Changes in cholesterol absorption and cholesterol synthesis caused by ezetimibe and/or simvastatin in men

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Abstract **This study evaluates changes in cholesterol balance in hypercholesterolemic subjects following treatment with an inhibitor of cholesterol absorption or cholesterol synthesis or coadministration of both agents. This was a randomized, double blind, placebo-controlled, four-period crossover study to evaluate the effects of coadministering 10 mg ezetimibe with 20 mg simvastatin (ezetimibe/simvastatin) on cholesterol absorption and synthesis relative to either drug alone or placebo in 41 subjects. Each treatment period lasted 7 weeks. Ezetimibe and ezetimibe/simvastatin decreased fractional cholesterol absorption by 65% and 59%, respectively ($P < 0.001$ for both relative to placebo). Simvastatin did not significantly affect cholesterol absorption. Ezetimibe and ezetimibe/simvastatin increased fecal sterol excretion (corrected for dietary cholesterol), which also represents net steady state cholesterol synthesis, by 109% and 79%, respectively ($P < 0.001$). Ezetimibe, simvastatin, and ezetimibe/simvastatin decreased plasma LDL-cholesterol by 20, 38, and 55%, respectively. The coadministered therapy was well tolerated.** **The decreases in net cholesterol synthesis and increased fecal sterol excretion yielded nearly additive reductions in LDL-cholesterol for the coadministration of ezetimibe and simvastatin.—** Sudhop, T., M. Reber, D. Tribble, A. Sapre, W. Taggart, P. Gibbons, T. Musliner, K. von Bergmann, and D. Lütjohann. **Changes in cholesterol absorption and cholesterol synthesis caused by ezetimibe and/or simvastatin in men.** ***J. Lipid Res.*** **2009.** 50: **2117–2123.**

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The understanding of the role of cholesterol in the human condition in relation to health or disease has improved over the last 50 years. With the identification of various pathways of cholesterol and bile acid metabolism as well as the discovery of new compounds that modulate cholesterol synthesis and intestinal cholesterol absorption, there is now a battery of lipid lowering medicinal products available. It is known that combining lipid lowering agents with different modes of action may enhance the lipid altering effect, but the impact of the combined treatment effects on the major cholesterol balance mechanisms is only poorly understood.

In addition to newer medications meant to treat hypercholesterolemia, there have been major improvements in the development of methods of measuring cholesterol balance. The use of nonradiolabeled isotope enrichment of tracer cholesterol and the nonabsorbed marker sitostanol and multiple selective ion monitoring gas chromatography-mass spectroscopy (GC-MS) have made it possible to make repeated treatments within a study, whereas, previously, the number of treatments and measurements was limited by exposure to radiation and/or the ability to detect the isotopes. Numerous studies of cholesterol absorption have demonstrated that there are wide interindividual variations in the fraction of cholesterol absorbed with a range of about 15–70% in normal healthy individuals (1–5).

This study investigated the influence of simvastatin and ezetimibe and the combination of simvastatin and ezetimibe on cholesterol balance by assessing fractional cholesterol absorption from the gastrointestinal tract by measuring the absorption of isotopic cholesterol compared with the nonabsorbed marker sitostanol (6) as well as cholesterol synthesis by mass balance (7).

Simvastatin and ezetimibe are approved cholesterol lowering medications that are prescribed individually or together in patients in need of plasma cholesterol reduction. Simvastatin has been previously characterized as an inhibitor of HMG-CoA reductase and as an LDL receptor enhancer (8), and ezetimibe, an inhibitor of cholesterol absorption from the gastrointestinal tract, has been characterized as an inhibitor of the Niemann-Pick C1-Like 1

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Abbreviations: CI, confidence interval; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

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protein transporter in intestine and possibly other tissues/cells (9).

