ORIGINAL RESEARCH

Changes in Lipoprotein Particle Number With Ezetimibe/Simvastatin Coadministered With Extended-Release Niacin in Hyperlipidemic Patients

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***Background*—**Combination therapy with ezetimibe/simvastatin (E/S) and extended-release niacin (N) has been reported to be safe and efficacious in concomitantly reducing low-density lipoprotein cholesterol and increasing high-density lipoprotein cholesterol in hyperlipidemic patients at high risk for atherosclerotic cardiovascular events. This analysis evaluated the effect of E/S coadministered with N on low-density lipoprotein particle number (LDL-P) and high-density lipoprotein particle number (HDL-P) as assessed by nuclear magnetic resonance (NMR) spectroscopy in patients with type IIa or IIb hyperlipidemia.

***Methods and Results*—**This was an analysis of a previously reported 24-week randomized, double-blind study in type IIa/IIb hyperlipidemic patients randomized to treatment with E/S (10/20 mg/day)+N (titrated to 2 g/day) or N (titrated to 2 g/day) or E/S (10/20 mg/day). Samples from a subset of patients (577 of 1220) were available for post hoc analysis of LDL-P and HDL-P by NMR spectroscopy. Increases in HDL-P (+16.2%) and decreases in LDL-P (−47.7%) were significantly greater with E/S+N compared with N (+9.8% for HDL-P and −21.5% for LDL-P) and E/S (+12.8% for HDL-P and −36.8% for LDL-P). In tertile analyses, those with the lowest baseline HDL-P had the greatest percent increase in HDL-P (N, 18.4/7.9/2.1; E/S, 19.3/12.2/5.3; and E/S+N, 26.9/13.8/6.9; all $P<0.001$). Individuals in the highest tertile of LDL-P had the greatest percent reduction in LDL-P (N, 18.3/23.1/24.6; E/S, 29.7/38.3/41.8; and E/S+N, 44.3/49.0/50.5; all $P<0.001$).

***Conclusions*—**These results suggest that E/S+N improves lipoprotein particle number, consistent with its lipid-modifying benefits in type IIa or IIb hyperlipidemia patients and may exert the greatest effect in those with high LDL-P and low HDL-P at baseline.

***Clinical Trial Registration*—**URL: Clinicaltrials.gov Identifier: NCT00271817 **(*J Am Heart Assoc.* 2013;2:e000037 doi: 10.1161/JAHA.113.000037)**

Key Words: ezetimibe • lipoprotein particle number • lipoproteins • niacin • NMR spectroscopy • statins

Current guidelines for the prevention and treatment of coronary heart disease (CHD) have identified reduction in low-density lipoprotein cholesterol (LDL-C) as the key lipid-related goal.[1–3] Raising high-density lipoprotein cholesterol (HDL-C) has also been shown to be associated with cardiovascular disease (CVD) risk reduction.[4] Ezetimibe/simvastatin (E/S), which has a dual effect on both absorption of dietary cholesterol and upregulation of LDL clearance, has been shown to be effective at lowering levels of LDL-C, non-HDL-C, and triglycerides (TG) in patients with hypercholesterolemia.[5] Niacin (N) is an effective agent available for raising HDL-C[6] and has also been reported to reduce levels of TG, LDL-C, and lipoprotein(a) in patients with combined dyslipidemia.[7,8] Statin-niacin combination therapy has been reported to be safe and efficacious in several studies with different statin formulations.[9–11]

Knowledge of lipoprotein particle number and size, in addition to lipid profile assessment in patients with mixed dyslipidemia, may aid in further predicting CVD risk assessment and in guiding therapy. Data from cross-sectional[12–16] as well as interventional[17] studies have indicated additional predictive value for both LDL particle number[12,18,19] (LDL-P) and HDL particle number[20,21] (HDL-P) on CVD risk, independent of cholesterol levels. Niacin in combination with statin

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Accompanying Tables S1–S3 are available at http://jaha.ahajournals.org/content/2/4/e001596/suppl/DC1

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therapy has been shown to improve both the atherogenic and the antiatherogenic lipoprotein profiles of patients with hyperlipidemia compared with atorvastatin alone.[22,23]

