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Original Articles

Initial combination therapy with metformin plus colesevelam improves lipoprotein particles in patients with early type 2 diabetes mellitus

Ronald B. Goldberg, MD*, Robert S. Rosenson, MD, Eric Hernandez-Triana, MD, Soamnauth Misir, PharmD, Michael R. Jones, PhD

Division of Endocrinology, Diabetes and Metabolism, University of Miami Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA (Dr. Goldberg); Mount Sinai School of Medicine, New York, NY, USA (Dr. Rosenson); Endocare Research Institute, Universidad del Rosario, Bogota, Colombia (Dr. Hernandez-Triana); and Daiichi Sankyo, Inc., Parsippany, NJ, USA (Drs. Misir and Jones)

KEYWORDS:
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BACKGROUND: The bile acid sequestrant colesevelam has been shown to significantly reduce low-density lipoprotein particle concentration (LDL-P) in adults with primary hyperlipidemia or type 2 diabetes mellitus (T2DM).

OBJECTIVE: To assess the effect of initial combination therapy with metformin plus colesevelam on lipoprotein particles in patients with T2DM (secondary efficacy variables).

METHODS: This 16-week, randomized, double-blind, placebo-controlled study enrolled drug-naïve adults with T2DM, glycated hemoglobin 6.5%–10.0%, low-density lipoprotein cholesterol (LDL-C) ≥100 mg/dL, and triglycerides <500 mg/dL. Patients were randomized 1:1 to either open-label metformin (titrated to 1700 mg/day) plus double-blind colesevelam 3.75 g/day or open-label metformin plus double-blind placebo.

RESULTS: In total, 286 patients were randomized (metformin plus colesevelam [n = 145]; metformin plus placebo [n = 141]). Compared with metformin plus placebo, the combination of metformin plus colesevelam significantly reduced LDL-C (mean treatment difference: −16.3%), total cholesterol (−6.1%), non–high-density lipoprotein cholesterol (−8.3%), and apolipoprotein (apo) B (−8.0%) and significantly increased triglycerides (median treatment difference: 18.6%) and apoA-I (mean treatment difference: 4.4%; all $P < .001$). Metformin plus colesevelam significantly reduced total LDL-P (mean treatment difference: absolute change –186 nmol/L [percent change −11.7%]; both $P < .0001$), largely attributable to a reduction in small LDL-P, and increased total very-low-density lipoprotein particle concentration (mean treatment difference: absolute change 6 nmol/L; $P = .03$ [percent change 8.3%; $P = .06$]) and total high-density lipoprotein particle concentration (1.0 μmol/L; $P = .03$ [4.5%; $P = .01$]) versus metformin plus placebo.

CONCLUSION: Initial combination therapy with metformin plus colesevelam improved the atherogenic lipoprotein profile of patients with early T2DM by significantly reducing LDL-P. ClinicalTrials.gov identifier: NCT00570739.

* Corresponding author.
E-mail address: rgoldber@med.miami.edu

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The bile acid sequestrant colesevelam decreases low-density lipoprotein cholesterol (LDL-C), improves hyperglycemia in patients with type 2 diabetes mellitus (T2DM),[1–3] and is approved by the U.S. Food and Drug Administration as an add-on antihyperglycemic agent to metformin-, sulfonylurea-, or insulin-based therapy. The presence of diabetes mellitus is considered a coronary heart disease risk equivalent and, as a result, LDL-C treatment targets are more aggressive in patients with diabetes.[4] Thus, an agent such as colesevelam that lowers increased LDL-C in addition to improving hyperglycemia provides a unique combination of therapeutic effects on the two major cardiovascular risk factors in patients with T2DM. In T2DM, LDL-C is not always elevated, but LDL, as well as high-density lipoproteins (HDLs), have altered properties that may influence atherogenicity.[4–6] Patients with T2DM have been shown to have an increased concentration of small LDL particles, even when LDL-C is not increased.[5,6] Evidence indicates that LDL particle concentration (LDL-P) may be a better indicator of cardiovascular risk than LDL-C.[7] Interest has increased in better characterizing the lipoprotein profile in conditions such as T2DM, where a discrepancy between LDL-C and the number of atherogenic particles is commonly present,[8,9] and in evaluating the effect of treatments on these measures in clinical trials.