METHODS

Study design

This study was a randomized, double-blind, placebo-controlled, four-period, balanced crossover study comparing the effects of 10 mg ezetimibe plus 20 mg simvastatin (ezetimibe/simvastatin), 10 mg ezetimibe alone, 20 mg simvastatin alone, or placebo on fractional cholesterol absorption and cholesterol synthesis in male subjects with mild hypercholesterolemia. The clinical phase of protocol number 050 was conducted between July 2003 and April 2004. The trial was registered with www.clintrials.gov under number NCT00652301.

Following a 2-week, single-blind, placebo run-in period, to allow the subjects to acclimate to the dietary requirements of the study and to the medication regimen, subjects, as they qualified, were assigned to the next available allocation number. Treatment sequences had been previously randomly assigned to the allocation numbers by a computer program run by the study statistician into one of four treatment sequences (balanced Latin square) involving four consecutive 7-week treatment periods: *A*) 10 mg ezetimibe plus 20 mg simvastatin, *B*) ezetimibe placebo plus 20 mg simvastatin, *C*) 10 mg ezetimibe plus simvastatin placebo, and *D*) ezetimibe placebo plus simvastatin placebo. The four treatment sequences are indicated in **Table 1**. The design is presented schematically in **Fig. 1**. The investigator, subjects, study personnel, laboratory analytical personnel, and study sponsor personnel were blinded to the treatment assigned to the sequence until the study was closed and the database locked.

Washout periods were not included between treatments, since the possibility of carryover drug effect from one measurement period to the next was expected to be negligible with the 7-week period between measurements, based on the findings of prior studies. The duration of the treatment periods was consistent with the standard 6-week washout period for statins with week 7 included in this study for execution of the cholesterol and mass balance measurements. It is also consistent with data from tracer studies that indicate that the rapidly miscible pool of cholesterol has equilibrated with the slower turnover pool within that time frame (10). This time interval is also more than adequate for washout of ezetimibe, which has an estimated half-life of about 24 h (9). Likewise, the duration of the treatment periods was expected to be sufficient to avoid overlapping effects of residual isotopic tracer used to measure fractional cholesterol absorption.

The study was conducted in accordance with the principles of the Declaration of Helsinki, the Good Clinical Practice Guidelines, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research. The study protocol was approved by the Ethical Committee of the Faculty of Medicine of the University of Bonn, Bonn, Germany, and all participants of the study provided written informed consent.

TABLE 1. Treatment sequences

Treatment	Period 1	Period 2	Period 3	Period 4
Sequence 1	A	B	D	C
Sequence 2	B	C	A	D
Sequence 3	C	D	B	A
Sequence 4	D	A	C	B

A: 10 mg Ezetimibe and 20 mg simvastatin. B: Ezetimibe placebo and 20 mg simvastatin. C: 10 mg Ezetimibe and simvastatin placebo. D: Ezetimibe placebo and simvastatin placebo.

Subjects

Subjects eligible for the study were men between the ages of 18 and 55, inclusive, in general good health, having a body mass index between 18 and 31 kg/m^2. Women were not included in this study because of potential shifts in fractional cholesterol absorption during the menstrual cycle (11). The acceptable ranges for laboratory plasma lipid values were LDL-cholesterol (LDL-C) concentrations between 130 mg/dl (3.36 mmol/L) and 180 mg/dl (4.65 mmol/L) and triglycerides <250 mg/dl (2.83 mmol/L). Additional criteria based on the specific sample collection and dietary consistency requirements of the study were as follows: At least one bowel movement, but not more than two per day, on average, without regular laxative use; good medication compliance defined by not having missed more than one tablet during the placebo run-in period; completion of placebo run-in food diary and a dietary intake of between 200 and 500 mg/day of cholesterol based on a calculated analysis of the 7-day food diary.

Individuals were not enrolled in the study if there was any condition that, in the opinion of the investigator, would be likely to render the individual unable to complete the study or for which study participation would produce significant risk or not be in the best interests of the subject.