Combination therapy with ezetimibe/simvastatin (E/S) and extended-release niacin (N) has been shown to be effective in concomitantly reducing LDL-C and TG and increasing HDL-C in patients with type IIa and type IIb hyperlipidemia during 24 weeks in a randomized, double-blind study.[24] The study showed that combination treatment with E/S plus N had a greater lipid-altering efficacy compared with E/S or N monotherapy in these study subjects. In the present analysis, the impact of these lipid therapies on the characteristics of LDL and HDL particles, in particular particle number and particle size, was assessed by nuclear magnetic resonance (NMR) spectroscopy.[25]

Methods

Study Design

This analysis of a previously reported 24-week multicenter, double-blind trial is based on a subset of 577 participants (316 men and 261 women) who had samples available from the original study cohort of 2697 patients.[24] Participants aged 18 to 79 years had LDL-C between 130 and 190 mg/dL, triglyceride levels ≤500 mg/dL, and metabolic and clinical stability (eg, euthyroid, creatinine <2 mg/dL, creatinine kinase ≤2×upper limit of normal [ULN], transaminases ≤1.5×ULN). After a 4-week washout period, 124 subjects were randomized to N (titrated to 2 g/day), 160 subjects to E/S (10/20 mg/day), and 294 subjects to the combination of E/S (10/20 mg)+N (titrated to 2 g/day). As previously reported, N was increased by 500 mg every 4 weeks up to 2 g/day from a starting dose of 500 mg/day. Patients were counseled to take N at bedtime with a low-fat snack and aspirin (325 mg), or ibuprofen (200 mg) 30 minutes before taking N and to avoid alcoholic and hot beverages near the time of taking N. Details of the study have been described elsewhere.[24]

Lipoprotein Analyses

The primary hypothesis of this subset analysis was that E/S+N would be superior to N with respect to percent change from baseline in LDL-P after 24 weeks of treatment. End points, assessed as percent changes from baseline to week 24, included LDL-P, LDL size, HDL-P, and HDL size. Lipoprotein particle concentrations were measured by NMR spectroscopy as described previously.[25] HDL-P and LDL-P (coefficient of variation <4%) are the sums of the particle concentrations determined for the respective subclasses on the basis of measured amplitudes of the distinct lipid methyl group that NMR signals emitted. Each lipoprotein subclass signal emanates from the aggregate number of methyl groups on the lipids contained within the particle. This number is largely dependent on the lipoprotein particle diameter; thus, the amplitude of each lipoprotein subclass signal is directly proportional to the number of subclass particles emitting the signal, irrespective of variation in lipid composition. Mean LDL and HDL particle sizes were calculated from the sum of the diameter of each subclass multiplied by their estimated relative mass percentages, as previously described.[12–14] Changes from baseline were also analyzed as stratified by tertiles of baseline LDL-P and HDL-P.

Statistical Analyses

All statistical analyses were performed using SAS for Windows (version 9.1). Results are presented as mean and standard deviation (SD) unless indicated otherwise. Data were checked for normality and equal variance prior to any analysis. The independent 2-sample *t* test was used to evaluate and compare the difference of treatment effect, and *P* values were reported. Participants were stratified by tertiles on the basis of either LDL-P or HDL-P as assessed at baseline. The significance of the changes in various parameters between the baseline (preintervention) and week 24 (postintervention) within each tertile was assessed by paired *t* tests. Two-way ANOVA (treatment and tertile classification) was conducted to further analyze the effect of treatment groups. For comparison with overall $P>0.05$, a post hoc Tukey's test was used for pairwise comparisons.

Results

Table 1 presents the baseline characteristics of the subset of patients included in the current analysis. There were no clinically meaningful differences in the baseline characteristics of this subset of participants, both among the treatment groups and in comparison with the entire study population. Table 2 summarizes the percent changes in the primary and secondary end points from baseline at week 24 and the significance of the treatment difference. For the subset of patients included in this analysis, the changes in lipid parameters observed with the different treatments were comparable to those previously reported for the entire cohort.[24] Combination E/S+N reduced LDL-C, total cholesterol, TG, non-HDL-C, and apolipoprotein B (apoB) more than E/S or N alone; changes in apoA-I and HDL-C were comparable to N alone and greater than those with E/S alone.