Colesevelam has been shown to reduce LDL-P in patients with T2DM taking other antidiabetes agents, including metformin, a sulfonylurea, or both.[10] Metformin, the recommended first choice antihyperglycemic therapy for most patients with T2DM, has been shown to have modest LDL-C−lowering effects,[11] as well as reducing LDL-P and increasing HDL particle concentration (HDL-P).[12] We have conducted a large, placebo-controlled study to evaluate the effects of initial combination of colesevelam and metformin compared with metformin alone in patients with T2DM.[13] Here we report the effects of metformin plus colesevelam on the concentrations and sizes of various lipoprotein particle subclasses from this study.

Methods

A 16-week, randomized, double-blind, placebo-controlled, parallel-group study was conducted from January 2008 to April 2009 at 16 sites in the United States, 10 in Mexico, 7 in Colombia, and 5 in India. Details of this study, including design, inclusion/exclusion criteria, and statistical methods, have been described previously.[13,14] To summarize, adults who had received a diagnosis of T2DM with glycated hemoglobin (HbA_{1c}) levels of 6.5% to 10.0%, LDL-C levels ≥100 mg/dL, and triglyceride (TG) levels <500 mg/dL were eligible. Patients included in the study had either never received antihyperglycemic treatment or had not received antihyperglycemic therapy within 3 months of screening. Patients were randomized 1:1 to receive open-label metformin plus double-blind colesevelam or open-label metformin plus double-blind placebo. All patients initiated metformin at 850 mg/day (1 × 850 mg tablet with the morning meal). After 1 week, the dosage was increased to 1700 mg/day (1 × 850 mg tablet with the morning and evening meal). If rapid titration was not tolerated, an additional 1 to 2 weeks were permissible for titration. If a patient could not tolerate metformin 1700 mg/day by week 4, he or she was permitted to continue in the study on metformin 850 mg/day. Colesevelam 3.75 g/day was taken either once daily (6 × 625 mg tablets with the evening meal) or twice daily (3 × 625 mg tablets with the noon and evening meals). Patients chose their preferred dosing schedule, which was to be maintained for the duration of the study.

The primary efficacy variable was the change in HbA_{1c} from baseline to week 16 with last (postbaseline) observation carried forward (LOCF) imputation. Secondary efficacy variables, which also were evaluated using week 16 LOCF analyses, included change and percent change in lipids, apolipoproteins, and lipoprotein particle concentration and size. Lipoprotein particle concentration and size were determined by nuclear magnetic resonance spectroscopy by LipoScience Inc. (Raleigh, NC).[15]

Statistical methods

The sample size was based on the primary efficacy variable. At a 2-sided level of significance of .05, assuming a common standard deviation of 1.2% and using a 2-sample *t* test, a sample size of 113 patients in each treatment group would have provided ≥80% power to detect a difference between the groups of ≥0.45% in the change from baseline in HbA_{1c} value. To allow for withdrawals, the number of patients was adjusted to 130 per group.

Analyses were carried out with the intent-to-treat population, which included all randomized patients who had taken at least 1 dose of study medication and had a baseline and at least 1 post-baseline efficacy variable measurement. An analysis of covariance (ANCOVA) model, which included treatment and country as fixed effects and the baseline value as a continuous covariate, was used. The treatment difference was expressed as the difference between the least-squares mean change for each treatment arm. Hypothesis testing was conducted at a 2-sided significance level of 5%.

Before fitting the ANCOVA model, we examined the normality assumption of the efficacy data by using Q-Q plots of the residuals versus quantiles of the standard normal distribution and the Shapiro-Wilk normality test. When significant departure from normality was observed, a log-transformation was performed, and Q-Q plots were again used on the transformed data to ensure the data followed a log-normal distribution. When log-transformation was used, log(endpoint) minus log(baseline) served as the dependent variable, with log(baseline) as the covariate for the ANCOVA models. The geometric least-squares mean ratio, as well as 2-sided 95% confidence intervals for the mean ratio of 2 treatment groups, was calculated. If significant departure from normality on the log-transformation of efficacy data was still observed, a nonparametric equivalent of ANCOVA (rank ANCOVA) stratified by country was applied.

