Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia

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Abstract Sixteen hyperlipidemic men were enrolled in a randomized, placebo-controlled, double-blind, cross-over study to evaluate the effect of ezetimibe 10 mg and simvastatin 40 mg, coadministered and alone, on the in vivo kinetics of apolipoprotein (apo) B-48 and B-100 in humans. Subjects underwent a primed-constant infusion of a stable isotope in the fed state. The coadministration of simvastatin and ezetimibe significantly reduced plasma concentrations of cholesterol (−43.0%), LDL-C (−53.6%), and triglycerides (−44.0%). Triglyceride-rich lipoproteins (TRL) apoB-48 pool size (PS) was significantly decreased (−48.9%) following combination therapy mainly through a significant reduction in TRL apoB-48 production rate (PR) (−38.0%). The fractional catabolic rate (FCR) of VLDL and LDL apoB-100 were significantly increased with all treatment modalities compared with placebo, leading to a significant reduction in the PS of these fractions. We also observed a positive correlation between changes in TRL apoB-48 PS and changes in TRL apoB-48 PR ($r = 0.85$; $P < 0.0001$) with combination therapy. Our results indicate that treatment with simvastatin plus ezetimibe is effective in reducing plasma TRL apoB-48 levels and that this effect is most likely mediated by a reduction in the intestinal secretion of TRL apoB-48. Our study also indicated that the reduction in LDL-C concentration following combination therapy is mainly driven by an increase in FCR of apoB-100 containing lipoproteins.— Tremblay, A. J., B. Lamarche, J-C. Hogue, and P. Couture. **Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia. *J. Lipid Res.* 2009.** 50: **1463–1471.**

Supplementary key words Apolipoprotein B-48 • apolipoprotein B-100 • cholesterol absorption and synthesis • gas chromatography/mass spectrometry • intestine • kinetic • liver

Several lines of evidence show that an elevated plasma LDL-cholesterol (LDL-C) concentration is a major risk factor for atherosclerosis (1, 2). Lowering plasma LDL-C has been shown to slow the progression of atherosclerosis and to reduce the rate of cardiovascular events and mortality; thus, reducing LDL-C is a primary objective in the prevention of coronary heart disease (CHD) (3, 4). Plasma cholesterol concentrations are regulated by feedback mechanisms between endogenous (synthesis) and exogenous (dietary intake and intestinal absorption) pathways. Data suggest that the efficiency of cholesterol absorption and the rate of cholesterol synthesis are key factors regulating plasma lipoprotein levels, and both may play an important role in CHD development (5–7).

Simvastatin inhibits 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate-limiting step in the cholesterol biosynthesis pathway. The reduction of plasma LDL-C levels by statins has been associated with decreased hepatic apoB secretion (8, 9) and to enhanced LDL receptor-mediated clearance of apoB-containing lipoproteins (10–13). On the other hand, ezetimibe selectively inhibits the intestinal uptake and absorption of dietary and biliary cholesterol at the brush border of small intestine enterocytes, confining cholesterol to intestinal lumen for subsequent excretion, without affecting the absorption of triglycerides or fat-soluble vitamins (14, 15). Recent data suggest that this drug binds to the Niemann-Pick C1 Like 1 protein, which plays a major role in the intestinal uptake of cholesterol and plant sterols (16–18). Clinical studies have shown that coadministration of ezetimibe with statins could provide as much as an additional 12–19% reduction in LDL-C in patients with primary hypercholesterolemia (19–22). Recent data showed that coadministration of simvastatin and ezetimibe in pigs decreased VLDL and LDL apoB-100 concentrations through reduced VLDL production and up-regulation of LDL receptor-mediated LDL clearance (23). However, the effect of the coadministration of simvastatin

This work is supported by an unrestricted research grant from Merck Frosst/Schering Company; a scholarship from Fonds de recherche en santé du Québec (P.C.); and a grant from the Heart and Stroke Foundation (J-C.H.). Benoît Lamarche is a Canada Research Chair in Nutrition and Cardiovascular Health.

Manuscript received 21 November 2009 and in revised form 6 February 2009 and in re-revised form 16 March 2009.

Published, JLR Papers in Press, March 22, 2009.
DOI 10.1194/jlr.P800061-JLR200

Abbreviations: Apo, apolipoprotein; BMI, body mass index; CHD, coronary heart disease; FCR, fractional catabolic rate; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; LDL-C, low density lipoprotein cholesterol; MTP, microsomal triglyceride transfer protein; PR, production rate; PS, pool size; TRL, triglyceride-rich lipoproteins.