The reduction in LDL-P as assessed by NMR spectroscopy was smaller with N treatment as compared with E/S in these patients, and the effect of E/S+N co-administration was nearly additive (Table 2). The changes from baseline and between-treatment changes from baseline group differences

Table 1. Baseline Characteristics of Randomized Patients

	N (n=124)	E/S (n=160)	E/S+N (n=294)
Age, y	58.2 (9.6)	58.4 (10.2)	56.8 (10.5)
Female, n (%)	47 (46.0)	69 (43.1)	136 (46.3)
Race, n (%)			
Asian	4 (3.2)	1 (0.6)	3 (1.0)
Black	5 (4.0)	9 (5.6)	14 (4.8)
Hispanic	9 (7.3)	2 (1.3)	15 (5.1)
Other	3 (2.4)	0 (0)	2 (0.7)
White	103 (83.1)	148 (92.5)	260 (88.4)
TC			
mmol/L (SD)	6.3 (0.7)	6.2 (0.7)	6.2 (0.7)
mg/dL (SD)	241.5 (27.1)	239.9 (28.1)	240.3 (26.8)
TG			
mmol/L (SD)	1.9 (0.9)	2.0 (1.0)	1.9 (0.9)
mg/dL (SD)	166.7 (80.6)	178.8 (89.3)	172.2 (75.4)
HDL-C			
mmol/L (SD)	1.3 (0.4)	1.3 (0.3)	1.2 (0.3)
mg/dL (SD)	49.8 (13.7)	48.4 (12.8)	46.7 (12.1)
LDL-C			
mmol/L (SD)	4.1 (0.6)	4.0 (0.6)	4.0 (0.6)
mg/dL (SD)	158.3 (22.1)	155.9 (21.3)	156.3 (22.9)
Non-HDL-C			
mmol/L (SD)	5.0 (0.7)	5.0 (0.7)	4.9 (0.7)
mg/dL (SD)	191.7 (27.8)	191.6 (26.6)	190.6 (25.4)
ApoB			
g/L (SD)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)
mg/dL (SD)	150.3 (19.7)	151.5 (21.8)	151.1 (20.4)
ApoA-I			
g/L (SD)	1.6 (0.3)	1.6 (0.3)	1.6 (0.3)
mg/dL (SD)	161.5 (25.7)	164.0 (27.7)	164.7 (26.2)
hsCRP			
mmol/L (SD)	21.0 (39.0)	18.1 (30.5)	22.9 (31.4)
mg/dL (SD)	2.2 (4.1)	1.9 (3.2)	2.4 (3.3)
LDL-P, nmol/L (SD)	1730.3 (333.1)	1758.2 (332.0)	1721.6 (302.3)
HDL-P, nmol/L (SD)	32.0 (6.0)	32.0 (6.0)	32.3 (6.1)
LDL-S, nm (SD)	21.0 (0.7)	20.9 (0.7)	20.9 (0.6)
HDL-S, nm (SD)	8.6 (0.4)	8.6 (0.4)	8.7 (0.4)

N indicates extended-release niacin (to 2 g/day); E/S, ezetimibe (10 mg/day)/simvastatin (20 mg/day); TC, total cholesterol; SD, standard deviation; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoB, apolipoprotein B; ApoA-I, apolipoprotein A-I; hsCRP, high-sensitivity C-reactive protein; LDL-P, low-density lipoprotein particle number; HDL-P, high-density lipoprotein particle number; LDL-S, low-density lipoprotein particle size; HDL-S, high-density lipoprotein particle size.

were statistically different. Changes in LDL size were small for all 3 treatments (Table 2). With N treatment there was a 2.1% increase in LDL size in contrast to a 1.2% reduction with E/S. Compared with N only, individuals randomized to E/S monotherapy and combination E/S+N had significant reductions in LDL size, whereas compared with E/S, the combination E/S+N produced a significant increase in LDL size.

Table 2. Percent Changes From Baseline in Lipids, LDL-P, HDL-P, and LDL and HDL Size