Cholesterol balance measurements

Fractional cholesterol absorption (the primary endpoint) was measured during the seventh week of each treatment period using a previously validated, continuous-feeding, dual-stable isotope method (6, 12, 13). Cholesterol synthesis (a secondary endpoint) was evaluated based on fecal sterol mass balance estimates (7). Typical clinical plasma lipid concentrations were also evaluated.

Fractional cholesterol absorption

Fractional cholesterol absorption (the primary endpoint) was assessed during the seventh week of each treatment period using the continuous-feeding, dual-stable isotope method. For this purpose, subjects ingested tracer capsules containing 3 mg of [$^2H_6$]cholesterol and 3 mg of [$^2H_4$]sitostanol three times daily for 7 days during the seventh week of each treatment period (days 36–42, respectively). During the final four days of each treatment period, stool samples were collected, and measurement of intestinal cholesterol absorption was performed by GC-MS from these samples as described previously (6).

Fecal sterol balance estimates

Fecal sterol balance estimates were based on sterol intake estimates from food diaries and fecal output measurements of neutral and acidic sterols (7). Subjects were instructed to maintain a consistent diet throughout the course of the study, and dietary intake was collected via 7-day food diaries during the final week of the placebo run-in period and for week 7 of each active treatment period. The use of dietary supplements containing agents that lower cholesterol or margarines or other products containing phytosterols/phytostanols was prohibited.

Dietary intake data were analyzed for cholesterol intake (mg/day) using computerized food composition and nutrition tables (Prodi 4.5; Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany). Neutral and acidic sterols in stool samples were evaluated by GC analysis of sample extracts following trimethyl silyl derivatization (7). Each daily stool sample provided an independent value; the median value of all stool samples was used to calculate fractional cholesterol absorption for each of the four treatment

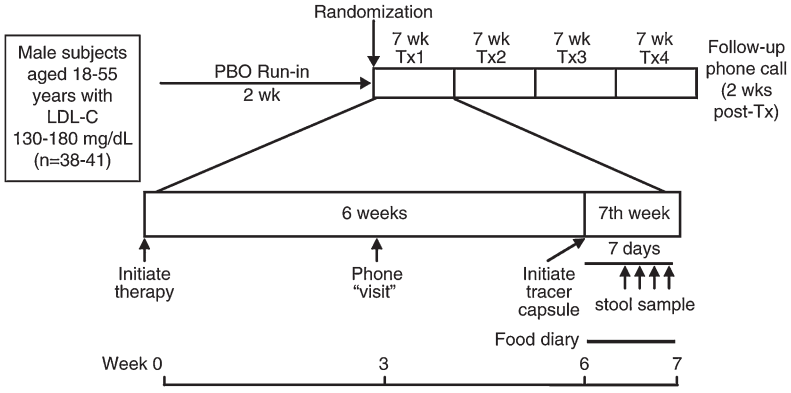

Fig. 1. Study flow chart.

periods. Cholesterol synthesis was calculated by subtracting dietary cholesterol intake from the sum of neutral and acidic sterol output on the final four days of the treatment periods. These calculations assume that steady state had been achieved (5).

Plasma lipids

Plasma total cholesterol (TC) and triglyceride (TG) concentrations were determined using standard clinical laboratory enzymatic methods. HDL-cholesterol (HDL-C) was measured after precipitation of the apolipoprotein B-containing lipoproteins (LDL and VLDL) in whole plasma by heparin-manganese chloride. NonHDL-C was calculated by subtracting the HDL-C value from the TC value. LDL-C was calculated according to the Friedewald equation: LDL-C = TC − (HDL-C + TG/5) (14).

Statistical analysis

While the data were still blinded, the following criteria were applied to the database to determine which subjects would be included in the primary analysis: therapy compliance as assessed by pill counts (⩾80%) (the average for compliance was >90% with no subject <85%); number of stool samples (⩾2) per treatment period (one subject was excluded based on this criterion); subjects were evaluated for extreme deviations from the recommended low-cholesterol, low-saturated-fat diet for possible exclusion from the primary analysis (no subject was excluded using these criteria).