Post-hoc correlation analyses were performed using the CORR procedure in SAS 9.1. The Pearson correlation coefficient (r) and associated P value from a test for nonzero correlation were determined. The week 16 measurements (LOCF) were used for the correlation analysis. The analyses were performed using the full analysis population.

Results

Demographic and baseline characteristics

In total, 286 patients with T2DM were randomized to receive initial treatment with metformin plus colesevelam (n = 145) or metformin plus placebo (n = 141). Demographic and baseline characteristics were similar between the treatment groups (Table 1). The majority of the randomized population was Hispanic (63%). At baseline, 11 patients (8%) in the metformin plus colesevelam and 9 (6%) in the metformin plus placebo group were receiving statin therapy, whereas three patients (2%) in the metformin plus colesevelam and two (1%) in the metformin plus placebo group were receiving fibrate therapy.

Efficacy

Treatment with metformin plus colesevelam, in comparison with metformin plus placebo, resulted in a significant mean reduction in HbA_{1c} (mean treatment difference: –0.3%; $P = .0035$; primary efficacy variable), LDL-C (–16.3%), total cholesterol (–6.1%), non–HDL-C (–8.3%), and apolipoprotein (apo) B levels (–8.0%), and a significant median increase in TG (median treatment difference: 18.6%) and mean increase in apoA-I levels (mean treatment difference: 4.4%) from baseline to Week 16 (all $P < .001$).[13]

Table 1 Demographic and baseline characteristics (randomized population)[14]*

	Metformin + colesevelam (n = 145)	Metformin + placebo (n = 141)	P value
Age, y	52.7 ± 11.5	53.9 ± 10.1	.34
Sex, no. (%)			
Male	69 (48)	56 (40)	.19
Female	76 (52)	85 (60)	
Race, no. (%)			
White	21 (15)	20 (14)	.87
Hispanic	89 (61)	90 (64)	
Asian	32 (22)	30 (21)	
Black	3 (2)	1 (1)	
Country, no. (%)			
United States	30 (21)	30 (21)	>.99
Mexico	58 (40)	56 (40)	
Colombia	26 (18)	25 (18)	
India	31 (21)	30 (21)	
Weight, kg	80.8 ± 15.53	77.3 ± 16.17	.06
BMI, kg/m^2	30.6 ± 4.67	29.8 ± 4.44	.14
Previously diagnosed, No. (%)			
T2DM	109 (75)	110 (78)	.82
Prediabetes	21 (15)	17 (12)	
No T2DM or prediabetes	15 (10)	14 (10)	

BMI, body mass index; T2DM, type 2 diabetes mellitus.
*All data are presented as mean ± SD unless otherwise stated.

LDL particles

At week 16 with metformin plus colesevelam compared with metformin plus placebo, total LDL-P showed a significantly greater mean absolute ($P < .0001$; Fig. 1) and percent ($P < .0001$; Table 2) reduction from baseline. Large and small LDL-P showed significant treatment differences in terms of absolute changes ($P < .01$; Fig. 1), but not percent changes (Table 2). LDL size was increased from baseline in both treatment groups at Week 16; the mean treatment difference was not significant (Fig. 1).

VLDL particles

At week 16, treatment with metformin plus colesevelam, compared with metformin plus placebo, resulted in a significantly greater mean absolute ($P = .03$; Fig. 1) and numerically greater percent ($P = .06$; Table 2) increase from baseline in total very-low-density lipoprotein particle concentration (VLDL-P). The absolute and percent change in both large and medium VLDL-P showed significant increases with metformin plus colesevelam versus metformin plus placebo ($P < .001$, Fig. 1; and $P \leq .01$, Table 2, respectively), but there were no significant between-group differences regarding change from baseline in small VLDL-P (Fig. 1; Table 2). VLDL size increased with metformin plus colesevelam but decreased with metformin plus placebo, resulting in a significant mean treatment difference at Week 16 ($P = .01$; Fig. 1).

