.8)	7.8 (2.2)	+28.5 (32.1)	0.0002	7.0 (1.6)	6.9 (1.6)	+2.5 (30.7)	0.0179
LDL-IVB (%)	7.4 (1.7)	8.3 (2.2)	+16.7 (40.4)	0.0392	6.5 (2.2)	7.3 (2.9)	+14.3 (30.8)	0.44	8.0 (2.2)	6.1 (1.8)	−16.7 (37.4)	0.002

Data are presented as mean (SD). Each group comprised $n = 24$ subjects.
[a]Wald χ^2 test after adjusting for age, body mass index, baseline LDL cholesterol, and triglycerides.

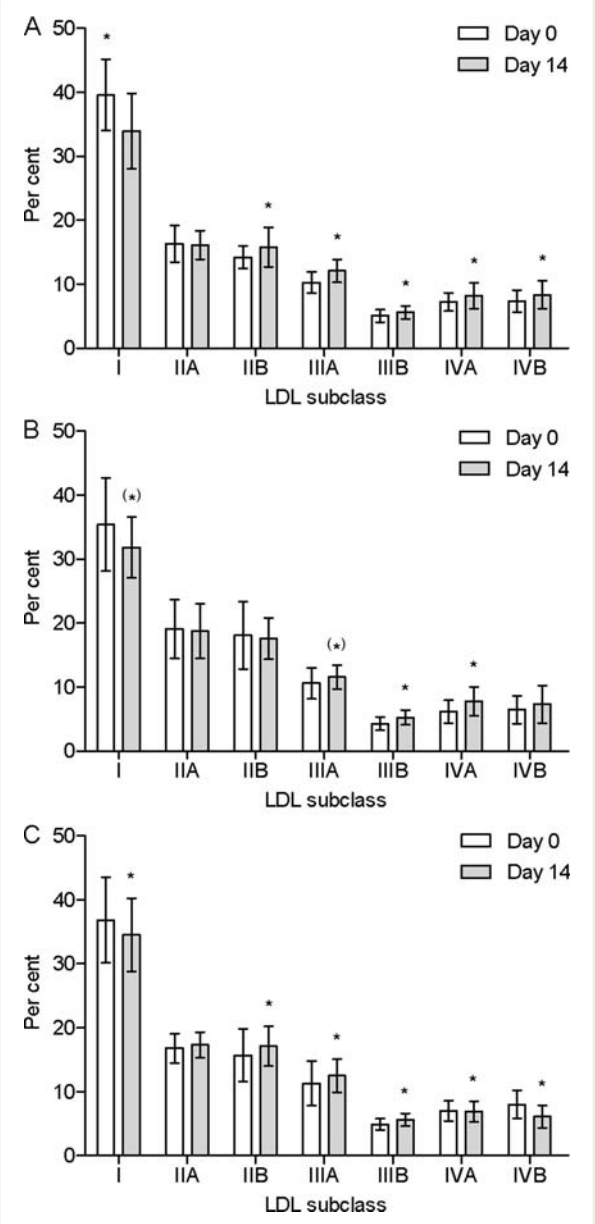

Figure 2 Low-density lipoprotein subclass distribution (in %) and changes from baseline. Low-density lipoprotein subclass distribution in the ezetimibe monotherapy group (*A*), combination treatment group (ezetimibe plus simvastatin) (*B*), and simvastatin monotherapy group (*C*). Significant changes, as determined by generalized estimating equations (Wald χ^2 *P*-values), adjusting for age, body mass index, baseline low-density lipoprotein cholesterol, and triglycerides, are indicated by asterisks [$^{*}P < 0.05$, $^{(*)}P < 0.1$]. Data shown are mean values (SEM).

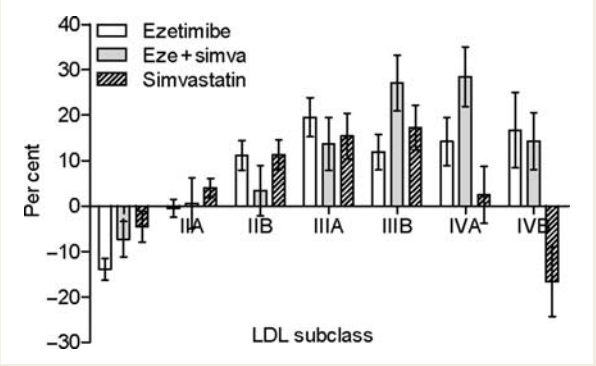

Figure 3 Per cent change from baseline in low-density lipoprotein subfractions in the three treatment groups. Data shown are mean values (SEM).