Primary and secondary objectives

The primary endpoint of the study was the fractional cholesterol absorption after 7 weeks of treatment. The secondary efficacy endpoint was the cholesterol biosynthesis rates (based on fecal sterol balance estimates) after 7 weeks of treatment. The analyses for the above endpoints were based on natural logarithm-transformed data. To provide a more robust treatment response estimate for the primary and secondary endpoints, each subject must have had at least two or more cholesterol absorption/biosynthesis measurements (i.e., stool samples) per period to be included in the analysis. For each subject, the median of the daily fractional cholesterol absorption and synthesis measurements per period was used as the response variable. As a test of sensitivity, the primary analysis was repeated using means of daily fractional cholesterol absorption measurements per period as a response variable.

An ANOVA model appropriate for a four-period, crossover design with terms for subject, period, treatment, and carryover was used to determine the effect of administration of 10 mg ezetimibe plus 20 mg simvastatin versus 20 mg simvastatin alone on fractional cholesterol absorption. The between-treatment carryover term was tested and removed from the model if not significant. The normality assumption was tested using the Shapiro-Wilk statistic. The homogeneity of variance assumption was tested using Hartley's Fmax test. Additionally, estimation of the relative difference between treatments in fractional cholesterol absorption was determined by a 90% confidence interval on the geometric mean ratio of treatment means. The confidence interval was calculated for the differences in treatment means in natural log units and the mean square error from the above ANOVA model. The upper and lower limits were exponentiated back to obtain the confidence interval for the geometric mean ratio. The secondary variable (cholesterol synthesis) and exploratory variables (plasma LDL-C, TG, TC, nonHDL, and HDL-C concentrations) were also assessed with the same model; however, the analysis for lipid endpoints was performed without log-transforming the data. Treatment group summary statistics and respective error intervals were provided for each of the endpoints.

Parametric analyses were corroborated by nonparametric analyses based on Tukey's normalized ranks. Due to the large variability in the TGs, nonparametric analyses base on Tukey's normalized ranks were used as the primary result. For the secondary general safety hypothesis, no inferential tests were performed due to the limited sample size available in this study. Instead, summary statistics were generated for the key safety parameters [creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and adverse experience counts].

Analysis of multiplicity

Multiplicity was not an issue for the single primary treatment group comparison in the analysis: change in fractional cholesterol absorption for 10 mg ezetimibe plus 20 mg simvastatin versus 20 mg simvastatin alone after 7 weeks of treatment. However, for key secondary efficacy comparisons (effect of ezetimibe/simvastatin versus placebo and of ezetimibe versus placebo on cholesterol absorption, effect of ezetimibe/simvastatin versus ezetimibe alone on cholesterol synthesis), the Hochberg method was used for multiplicity adjustment to control the overall α level at $\alpha = 0.05$. No multiplicity adjustments were prespecified for exploratory endpoints.

Sample size and power

Based on a sample size of n = 32 subjects, this study would have 90% (80%) power to detect a 38% (33%) difference between groups in fractional cholesterol absorption. This calculation was based on a within-subject coefficient of variation of 46% estimated from data collected in a previous cholesterol absorption study of similar design (2).

Safety evaluations

All randomized subjects were used in the safety analysis. Safety and tolerability were assessed by statistical and/or clinical review of all safety parameters, including adverse experiences, laboratory

results, ECG, physical exams, and vital signs. Adverse experiences were categorized as either "clinical," derived from signs, symptoms, and observations, or "laboratory," which indicates a laboratory test result that the investigator considered to be an adverse experience. The safety data were categorized according to the four treatments each subject received during the study.