HDL particles

Total HDL-P was increased from baseline in both treatment groups at week 16, although the increase was significantly greater with metformin plus colesevelam compared with metformin plus placebo in terms of both absolute change ($P = .03$; Fig. 1) and percent change ($P = .01$; Table 2). Significant absolute ($P < .05$; Fig. 1) but not percent (Table 2) increases in large and medium HDL-P were noted in the metformin plus colesevelam group versus the metformin plus placebo group, but there were no significant between-group differences regarding change from baseline in small HDL-P (Fig. 1; Table 2). HDL size was increased from baseline in both treatment groups at week 16, although the increase was significantly greater with metformin plus colesevelam versus metformin plus placebo ($P = .002$; Fig. 1).

Correlation analysis

In the group receiving combination therapy, but not in the metformin plus placebo group, there was a direct weak, but statistically significant, correlation between changes in HbA_{1c} and changes in total LDL-P ($r = 0.212$; $P = .02$), suggesting a possible mechanistic connection between the two

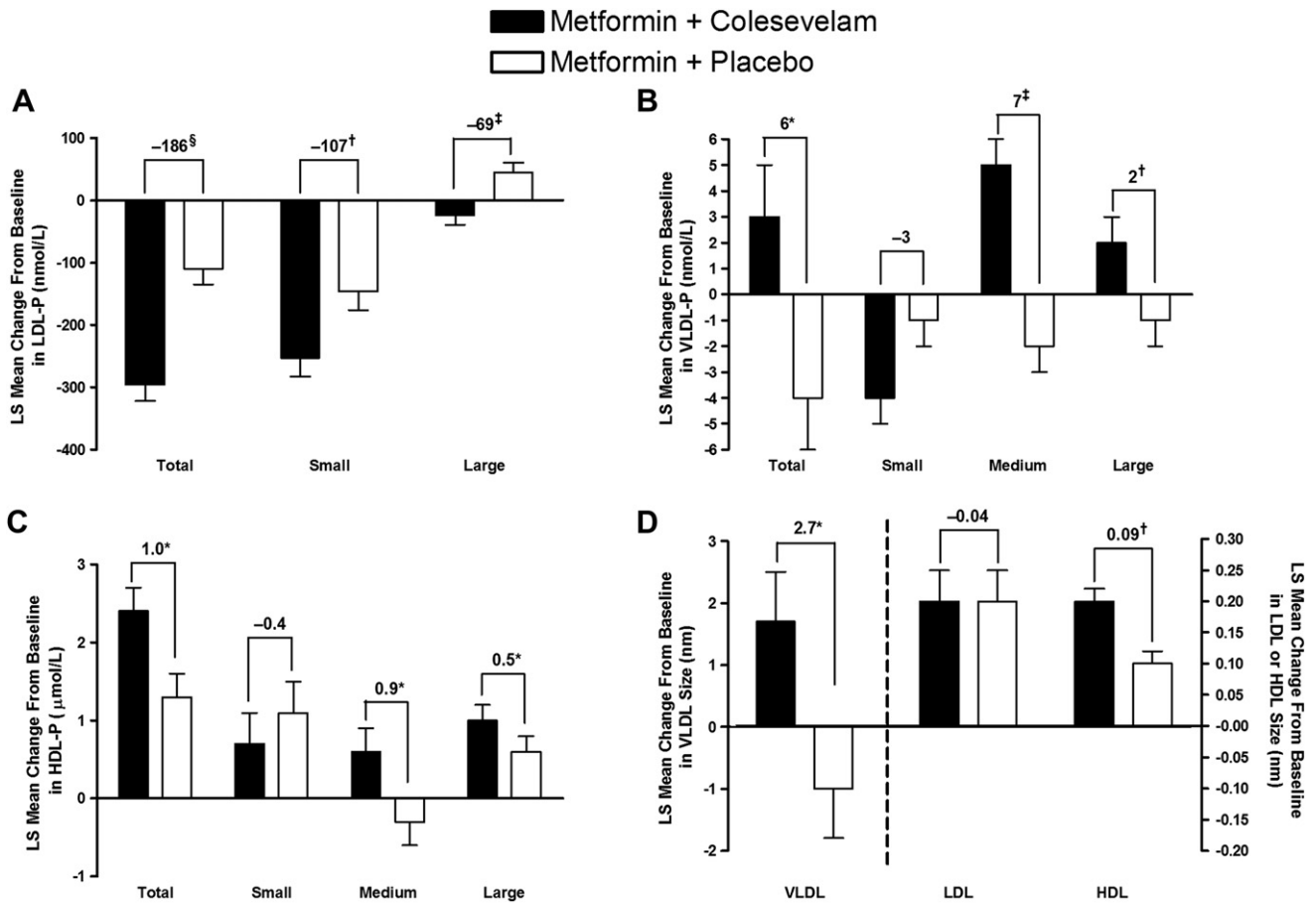

Figure 1 Least squares (LS) mean change from baseline to week 16 and LS mean treatment difference for (A) LDL-P, (B) VLDL-P, (C) HDL-P, and (D) lipoprotein size in patients with T2DM (intent-to-treat population). *$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$ for treatment difference (metformin + colesevelam – metformin + placebo).

effects. Not surprisingly, VLDL-P values were correlated with changes in TG, the strongest correlation being with large VLDL ($r = 0.736$–0.832), but the direction and magnitude of these correlations were similar in the two treatment groups. Changes in TG, however, were correlated with intermediate-density lipoprotein particle concentration in the combination group ($r = 0.188$; $P = .03$) but not in the metformin plus placebo group. Changes in TG in the combination group were not correlated with changes in total LDL-P or any of the subfractions. In the metformin plus placebo group, on the other hand, changes in TG were inversely