compared with fluvastatin alone. Tomassini *et al.*[43] in a 6-week trial compared the effects of ezetimibe/simvastatin with atorvastatin on lipoprotein subfractions in patients with type 2 diabetes and hypercholesterolaemia. Ezetimibe monotherapy was not evaluated. They found that neither the combination of ezetimibe/simvastatin nor atorvastatin alone significantly affected the LDL-IV cholesterol subfraction. However, when subjects with triglyceride levels <200 mg/dL were examined, a population similar to that of our study, they also observed an increase, albeit not significant, of the LDL-IV subfraction in the ezetimibe/simvastatin-treated patients. To our knowledge, the present study is the first one to examine whether ezetimibe modulates LDL size and subfraction distribution in healthy individuals, a model which in a sense reflects ezetimibe's 'true' effects on a normal metabolic background.

In the present study, we investigated a group of healthy men to assess the effects of ezetimibe, simvastatin, and their combination on LDL particle size and subfraction distribution. We were able to show that treatment with ezetimibe alone or in combination with a statin did not alter LDL particle size but altered the LDL subfraction distribution towards increased concentrations of atherogenic small dense particles. Although simvastatin alone also increased LDL-III subfractions, it significantly decreased the smallest, most dense LDL fraction (LDL-IVB), which has been found to be the best lipoprotein predictor of atherosclerotic disease progression, even if it represents only a minor fraction of total LDL.[12] This potentially atheroprotective effect of simvastatin was offset when ezetimibe was co-administered. These findings may, at least partially, explain the lack of additional benefit of ezetimibe added to simvastatin on atherosclerosis progression, measured as changes in IMT, despite a significant additional reduction in LDL-C levels, observed in the ENHANCE study.[15] Although there is still no consensus on the clinical significance of surrogate markers of cardiovascular risk, such as IMT,[44] it should be pointed out that data from a subsequent study with ezetimibe were also disappointing; the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study showed that treatment with ezetimibe (10 mg/day) plus simvastatin (40 mg/day) significantly reduced LDL-C concentrations in patients with aortic stenosis, compared with placebo, but did not affect the composite primary endpoint of aortic-valve events and ischaemic events.[45] Furthermore, the recently published trial ARBITER6-HALTS casts some more doubts on the clinical benefits of ezetimibe.[46] In specific, the trial showed that while extended-release niacin added to a statin causes a smaller LDL-C reduction compared with ezetimibe added to a statin, niacin had greater efficacy than ezetimibe regarding beneficial changes in IMT. The findings of our trial may at least

partially explain the lack of antiatherosclerotic effects of ezetimibe, despite its stronger LDL-C lowering than the comparators.

Interestingly, we found that under treatment with ezetimibe alone, a significant association existed between baseline LDL-C concentrations and the pro-atherogenic changes of the LDL subfractions. Considering that the population of the current study was normocholesterolaemic and that ezetimibe is prescribed to patients with much higher LDL-C levels, it can be postulated that the pro-atherogenic effects of ezetimibe would be even more pronounced in the latter population. Although it could be argued that ezetimibe is usually prescribed along with a statin, a group in which such an association was not observed, it should be pointed out that ezetimibe monotherapy is a widely used alternative for the treatment of hypercholesterolaemia in patients with statin intolerance.[47]

Limitation of the study is the fact that the clinical relevance of our findings remains to be established. Another limitation is the fact that no a priori power calculations were made for changes in LDL subfractions because the primary outcome parameter of the study was change in LDL-C. Therefore, statistical between-group changes did not reach significance due to the overall small sample size. Strengths of the study include its randomized design and robust statistical methodology, the blinded measurements of LDL subclasses, and the use of a 'drug-naïve' population, devoid of co-medications and co-morbidities, which could potentially alter lipid metabolism, and excellent treatment adherence. Treatment duration was relatively short, which does not exclude that the observed effects could be even more pronounced during long-term treatment, especially considering the different plasma residence times of light LDL (1.7–2 days) and dense LDL (2.4–5 days).[48,49]

A 2-week treatment duration was chosen for this study since the lipid-lowering effects of simvastatin reach maximum at day 14 and remain stable thereafter.[50] This was first shown in 2001 by the group of Michael Davidson and was later confirmed for simvastatin and other statins.[50–53] Regarding ezetimibe, Bays *et al.*[54] first showed that the maximum LDL-C-lowering effect is present after 2 weeks of treatment, after which it remains stable. This finding was confirmed also by others.

In conclusion, our findings suggest that treatment with ezetimibe alone is associated with the development of a pro-atherogenic LDL subfraction profile. Moreover, potentially atheroprotective effects of simvastatin are offset by ezetimibe when co-administered. Cardiovascular event outcome trials, which are underway, will hopefully provide additional insights into the effects of ezetimibe on cardiovascular events.

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