Parameter	N (n=124)	E/S (n=160)	E/S+N (n=294)	Treatment Difference E/S+N vs N	E/S+N vs E/S	E/S vs N
LDL-P						
Baseline mean (SD), nmol/L	1725.4 (333.4)	1758.2 (332.0)	1721.6 (302.3)	—	—	—
Study-end mean (SD), nmol/L	1341.4 (346.4)	1095.4 (255.9)	890.2 (355.3)	—	—	—
% Change from baseline	−21.5§	−36.8§	−47.7§	−26.1‡	−10.9‡	−15.2‡
LDL-S						
Baseline mean (SD), nm	21.0 (0.7)	20.9 (0.6)	20.9 (0.6)	—	—	—
Study-end mean (SD), nm	21.4 (0.5)	20.6 (0.5)	20.9 (0.5)	—	—	—
% Change from baseline	2.1§	−1.2§	0.1‖	−2.0‡	1.3‡	−3.3‡
HDL-P						
Baseline mean (SD), nmol/L	32.0 (6.0)	32.0 (6.0)	32.0 (6.0)	—	—	—
Study end mean (SD), nmol/L	34.7 (5.8)	35.7 (6.0)	37.0 (6.3)	—	—	—
% Change from baseline	9.8§	12.8§	16.2§	6.3†	3.3†	3.0‖
HDL-S						
Baseline mean (SD), nm	8.6 (0.4)	8.6 (0.4)	8.7 (0.4)	—	—	—
Study-end mean (SD), nm	9.2 (0.6)	8.7 (0.4)	9.3 (0.6)	—	—	—
% Change from baseline	5.9§	1.6§	7.5§	1.6†	5.9‡	−4.3‡
LDL-C						
Baseline mean (SD), mmol/L	4.1 (0.6)	4.0 (0.6)	4.0 (0.6)	—	—	—
Study-end mean (SD), mmol/L	3.2 (0.7)	1.9 (0.5)	1.6 (0.7)	—	—	—
% Change from baseline	−20.3§	−53.7§	−58.9§	−38.6†	−5.2*	−33.3‡
HDL-C						
Baseline mean (SD), mmol/L	1.3 (0.4)	1.3 (0.3)	1.3 (0.3)	—	—	—
Study-end mean (SD), mmol/L	1.6 (0.4)	1.3 (0.3)	1.6 (0.7)	—	—	—
% Change from baseline	28.1§	7.9§	29.4§	1.3‖	21.6†	−20.3‡
ApoB						
Baseline mean (SD), g/L	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	—	—	—
Study-end mean (SD), g/L	1.2 (0.2)	0.9 (0.2)	0.6 (0.2)	—	—	—
% Change from baseline	−19.7§	−40.0§	−48.3§	−28.6‡	−8.4‡	−20.2‡
ApoA-I						
Baseline mean (SD), g/L	1.6 (0.3)	1.6 (0.3)	1.6 (0.3)	—	—	—
Study-end mean (SD), g/L	1.8 (0.3)	1.7 (0.3)	1.8 (0.3)	—	—	—
% Change from baseline	11.2§	3.2†	10.4§	−0.8‖	7.1‡	−7.9‡
Non-HDL-C						
Baseline mean (SD), mmol/L	5.0 (0.7)	5.0 (0.7)	4.9 (0.7)	—	—	—
Study-end mean (SD), mmol/L	3.8 (0.9)	2.6 (0.6)	2.2 (0.9)	—	—	—
% Change from baseline	−22.5§	−47.6§	−55.8§	−33.3†	−8.2†	−25.1‡
TG						
Baseline mean (SD), mmol/L	1.9 (0.9)	2.0 (1.0)	1.9 (0.9)	—	—	—
Study-end mean (SD), mmol/L	1.3 (0.6)	1.6 (0.7)	1.2 (0.6)	—	—	—
% Change from baseline	−26.4§	−15.7§	−36.6§	−10.2†	−20.9†	10.7*

Continued

Table 2. Continued

Parameter	N (n=124)	E/S (n=160)	E/S+N (n=294)	Treatment Difference E/S+N vs N	E/S+N vs E/S	E/S vs N
TC						
Baseline mean (SD), mmol/L	6.3 (0.7)	6.2 (0.7)	6.2 (0.7)	—	—	—
Study-end mean (SD), mmol/L	5.5 (0.7)	3.9 (0.7)	3.8 (0.8)	—	—	—
% Change from baseline	−12.1§	−36.7§	−38.5§	−26.4‡	−1.8‖	−24.6‡

To convert SI units to conventional units, multiply by 0.0259 for LDL-C, HDL-C, non-HDL-C, and TC; by 0.01 for apoB and apoAI, and by 0.0113 for TG. N indicates extended-release niacin (to 2 g/day); E/S, ezetimibe (10 mg/day)/simvastatin (20 mg/day); LDL-P, low-density lipoprotein particle number; HDL-P, high-density lipoprotein particle number; SD, standard deviation; LDL-S, low-density lipoprotein size; HDL-S, high-density lipoprotein size; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ApoB, apolipoprotein B; ApoA-I, apolipoprotein A-I; TG, triglycerides; TC, total cholesterol.
*$P<0.05$; †$P<0.01$; ‡$P<0.001$; §$P<0.001$; ‖$P>0.05$.