RESULTS

Subject disposition

Forty-one subjects were enrolled in the study. Of these 41 subjects, 40 completed the trial, and 39 met all criteria for inclusion in the per protocol data analysis. One subject who was dropped from the study before completion of all four periods required treatment with metronidazole, an antibiotic that has demonstrated lipid altering effects (15). The other subject was excluded from the analysis because of only having one stool sample during period 4. At baseline (n = 41), mean age was 39.6 years (range 23–55), mean body weight was 84 kg (range 68–109), and mean body mass index was 25.5 kg/m^2 (range 21.1–30.4).

Fractional cholesterol absorption

The primary endpoint for the study was the effect of the drugs on fractional cholesterol absorption. The geometric means for fractional cholesterol absorption were 20.0% for 10 mg ezetimibe plus 20 mg simvastatin, 50.7% for simvastatin, 17.0% for 10 mg ezetimibe, and 48.7% for placebo (**Table 2**). No significant sequence or period effects were found. The test for carry-over effect was not significant. The within-subject coefficient of variation was 41%. Since this variation was below the level used to estimate the number of subjects to meet a 90% power, the results for the fractional cholesterol absorption are valid at this level.

The geometric mean ratio for fractional cholesterol absorption with 10 mg ezetimibe plus 20 mg simvastatin versus 20 mg simvastatin was 0.39 [90% confidence interval (CI): 0.35 and 0.44], which represents a 61% reduction attributable to ezetimibe, which was statistically significant ($P < 0.001$). Examination of individual values indicated that each of the 39 subjects exhibited a reduction in fractional cholesterol absorption on coadministration therapy compared with 20 mg simvastatin and placebo (**Fig. 2**).

As a secondary endpoint, the geometric mean ratio for 10 mg ezetimibe plus 20 mg simvastatin versus placebo was 0.41 (90% CI: 0.36 and 0.46), which represents a 59% reduction in the fraction of cholesterol absorbed relative to placebo, which was significant ($P < 0.001$). For the comparison of ezetimibe to placebo, the geometric mean ratio for 10 mg ezetimibe versus placebo was 0.35 (90% CI: 0.31 and 0.39), which represents a 65% reduction in fractional cholesterol absorption relative to placebo, which was significant ($P < 0.001$). The effects of simvastatin and placebo on fractional absorption were not significantly different (90% CI: 0.92 and 1.17).

Cholesterol synthesis determined by fecal balance

As a secondary endpoint, the cholesterol synthesis rates (mg/day), determined by the mass balance method after 7 weeks of treatment, were 1,591 mg/day for 10 mg ezetimibe plus 20 mg simvastatin, 787 mg/day for 20 mg simvastatin, 1,851 mg/day for 10 mg ezetimibe, and 884 mg/day for placebo when expressed as geometric means (Table 2). The prespecified calculations of cholesterol synthesis assumed that steady-state conditions would be present during the seventh week of treatment.

The geometric mean ratio for 10 mg ezetimibe plus 20 mg simvastatin versus 10 mg ezetimibe was 0.86 (90% CI: 0.79 and 0.93), which represents a 14% reduction in synthesis of cholesterol relative to 10 mg ezetimibe. The geometric mean ratio for 10 mg ezetimibe plus 20 mg simvastatin versus placebo was 1.79 (90% CI: 1.65 and 1.94), which represents a 79% increase in synthesis of cholesterol for treatment with 10 mg ezetimibe plus 20 mg simvastatin relative to placebo. The corresponding geometric mean ratio for 20 mg simvastatin versus placebo was 0.89 (90% CI: 0.82 and 0.97). All three secondary comparisons were significant at the overall α level of $\alpha = 0.050$ after adjusting for multiplicity by the Hochberg method.