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and ezetimibe on the lipoprotein metabolism in humans has not been characterized yet. Therefore, the objective of the present study was to investigate the impact of ezetimibe and simvastatin, coadministered and alone, on the in vivo kinetics of apolipoprotein B–containing lipoproteins labeled with a stable isotope [L-(5,5,5-D_3)-leucine] in 16 males with primary hypercholesterolemia. We hypothesized that inhibition of HMG-CoA reductase and cholesterol absorption by dual therapy could decrease plasma levels of intestinally-derived lipoproteins by reducing the secretion of these particles, and alternatively, by enhancing their catabolism. The effects of ezetimibe and simvastatin on the plasma surrogates of cholesterol biosynthesis (lathosterol) and intestinal cholesterol absorption (campesterol, β-sitosterol) were also assessed.

METHODS

Subjects

Sixteen men with plasma LDL-C levels above the 50th percentile for their age were recruited in the Quebec City area to participate in the study (24). Subjects were excluded if they had persistent elevations of serum transaminases; monogenic hyperlipidemia, such as familial hypercholesterolemia; plasma triglyceride levels greater than 4.5 mmol/L; a recent history of alcohol or drug abuse; diabetes mellitus; or a history of cancer. Furthermore, all participants were unrelated at the first and second degree. All eligible subjects had to be withdrawn from lipid-lowering medications for at least six weeks before the beginning of the study. The study consisted of a one-week screening period and a four-week placebo run-in period, followed by four six-week double-blind treatment periods with ezetimibe 10 mg plus simvastatin 40 mg; ezetimibe 10 mg; simvastatin 40 mg; and placebo in random order. Kinetic studies using primed-constant infusion of deuterated leucine were performed following each phase of treatment. Participants were instructed to take one capsule in each bottle at the time of their evening meal. Compliance was assessed by pill counting. The research protocol was approved by the Laval University Medical Center ethical review committee and written informed consent was obtained from each subject.

Experimental protocol for in vivo stable isotope kinetics

To determine kinetics of triglyceride-rich lipoprotein (TRL) apoB-48, VLDL, IDL, and LDL apoB-100, subjects underwent a primed-constant infusion of L-[5,5,5-D_3] leucine while they were in a constantly fed state. Starting at 7 AM, the subjects received 30 identical small cookies every 30 min for 15 h, each equivalent to 1/30th of their estimated daily food intake based on the Harris-Benedict equation (25), with 15% of calories as protein; 45% carbohydrate; 40% fat (7% saturated, 26% monounsaturated, 7% polyunsaturated); and 85 mg of cholesterol/1000 kcal. At 10 AM, with two intravenous lines in place, one for the infusate and one for blood sampling, L-[5,5,5-D_3] leucine (10 μmol/kg body wt) was injected as a bolus IV and then by continuous infusion (10 μmol · kg body wt^{-1} · h^{-1}) over a 12-h period. Blood samples (20 ml) were collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 11, and 12 h.

Characterization of plasma lipids and lipoproteins

Twelve-hour fasting venous blood samples were obtained from an antecubital vein into Vacutainer tubes containing EDTA (0.1% final concentration) prior to the beginning of the kinetic study. Plasma was separated from blood cells by centrifugation at 3000 rpm for 10 min at 4°C. Plasma cholesterol and triglyceride concentrations were determined with an Analyzer RA-1000 (Technicon Instruments Corporation, Tarrytown, NY), as previously described (26). VLDL (TRL) ($d < 1.006$ g/ml), IDL ($d =$ 1.006–1.019 g/ml) and LDL ($d = 1.019$–1.063 g/ml) fractions were isolated from fresh plasma by sequential ultracentrifugation (27), and HDL-cholesterol was measured as previously described (28). Plasma concentrations of lathosterol, a precursor in the biosynthesis of cholesterol, and of the plant sterols campesterol and β-sitosterol, used as plasma surrogates of intestinal cholesterol absorption, were quantified using a gas chromatography method similar to that previously described (29). Because the noncholesterol sterols are transported in plasma by lipoproteins, their concentrations have been expressed relative to the concentration of total cholesterol (mmol/mol of cholesterol) to correct for the differing number of lipoprotein acceptor particles.