correlated with changes in large LDL-P ($r = -0.306$; $P = .0003$) and directly correlated with changes in small LDL-P ($r = 0.264$; $P = .002$). There was also a significant positive correlation between change in TG and change in total HDL-P ($r = 0.202$; $P = .02$) in the group receiving metformin plus colesevelam, but not in the group receiving metformin plus placebo.

Discussion

In the current study, metformin plus colesevelam treatment resulted in favorable changes to lipid fractions more strongly related to cardiovascular disease (CVD) in patients with early T2DM. Total cholesterol, LDL-C, non–HDL-C, and apo B levels were reduced with metformin plus colesevelam treatment, although TG levels were significantly increased. In addition, total LDL-P was significantly reduced, largely as the result of a decrease in small LDL-P. Furthermore, total HDL-P was significantly increased as the result of elevations of both large and medium HDL-P. The data therefore demonstrate that colesevelam, when combined with metformin, may be an effective treatment for reducing LDL-P, and especially small LDL-P, in patients with T2DM. The reductions in both LDL-C and LDL-P in essence reflect a less atherogenic profile. In the present study, the addition of colesevelam to metformin treatment led to a greater reduction in total and small LDL-P than did treatment with metformin plus placebo, while LDL size increased equally with both treatments. There is conflicting information regarding whether LDL size independently predicts CVD risk.[16–20] These findings are in support of previous colesevelam studies in patients with primary hyperlipidemia[21] and in combination with metformin and/or sulfonylurea therapy in patients with T2DM.[10] The reduction in LDL-P in the present study occurred despite an increase in TG and VLDL-P, which is usually accompanied by an increase in small LDL-P.[22] Although there was a small, but statistically significant, increase in VLDL-P, there was a decrease in LDL-P that was approximately 30-fold greater. Thus, the

Table 2 Lipoprotein particle concentrations and sizes at baseline and week 16 (intent-to-treat population)