Plasma lipids

Although plasma lipids were an exploratory endpoint for the study, they represent typical clinical endpoints that are often measured to determine the effects of lipid altering drugs, such as ezetimibe and/or simvastatin. The least squares mean percentage changes in the LDL-C from baseline were −55.0% for ezetimibe/simvastatin, −38.3% for simvastatin, −20.0% for ezetimibe, and 2.6% for placebo (**Table 3**). Coadministration therapy was significantly superior to the monotherapy with either drug in lowering LDL cholesterol. Similarly, highly significant between-treatment differences were observed for TC, TG, and nonHDL-C. Coadministration therapy was borderline

TABLE 2. Determination of cholesterol synthesis from steady state mass balance after 7 weeks of treatment

Treatment (n = 39)	Placebo	10 mg Ezetimibe	20 mg Simvastatin	10 mg Ezetimibe + 20 mg Simvastatin
Parameter	Geometric Mean ± SD			
Fractional cholesterol absorption (%)	48.7^a ± 11.6	17.0^b ± 19.4	50.7^a ± 2.2	20.0^b ± 14.5
Dietary cholesterol (mg/day)	324^a ± 103	341^a ± 107	322^a ± 98	324^a ± 75
Neutral sterol excretion (mg/day)	1,009^a ± 550	1,919^b ± 729	893^a ± 464	1,715^c ± 691
Acidic sterol excretion (mg/day)	219^a ± 101	239^a ± 135	215^a ± 123	220^a ± 106
Cholesterol synthesis (mg/day)	884^a ± 649	1,851^b ± 798	787^a ± 567	1,591^c ± 755

Within each parameter, if the superscript letters for the means of treatments are the same, they are not statistically significantly different. If the letters are different, the means are significantly different at the $P \leq 0.001$ level, with the exception of c versus b, where $P \leq 0.02$.

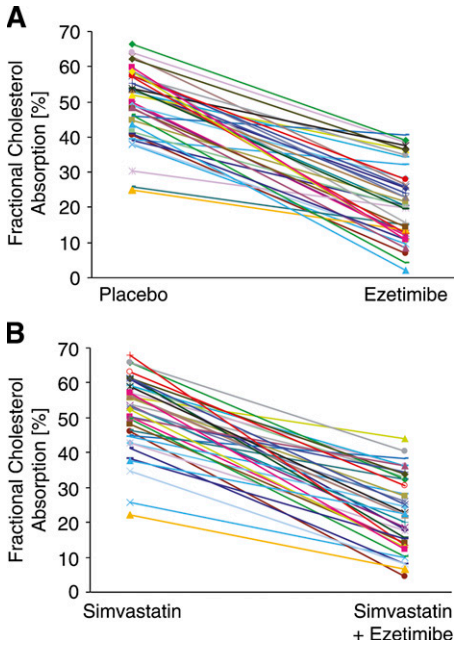

Fig. 2. Individual changes in fractional cholesterol absorption between placebo and ezetimibe (A) and between simvastatin and ezetimibe/simvastatin (B) treatment.

significantly different from simvastatin monotherapy for HDL-C (Table 3).

Safety

No subject experienced a consecutive elevation of the liver function tests >3 times the upper limit of normal during the course of the study. One subject experienced an elevation of CK ⩾10 times upper limit of normal on 20 mg simvastatin, which resolved without changing treatment. Treatment-related adverse experiences were observed in three subjects for the placebo and simvastatin treatments, one for ezetimibe treatment, and none for the combination treatment. There were no serious treatment-related adverse experiences. No subjects died, and one discontinued from the study. All treatments administered during this study were well tolerated.

DISCUSSION

The goal of this study was to measure various aspects of cholesterol balance within the body to provide quantitative values for important characteristics of cholesterol balance and disposition, especially when agents that modify two distinctly different aspects of cholesterol absorption and production perturb that balance. The results of the summation of these changes are the clinically relevant differences observed in plasma cholesterol levels.