Quantification and isolation of apoB-48 and apoB-100

ApoB concentration in TRL, IDL, and LDL were determined by noncompetitive ELISA using immuno-purified polyclonal antibodies (Alerchek Inc., Portland, ME) to calculate their respective pool size (PS). The coefficient of variation for the apoB assay was 6–10% depending upon the region of the standard curve. ApoB-100 and apoB-48 were then separated by SDS polyacrylamide slab gel electrophoresis according to standardized procedures (30). Briefly, 50 μl of TRL, IDL, or LDL fractions were mixed with 50 μl of 3% SDS sample buffer and subjected to electrophoresis in 3–10% linear gradient polyacrylamide slab mini gels. Gels were stained overnight in 0.25% Coomassie Blue R-250, then destained for 7–8 h. Based on the assumption that both apoB-100 and apoB-48 have the same chromogenicity, the relative proportion of apoB-100 and apoB-48 was assessed by scanning each gel with laser densitometry (31). We scanned lipoprotein fractions from three different time points to calculate ratios and estimate the average concentrations of apoB-100 and apoB-48 using the total apoB concentration.

Isotopic enrichment determinations

ApoB-48 and apoB-100 bands were excised from polyacrylamide gels, and bands were hydrolyzed in 6N HCl at 110°C for 24 h (32). Trifluoroacetic acid and trifluoroacetic anhydride (1:1) were used as derivatization reagents for the amino acids before analysis on a Hewlett-Packard 6890/5973 gas chromatograph/mass spectrometer (33). Isotope enrichment (%) and tracer/tracee ratio (%) were calculated from the observed ion current ratios (34). The isotopic enrichment of leucine in the apolipoproteins was expressed as tracer/tracee ratio (%) using standarized formulas (34).

Kinetic analysis

Kinetics of TRL apoB-48 were derived by a multicompartmental model as previously described (35). We assumed a constant enrichment of the precursor pool and used the TRL apoB-48 plateau tracer-tracee ratio data as the forcing function to drive the appearance of tracer into apoB-48 (32). Kinetics of apoB-100 in VLDL, IDL, and LDL fractions were derived by a multicompartmental model as previously described (36), with each compartment representing a group of kinetically homogenous particles. Briefly, compartment 1 represents the plasma amino acid pool. Compartment 2 is an intracellular delay compartment representing the synthesis of apo B in the liver. Compartments 4, 5, and 6 are used to account for the kinetics of the VLDL apoB-100 fraction and represent a classical delipidation chain. The delipidation chain in this model accounts for VLDL particles that turn over more slowly, whereas compartment 3 represents more rapidly turning–over VLDL particles. The rate constants between compartments 4, 5, and 6

are arbitrarily set as being equal, as previously described (37). The rate constants between removal from compartments 4, 5, and 6 are also set as being equal. Compartment 7 represents IDL; and compartment 8, LDL. It is assumed that plasma leucine (compartment 1) is the source of the leucine that is incorporated into apo B and that all apo B enters plasma via compartment 3. Therefore, transport rates into compartment 3 correspond to total apoB-100 production. We also assumed a constant enrichment of the precursor pool and used the VLDL apoB-100 plateau tracer-tracee ratio data as the forcing function to drive the appearance of tracer into apoB-100 as previously described (32). Under steady-state condition, the fractional catabolic rate (FCR) is equivalent to the fractional synthetic rate. ApoB production rates (PR) were determined by the formula PR ($mg \cdot kg^{-1} \cdot d^{-1}$) = [FCR (pools/d) × apoB concentration (mg/dl) × plasma volume (L) × 10]/body wt (kg) (38). Plasma volume was estimated as 4.5% of body weight. The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the observed tracer data.

Statistical analysis

Data were analyzed by using the PROC MIXED procedure for repeated measures in SAS software (version 8.02; SAS, Inc., Cary, NC). The structure of the covariance matrix for each variable (intrasubject autocorrelation across repeated measures) was taken into account in all analyses to ensure the most adequate statistical fit and power. The Tukey adjustment was used to account for the multiple comparisons of the four treatments. Carryover effects were tested by introducing terms reflecting the interaction between the sequence of treatments and the treatments per se. Triglyceride concentrations were logarithmically transformed before statistical analysis. Spearman correlation coefficients were determined to assess the significance of associations. Differences were considered significant at $P \leq 0.05$.