Lipoprotein	Baseline, mean ± SD		Week 16 LOCF, mean ± SD		Percent change from baseline, mean ± SD		Treatment difference,* LS mean ± SE (95% CI)	*P* value
	Metformin + colesevelam	Metformin + placebo	Metformin + colesevelam	Metformin + placebo	Metformin + colesevelam	Metformin + placebo		
VLDL-P, nmol/L								
Total	81 ± 32	81 ± 31	84 ± 37	78 ± 29	9.8 ± 3.2	1.5 ± 3.2	8.3 ± 4.5 (−0.5, 17.1)	.06
Large/chylomicrons	6 ± 6	6 ± 6	8 ± 8	6 ± 6	137.7 ± 27.7	38.8 ± 27.6	99.0 ± 38.2 (23.8, 174.1)	.01
Medium	33 ± 21	33 ± 20	38 ± 23	31 ± 20	62.7 ± 12.9	13.6 ± 12.8	49.1 ± 17.7 (14.2, 83.9)	.006
Small	42 ± 17	42 ± 17	38 ± 18	41 ± 17	8.0 ± 7.1	7.7 ± 7.0	0.2 ± 9.8 (−19.0, 19.5)	.98
VLDL size, nm	53.8 ± 9.5	54.3 ± 8.6	56.1 ± 10.6	53.6 ± 10.2	NA	NA	NA	NA
LDL-P, nmol/L								
Total	1623 ± 431	1670 ± 395	1336 ± 414	1551 ± 359	−16.6 ± 1.6	−4.9 ± 1.6	−11.7 ± 2.2 (−16.1, −7.3)	<.0001
Large	329 ± 229	336 ± 237	311 ± 217	380 ± 242	−1.2 ± 58.3	111.8 ± 57.3	−113.0 ± 79.9 (−270.5, 44.4)	.16
Small	1212 ± 522	1247 ± 491	961 ± 478	1094 ± 453	−10.4 ± 5.1	−7.6 ± 5.0	−2.8 ± 7.0 (−16.6, 11.0)	.69
LDL size, nm	20.4 ± 0.8	20.3 ± 0.8	20.5 ± 0.9	20.5 ± 0.8	NA	NA	NA	NA
HDL-P, μmol/L								
Total	28.7 ± 5.8	28.4 ± 4.8	31.1 ± 5.5	29.9 ± 4.9	10.4 ± 1.3	5.9 ± 1.3	4.5 ± 1.8 (1.0, 7.9)	.01
Large	4.9 ± 2.8	4.6 ± 2.9	6.0 ± 2.8	5.2 ± 2.8	55.4 ± 14.4	39.5 ± 14.3	15.9 ± 19.8 (−23.1, 54.9)	.42
Medium	2.6 ± 2.8	2.5 ± 3.3	3.3 ± 3.8	2.3 ± 3.3	98.7 ± 37.8	78.8 ± 39.0	19.9 ± 52.8 (−84.1, 124.0)	.71
Small	21.2 ± 5.2	21.3 ± 4.8	21.8 ± 5.3	22.4 ± 5.0	6.0 ± 2.1	8.7 ± 2.1	−2.7 ± 2.9 (−8.4, 3.0)	.36
HDL size, nm	8.6 ± 0.3	8.6 ± 0.3	8.8 ± 0.4	8.7 ± 0.4	NA	NA	NA	NA

CI, confidence interval; HDL, high-density lipoprotein; HDL-P, HDL particle concentration; LDL, low-density lipoprotein; LDL-P, LDL particle concentration; LOCF, last observation carried forward; LS, least squares; VLDL, very-low-density lipoprotein; VLDL-P, VLDL particle concentration; NA, not applicable.

*Difference is displayed as metformin + colesevelam – metformin + placebo.

small increase in potentially atherogenic VLDL-P was greatly outnumbered by the decrease in LDL-P. The increase in VLDL-P was attributable to changes in large and medium VLDL-P, and was not associated with a reduction in HDL-P, which rose favorably. Thus, there was an overall decrease in the total number of potential atherogenic particles (VLDL, intermediate-density lipoprotein, and LDL) with metformin combined with colesevelam versus metformin plus placebo.

These changes are also of interest in view of the tendency of bile acid sequestrants to increase TG levels. We confirmed that compared with metformin plus placebo, metformin plus colesevelam increased total, large, and medium VLDL-P and VLDL size, consistent with evidence that decreased hepatic farnesyl X receptor (FXR) activity resulting from bile acid sequestration leads to increased VLDL synthesis and secretion.[23,24] Typically, increasing TG levels are accompanied by cholesteryl ester transfer protein and hepatic lipase–mediated remodeling of LDL and HDL, leading to increases in smaller, denser particles, which have been suggested to be proatherogenic.[25,26] In the present study, despite the significant elevation in TG levels and VLDL-P accompanying add-on colesevelam treatment, both small and large LDL particles were reduced, with a net increase in LDL size due to the relatively greater reduction in small versus large LDL-P.