The duration of each period of therapy in this crossover study was 7 weeks, with measurement of endpoint parameters made during the last week of each period. Based on prior studies of the half-lives, time course to maximal lipid changes, and time course of their reversal upon withdrawal of therapy, it can be assumed that steady state, in terms of treatment effects, was achieved by the time the key measurements were performed during each period (16–18). This was supported by the absence of any observed carryover effects.

The fractional absorption of cholesterol in the placebo group was measured at about 49%, which agrees well with similar values in the literature (2, 13, 16). Therefore, in normal individuals, the amount of cholesterol leaving the body on a daily basis via the gastrointestinal tract is about a gram of cholesterol with a relatively large range of approximately 0.25–4 g a day in the subjects in this study. This range of absorption is comparable to that seen in previous studies (1–5).

Ezetimibe reduced the fraction of cholesterol absorbed to <20%, which is less than half that in the untreated state. This inhibition of absorption is directly associated with a substantial increase in neutral sterol excretion. Simvastatin and ezetimibe together gave approximately the same absorbed fraction as ezetimibe alone. Simvastatin by itself had no significant effect on absorption at the dose and duration of treatment studied. The range of the fraction of cholesterol absorbed for ezetimibe compared with placebo in this relatively small population was considerable, varying from about 25–70% for placebo and 2–47% for ezetimibe, as seen in Fig. 2. Also shown in Fig. 2, the range of fraction absorbed for ezetimibe with simvastatin compared with simvastatin alone was similar, ranging from about 2–47% and 21–70%, respectively. It is notable that ezetimibe reduced the fraction of cholesterol absorbed in every individual, although the amount of the decrease varied markedly between individuals. Because we do not have a direct measure of the absolute amount of cholesterol at the site of absorption, we do not have a measured value for the absolute amount of cholesterol absorbed each day.

TABLE 3. Plasma cholesterol, TGs, and cholesterol precursor at baseline and after 7 weeks of treatment

Treatment (n = 39)	Baseline	Placebo	10 mg Ezetimibe	20 mg Simvastatin	10 mg Ezetimibe + 20 mg Simvastatin
Parameter (mg/dL)		Least Squares Mean Percentage Change from Baseline (±SE)			
TC	228.6	1.7^{a} (10.4)	-13.8^{b} (8.70)	-26.4^{c} (9.6)	-38.1^{d} (8.6)
LDL-C	152.1	2.6^{a} (12.9)	-20.0^{b} (10.6)	-38.1^{c} (12.2)	-55.0^{d} (10.4)
NonHDL-C	177.7	1.6^{a} (12.8)	-19.3^{b} (8.9)	-35.5^{c} (11.1)	-57.7^{d} (10.1)
HDL-C	50.9	2.4^{a} (10.9)	5.2^{a} (12.5)	6.0^{b} (12.3)	9.6^{b} (11.5)
		Median Percentage Change from Baseline (±SE)			
TG	128.5	-7.1^{a} (38.5)	-10.7^{a} (23.6)	-21.0^{b} (29.2)	-30.8^{c} (23.0)

Within each parameter, if the superscript letters for the means of treatments are the same, they are not statistically significantly different. If the letters are different, the means are significantly different at the $P \leqslant 0.001$ level.

The decreased fraction of cholesterol absorbed was associated with an increase in the excretion of neutral sterol, but there was little, if any, change in acidic sterol excretion. Thus, it is most likely attributable to the reduced absorption of cholesterol as a direct effect of ezetimibe on the intestinal cholesterol transport process.

Because the mass balance method applied at steady state estimates the daily synthesis of cholesterol, we have a measure of the ability of the body to replace the cholesterol lost each day as the sum of acidic sterols (bile acids) and neutral sterols (which include biliary cholesterol, gastrointestinal bacterial metabolites of cholesterol, and cholesterol lost via sloughing of intestinal cells). It is noted that mass balance is a whole-body issue, and there may be instances where there are local changes in cholesterol within tissues in the body after the whole body has achieved a new steady state. It is also likely that net changes specifically in cholesterol deposited in atherosclerotic plaques or xanthomas, which are believed to occur slowly and in small quantities, cannot be assessed using mass balance techniques.