There were statistically significant increases in HDL-P in all 3 treatment groups (Table 2). When E/S was coadministered with N, there was an additional 6% increase in HDL-P compared with N only and no additional increase when compared with E/S monotherapy. Similarly, the between-treatment difference effect for E/S versus N monotherapies on HDL-P were comparable. Statistically significant increases in HDL size were also observed with all 3 treatments (Table 2). Combination E/S+N had a strong additive effect on HDL size compared with N monotherapy and E/S alone. The increase in HDL size with E/S treatment was significantly smaller than that with N treatment.

When stratified by baseline LDL-P tertile, N monotherapy was least effective in reducing LDL-C in the highest tertile, whereas E/S monotherapy and E/S+N combination therapy were more effective in patients with greater baseline LDL-P (Figure 1A, Table S1). Individuals in the highest LDL-P tertile exhibited the greatest reduction in LDL-P with all 3 treatments, and when N was coadministered with E/S, this effect was additive (Figure 1B and Table 3).

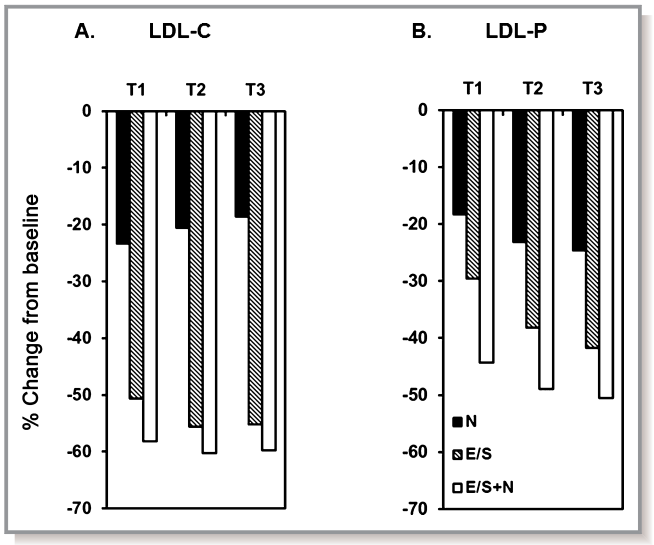

Figure 1. Percent changes from baseline in LDL-C (A) and LDL-P (B) as stratified by tertiles of LDL-P. All 3 treatments are presented as indicated. LDL-C indicates low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein cholesterol particle number; N, extended-release niacin; E/S, ezetimibe/simvastatin; T1-3, baseline LDL-P tertile.

When stratified by baseline HDL-P tertile, N and E/S+N therapies increased HDL-C substantially more than E/S monotherapy (Figure 2A and Table S2). E/S monotherapy was most effective in raising HDL-C in the subset of patients with the lowest HDL-P at baseline. Although statistically significant, the HDL-C increases with N monotherapy and E/S+N combination therapy were lower in patients with the highest baseline HDL-P. All 3 treatments increased HDL-P the most in patients with the lowest HDL-P baselines. The increase in HDL-P was largest for combination E/S+N therapy (26.9%), and increases with N (18.4%) and E/S (19.4%) monotherapies were similar (Figure 2B and Table 4). In patients with high baseline HDL-P, increases in HDL-P were substantially lower, although significant, with E/S and E/S+N, whereas the effect with N was minimal and nonsignificant.

Changes in LDL size varied slightly among baseline LDL-P tertiles (Table S3). Treatment with N increased LDL size, and this effect was greatest among individuals in the highest tertile of LDL-P (0.8%, 2.3%, and 3.4% from low to high tertiles). With E/S, there was a reduction in LDL size, and the greatest reductions occurred in individuals in the 2 lowest tertiles of LDL-P (−2.3%, −1.2%, and −0.3% from low to high tertiles). For the combination E/S+N, the change in LDL size was <1% across tertiles (−0.8%, 0.2%, 0.7% from low to high tertiles).

Both N and combination E/S+N therapies were associated with significant increases in HDL size, regardless of baseline HDL-P (Table S3). Treatment with N alone increased HDL-S similarly by 5.9%, 6.8%, and 5.4% from low to high HDL-P tertiles. With E/S only, significant increases in HDL size were observed in individuals in the lower HDL-P baseline tertiles (1.7% and 2.1%), whereas individuals in the highest tertile showed no significant increase in HDL size (0.7%).