In addition, colesevelam treatment was associated with an increase in total HDL particles and individually medium and large HDL-P subclasses with an overall increase in HDL size. Increased HDL-P and large HDL have been associated with a protective effect against heart disease.[17,27,28] The HDL findings therefore demonstrate that despite the rise in TG levels, colesevelam treatment does not lead to a decrease in HDL-C, in contrast to the typical inverse relationship observed between TG and HDL. The positive association seen in the correlation analysis between TG and total HDL-P in the metformin plus colesevelam treatment group implies an important effect of colesevelam on increasing HDL-P that is not mitigated by the increase in TG. In fact, apoA-I levels were increased, which also implies an increase in the number or size of HDL particles. These findings thus demonstrate that the colesevelam-induced increase in TG levels and VLDL-P is not associated with the typical decreases in LDL and HDL size that accompany other causes of TG elevation. This is further supported by our findings that while changes in TG and VLDL, especially large VLDL, were similarly correlated in both treatment groups, change in TG was inversely associated with a change in LDL size only in the metformin plus placebo group. Overall, however, these findings argue against the colesevelam-induced increase in TG levels as involving potentially proatherogenic lipoprotein remodeling alterations.

The mechanism for the differential effects of colesevelam on LDL and HDL particle number is not well understood. The decreases in LDL-C, apo B and LDL-P are thought to result from bile acid sequestrant–induced upregulation of hepatic LDL receptors. Another bile acid sequestrant, cholestyramine, has also been shown to decrease cholesteryl ester transfer protein activity, which could explain the reduction in small LDL-C through reduced remodeling, despite the increase in TG.[29] In addition, FXR activation has been reported to suppress apoA-I and hepatic lipase expression,[30] so that bile acid sequestrant–induced reduction in FXR activity might lead to an increase in total and large HDL-P. Whatever the mechanism, it does appear that the effects of colesevelam on LDL are in part related to its action in improving the HbA_{1c} value. The results of the correlation analyses showed a significant correlation between the changes in HbA_{1c} and LDL-P.

This finding is important, as it is possible that the HbA_{1c} lowering and LDL-P lowering could have been attributable to different subgroups of patients within each treatment group; thus, the mean values for both variables could have decreased overall without any relationship between them. However, the results of the correlation analyses suggest that the changes in HbA_{1c} and LDL-P occurred in the same patients. It is possible that some of the study findings could have been influenced by concomitant metformin therapy. These preliminary data indicate that metformin is associated with a reduction in LDL-P and an increase in both small and large HDL-P, consistent with the effects of metformin as observed in the Diabetes Prevention Program.[12] Thus, our findings show that colesevelam provides an additional decrease in LDL-P and increase in HDL-P over that achieved with metformin alone. This advantage over metformin is quite remarkable, as a retrospective study of metformin use showed more favorable lipids at 12 months compared with sulfonylureas or thiazolidinediones.[31]

Potential limitations of the study were that our randomization was not stratified by sex, ethnicity, or country, and that the study population consisted mostly of Hispanic females, which could limit the overall generalizability of these results. However, given the lack of clinical trial data in nonwhite populations, these data serve to expand our knowledge in patients of different ethnicity.

Conclusion

Initial combination therapy with metformin plus colesevelam improved the atherogenic lipoprotein profile by significantly reducing LDL-P, especially small LDL-P, and increasing HDL-P in patients with early T2DM. The regimen induced a moderate increase in TG, which was mediated through increases in large particle sizes of VLDL, but these changes were not associated with any deleterious effects on LDL or HDL or on total atherogenic particle number. Whether these favorable lipoprotein changes lead to a reduction in the risk of CVD must await long-term studies of this treatment.

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Dr. Hernandez-Triana has served on scientific advisory boards for Eli Lilly and Company, Novo Nordisk, Pfizer Inc, and Roche, and received consulting fees and research grants from Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Johnson & Johnson, MSD, Novartis, and sanofi-aventis. Dr. Hernandez-Triana and his immediate family do not have ownership interest and or stocks of any pharmaceutical or device company.

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