Ezetimibe affects mass balance by reducing the amount of cholesterol returning to the liver via enterohepatic circulation resulting in compensatory changes. The pathways explored in animal studies are very similar to those operating in man and undoubtedly help in understanding the compensatory processes in man (19–21). These studies indicate that there is an increase in cholesterol synthesis in the liver and perhaps more importantly, in the intestinal tract. In addition, there is an upregulation of hepatic LDL receptors (19–21).

The addition of the cholesterol synthesis inhibitor simvastatin in the presence of ezetimibe reduces the ability of the body to replace cholesterol through synthesis; however, net synthesis remains increased relative to placebo. It is not clear whether the modest reduction in whole-body synthesis by simvastatin either alone or with ezetimibe reflects a change in synthesis in the liver and/or in other organs.

The incremental decrease in plasma LDL-C is believed to primarily reflect upregulation of LDL receptors (22, 23). Additionally, it is also possible to speculate that there would be a net flux of cholesterol from the periphery into plasma and eventually into the liver. Although tissue cholesterol would be balanced to maintain a healthy, steady state, any excess that has accumulated on red blood cells, arterial walls, adipose tissue, and macrophages could be removed by HDL and shunted via chylomicrons, VLDLs, and/or LDL for transport back to the liver (24).

The addition of simvastatin decreased the amount of cholesterol as cholesterol or cholesterol metabolites excreted (calculated by the mass balance method) by 14% compared with ezetimibe treatment alone and by 11% compared with placebo. Given that ezetimibe had increased the excretion by 109%, the 14% reduction still represents an 80% increase in cholesterol output compared with placebo.

The doubling of the amount of cholesterol and its metabolites excreted at steady state tell us that there was a concomitant increase in cholesterol synthesis when the subjects were taking ezetimibe alone and a concomitant decrease when they were taking simvastatin (5). When taking the two together, the mass balance findings clearly indicate that cholesterol synthesis remains increased, in contrast to the small decrease observed during simvastatin cotreatment. Thus, the ezetimibe effect on this parameter predominates.

It is apparent that the body is capable of maintaining the amount of cholesterol synthesis necessary to sustain a new steady state when only about half or less than half of the normal amount of cholesterol in the enterohepatic circulation returns to the liver. This occurs in conjunction with a reduction of plasma cholesterol and with maintenance of the capacity of bile to support digestion in general and to support the hydrolysis of TG and absorption of the resulting free fatty acids in the intestine.

The literature contains several studies suggesting that statins increase cholesterol absorption (25). In this study, it was observed that simvastatin, at a dose of 20 mg taken for 7 weeks, increased the fractional cholesterol absorption by about 4%, which was not statistically significantly different from placebo. The literature acknowledges that the amount of cholesterol in biliary bile was in fact reduced by 41% with simvastatin treatment; thus, the amount of cholesterol absorbed by the enterocyte would be reduced because of a smaller intraluminal pool size, if there was no change in the fraction absorbed and dietary cholesterol (26). Although speculative, this result would mean reduced chylomicron cholesterol reaching the liver, which would likely have a positive effect on hepatic LDL receptor activity. It should be noted that this result would be expected for statins in general, perhaps with quantitative differences among the statins, as a reduction of biliary cholesterol has been demonstrated (27, 28). A recent review giving a more thorough discussion of the processes of cholesterol absorption generally supports this hypothesis, which is drawn from the data available from this study (29).

In conclusion, ezetimibe and simvastatin maintain their respective independent effects on fractional cholesterol absorption and cholesterol synthesis when coadministered. By inhibiting both intestinal absorption and cholesterol synthesis, ezetimibe when administered with simvastatin altered the balance of cholesterol within the body, such that it resulted in a decrease in plasma concentrations of LDL-C by >50%.

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