Table 3. Mean Baseline and Study-End Levels and Change From Baseline in LDL-P in Baseline LDL-P Tertiles

Treatment	Baseline LDL-P Tertiles													
	T1				T2				T3					
	N	Mean (SD), nmol/L		Mean Change From Baseline		N	Mean (SD), nmol/L		Mean Change From Baseline		N	Mean (SD), nmol/L		Mean Change From Baseline
		Week 0	Week 24	%	*P* Value for Treatment Difference		Week 0	Week 24	%	*P* Value for Treatment Difference		Week 0	Week 24	%
N only	46	1392 (138.7)	1138 (260.1)	−18.3*	<0.001	35	1712 (72.3)	1316 (272.5)	−23.1*	<0.0001	43	2104 (186.5)	1586 (335.9)	−24.6*
E/S only	51	1380 (134.0)	971 (250.8)	−29.7*	<0.0005	49	1733 (77.7)	1069 (189.0)	−38.3*	<0.0003	60	2100 (185.1)	1223 (252.0)	−41.8*
E/S+N	96	1404 (159.5)	782 (322.1)	−44.3*	<0.0001	108	1716 (74.3)	876 (306.4)	−50.5*	<0.0001	89	2070 (190.5)	1024 (402.0)	−49.5*
E/S+N vs N		—	—		<0.001		—	—		<0.0001		—	—	<0.0001
E/S+N vs E/S		—	—		<0.0005		—	—		<0.0003		—	—	<0.004
E/S vs N		—	—		<0.0001		—	—		<0.0001		—	—	<0.0001

LDL-P indicates low-density lipoprotein particle number; T1 to T3, baseline LDL-P tertile; SD, standard deviation; N, extended-release niacin (to 2 g/day); E/S, ezetimibe (10 mg/day)/simvastatin (20 mg/day).
*$P<0.0001$.

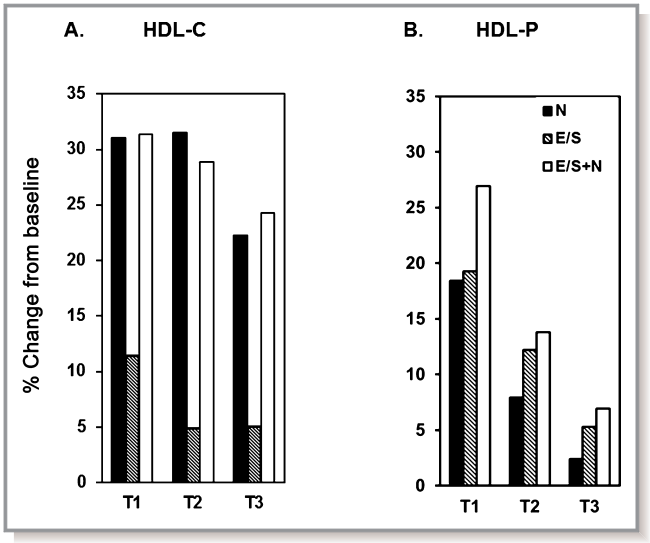

Figure 2. Percent changes from baseline in HDL-C (A) and HDL-P (B), as stratified by tertiles of HDL-P. All 3 treatments are presented as indicated. HDL-C indicates high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein cholesterol particle number; N, extended-release niacin; E/S, ezetimibe/simvastatin; T1 to T3, baseline HDL-P tertile.

Combination E/S+N resulted in the largest increases in HDL size (7.5%, 7.8%, and 7.2% from low to high tertiles).

Discussion

This study showed that coadministration of E/S and N therapies reduced LDL-P and increased HDL-P and HDL size substantially more than E/S or N alone in patients with type IIa and type IIb hyperlipidemia. These effects were consistent with the known LDL-C-lowering and HDL-C-raising properties of E/S and N therapies. Moreover, the effects were additive for the activities elicited by the component E/S and N monotherapies in this analysis. There was no change in LDL size with the combination, attributed to the observed inverse effects of N and E/S. Overall, these results suggest that combination E/S+N has a favorable impact on lipoprotein particle number, consistent with its lipid-modifying benefits in these patients.

These findings are consistent with previous reports that niacin+simvastatin reduced LDL-P and increased HDL-P to a greater extent than statin monotherapy.[22,23] To our knowledge, the effects of E/S therapy on LDL-P and HDL-P have not been previously reported using NMR spectroscopy. However, in several studies, E/S reduced the cholesterol content of all LDL subclasses but had minimal effect on subclass distribution, aside from significant reductions in small, dense LDL in patients with primary dyslipidemia and elevated TG concentrations.[26–32] In these studies, LDL subclasses and distribution were measured by a number of methods, including

Table 4. Mean Baseline and Study-End Levels and Change From Baseline in HDL-P in Baseline HDL-P Tertiles

Treatment	Baseline HDL-P Tertiles													
	T1				T2				T3					
	N	Mean (SD), nmol/L		Mean Change From Baseline	N	Mean (SD), nmol/L		Mean Change From Baseline	N	Mean (SD), nmol/L		Mean Change From Baseline
		Week 0	Week 24	%		Week 0	Week 24	%		Week 0	Week 24	%
N only	44	25.8 (2.7)	30.5 (4.9)	18.4**	32	31.8 (1.4)	34.3 (4.1)	7.9**	48	37.8 (3.8)	38.6 (4.7)	2.1*
E/S only	51	26.0 (2.5)	31.0 (3.7)	19.4**	62	31.7 (1.4)	35.6 (3.7)	12.2**	47	39.0 (4.8)	41.1 (5.9)	5.3*
E/S+N	96	25.9 (2.6)	32.8 (5.1)	26.9**	99	31.7 (1.3)	36.1 (4.4)	13.8**	98	39.1 (4.1)	41.9 (5.8)	6.9**
				P Value for Treatment Difference				*P* Value for Treatment Difference				*P* Value for Treatment Difference
E/S+N vs N		—	—	>0.05		—	—	>0.05		—	—	>0.05
E/S+N vs E/S		—	—	<0.009		—	—	>0.05		—	—	>0.05
E/S vs N		—	—	<0.05		—	—	>0.05		—	—	>0.05

HDL-P indicates high-density lipoprotein particle number; T1 to T3, baseline HDL-P tertile; SD, standard deviation; N, extended-release niacin (to 2 g/day); E/S, ezetimibe (10 mg/day)/simvastatin (20 mg/day).
*$P<0.01$; **$P<0.001$.

ultracentrifugation–vertical autoprofile (VAP), nondenaturing polyacrylamide gradient gel electrophoresis, and uniform nondenaturing tube gel electrophoresis.[26–32] Increases in the HDL_2 and HDL_3 subclasses were generally comparable for E+statin and statin monotherapy, although in diabetic patients E/S increased HDL_3 more than atorvastatin.[26,31,32] In addition, E/S+N therapy significantly improved changes in the cholesterol content of most apoB-containing lipoproteins and most HDL_2 and HDL_3 subclasses when assessed by VAP compared with N and E/S alone at 24 weeks in a prespecified analysis of this clinical study.[33]

Although LDL-C is the primary target of lipid-lowering therapy,[34] LDL particles vary in cholesterol content among individuals because of patient characteristics and are associated with plasma LDL-C concentration, TG levels, and various metabolic factors.[35] ApoB measurement has been used as a surrogate for LDL particle number and is a better predictor of CVD risk than LDL-C in various populations.[36] ApoB also includes the contribution of very-low-density lipoproteins, which may be significant in patients with mixed dyslipidemia. LDL particle number assessed by NMR spectroscopy has been shown to be more highly associated with CVD than LDL-C in several studies, in particular in the setting of LDL-C and LDL-P discordance.[13,18,37] In several statin intervention studies, the magnitudes of LDL-P and apoB reduction have been shown to be less than those for LDL-C and non-HDL-C in various populations, and it has been suggested that LDL-P may provide a better assessment of on-treatment residual risk, particularly in patients with cardiometabolic risk.[20,38,39] This discordance may be attributed to the predominance of small, dense LDL, that is, higher LDL particle number, a characteristic that is not reflected in measurement of LDL-C or non-HDL-C.

We observed greater reductions in LDL-P for individuals with higher baseline LDL-P across all 3 treatments, whereas LDL-C reductions were more similar regardless of initial LDL-P levels. There were also interesting differences in how these treatments affected lipoprotein lipids, lipoprotein particle numbers, and size distribution. As expected, N monotherapy resulted in the smallest reductions in LDL-C, and individuals with the highest LDL-P at baseline appeared to benefit the least from N monotherapy. In contrast, patients with the highest LDL-P at baseline appeared to benefit the most from either E/S monotherapy or the combination E/S+N. Changes in LDL size varied depending on baseline LDL-P, with a trend toward small increases in LDL size with both N treatments in the higher 2 LDL-P tertiles.

It should be noted that niacin monotherapy has been associated with increased LDL size and that combination niacin+simvastatin therapy increased LDL size more than atorvastatin alone.[22] The effects of statins[40,41] and ezetimibe[26,28–32,42] on LDL size have been variable, attributed to

differing patient populations studied, study sizes, baseline lipid profiles, and methodologies used in the lipoprotein assessments. Statins and ezetimibe have been shown to have the greatest effects on increasing LDL size in patients with high TG, presumably because of the higher levels of small, dense LDL.[30,43] Similarly, in our study, more pronounced increases in LDL size were observed in patients with high baseline LDL-P.

Some studies have also suggested that HDL-P may be a better predictor of CVD risk than HDL-C.[18,44,45] Although improvements in both HDL-P and HDL-C have been shown to be related to CVD risk reduction, the contribution of HDL-P appears to be more consistent after adjustments for baseline and metabolic parameters, including baseline levels of LDL-P and HDL-P.[44,45] Thus, NMR-derived HDL particle number may potentially be a more suitable surrogate marker for assessment of CVD risk and HDL-directed therapies than HDL-C. The few studies that have evaluated the effects of intervention on HDL-P have shown that niacin raises HDL-C more than HDL-P, whereas statins increase HDL-P more than HDL-C in patients with CHD risk.[20,46] In our study, both N and E/S+N treatments increased HDL-C more than HDL-P, and these effects were most pronounced in patients with higher HDL-P levels at baseline, whereas E/S treatment increased HDL-P more than HDL-C, mainly in the 2 lower HDL-P tertiles. Increases in HDL-C were somewhat attenuated in the highest HDL-P tertiles with all 3 therapies. The improvement in HDL profile with N monotherapy and E/S+N combination therapy for individuals with the lowest HDL-P at baseline is accounted for by an increase in both HDL-P and HDL size. In contrast, individuals with the highest HDL-P at baseline exhibited an increase in HDL size with minimal increase in HDL-P on these therapies.

It should also be noted that although particle number, both LDL and HDL, as assessed by NMR spectroscopy, has been shown to be associated with cardiovascular disease risk, the relationship of particle size to CVD risk is less definitive.[38] In part, this may be because plasma LDL-C and HDL-C represent a broad spectrum of particle sizes and because LDL size, estimated from mass-weighted mean particle diameters, may not be the best approach to representing this heterogeneity. Although reductions in cholesterol can shift the distribution of LDL particles, these changes result in minimal effects on mean particle diameter. Subgroup analysis of individuals matched for particle number may be required to demonstrate the contribution of particle size. It is possible that some indices of particle size distribution may be better predictors than the mass–weighted mean diameter that is currently being used.

A limitation of our study is that the samples analyzed were not randomly selected and were those available from the original clinical trial; however, the generally similar baseline characteristics across the E/S+N, E/S, and N treatment groups indicated that there was no selection bias in the samples that were analyzed. Furthermore, the effect of the different treatments on traditional end points (TC, TG, HDL-C, and LDL-C) in the subset was comparable to that observed in the original trial. In addition, our analysis was exploratory in nature, and as with any post hoc analysis, the results should be interpreted carefully. Nonetheless, our study results are consistent with the limited prior reports of these agents on LDL and HDL subfractions. Furthermore, this is the first analysis of combination E/S+N therapy on LDL and HDL particle number/size by NMR spectroscopy and provides new knowledge regarding lipid-lowering combination therapy.

In conclusion, E/S+N therapy reduced LDL-P and increased HDL-P more than N or E/S monotherapy in patients with mixed hyperlipidemia. The effects on LDL and HDL particle numbers were consistent with the lipid changes observed with the combination in these patients and may be most important in patients with high LDL-P and low HDL-P at baseline. Overall, these results indicate that assessing lipoprotein particle number in high-risk individuals may aid in better understanding the lipid profile in these patients. Additional studies are needed to further define the roles of LDL-P and HDL-P in clinical practice. It should also be noted that presently there is no definitive evidence that combination therapy with niacin and statins reduces CVD events more than statins alone[47,48]; thus, the clinical impact of these results is not known.

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