RESULTS

Characteristics of subjects

The mean age and body mass index (BMI) of participants were 35.4 ± 8.8 years and 26.5 ± 2.4 kg/m^2, respectively. Three out of the 16 participants had plasma triglyceride levels above 2.5 mmol/L. As indicated in **Table 1**, the mean plasma triglyceride levels was 1.91 mmol/L, a value corresponding to the 70th percentile for men aged 35–44 years (24). On the other hand, the mean plasma LDL-C level after placebo treatment was 3.64 mmol/L, a value corresponding to the 60th percentile. Subjects maintained their weight throughout the study. Table 1 shows the lipid/lipoprotein profile of subjects following each treatment phase with ezetimibe (10 mg/d) plus simvastatin (40 mg/d); ezetimibe (10 mg/d); simvastatin (40 mg/d); and placebo. Ezetimibe alone led to significant reductions in the concentrations of plasma cholesterol (−19.2%), triglycerides (−24.1%), apoB (−19.8%), VLDL-C (−38.8%), and LDL-C (−21.4%). Similarly, monotherapy with simvastatin compared with placebo significantly reduced plasma cholesterol (−30.4%), triglycerides (−22.0%), apoB (−34.5%), VLDL-C (−37.3%), and LDL-C (−39.0%) concentrations. Combination therapy was associated with the greatest reductions in plasma cholesterol (−43.0%), triglycerides (−44.0%), apoB (−47.4%), VLDL-C (−62.7%), LDL-C (−53.6%) among all treatments. Compared with placebo, however, combination therapy and monotherapy with either simvastatin or ezetimibe had no significant impact on plasma HDL-C and apoA-I concentrations.

Kinetics of TRL apoB-48

Plasma leucine enrichment with deuterated leucine as well as plasma triglyceride and TRL apoB-48 levels remained constant during the course of the infusion (data not shown). **Table 2** shows PS, FCR, and PR of TRL apoB-48 following each treatment phase. TRL apoB-48 PS was significantly decreased after ezetimibe treatment (−33.0%, $P = 0.0009$), after simvastatin treatment (−37.6%, $P = 0.0002$), and following combination therapy (−48.9%, $P < 0.0001$) compared with placebo. There was no significant difference in

TABLE 1. Lipid/lipoprotein profile after a six-week treatment

	Placebo	Ezetimibe	%Δ Placebo[d]	Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	Ezetimibe + Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	%Δ Simvastatin[d]
Plasma										
Cholesterol, mmol/L	5.37 ± 0.57	4.34 ± 0.51[a]	−19.2	3.74 ± 0.45[a,b]	−30.4	−13.8	3.06 ± 0.36[a,b,c]	−43.0	−29.5	−18.2
Triglycerides, mmol/L	1.91 ± 1.11	1.45 ± 0.68[a]	−24.1	1.49 ± 0.83[a]	−22.0	+2.8	1.07 ± 0.49[a,b,c]	−44.0	−26.2	−28.2
Apolipoprotein B, g/L	1.16 ± 0.16	0.93 ± 0.12[a]	−19.8	0.76 ± 0.11[a,b]	−34.5	−18.3	0.61 ± 0.08[a,b,c]	−47.4	−34.4	−19.7
VLDL										
Cholesterol, mmol/L	0.67 ± 0.44	0.41 ± 0.22[a]	−38.8	0.42 ± 0.27[a]	−37.3	+2.4	0.25 ± 0.15[a,b,c]	−62.7	−39.0	−40.5
Triglycerides, mmol/L	1.52 ± 1.05	1.10 ± 0.65[a]	−27.6	1.14 ± 0.80[a]	−25.0	+3.5	0.77 ± 0.46[a,b]	−49.3	−30.0	−32.5
Apolipoprotein B, g/L	0.16 ± 0.08	0.13 ± 0.03	−18.8	0.11 ± 0.04[a]	−31.3	−15.4	0.08 ± 0.03[a,b]	−50.0	−38.5	−27.3
LDL										
Cholesterol, mmol/L	3.64 ± 0.56	2.86 ± 0.41[a]	−21.4	2.22 ± 0.38[a,b]	−39.0	−22.4	1.69 ± 0.27[a,b,c]	−53.6	−40.9	−23.9
Triglycerides, mmol/L	0.22 ± 0.06	0.20 ± 0.04	−9.1	0.18 ± 0.05[a]	−18.2	−10.0	0.16 ± 0.03[a,b]	−27.3	−20.0	−11.1
Apolipoprotein B, g/L	1.00 ± 0.14	0.80 ± 0.11[a]	−20.0	0.65 ± 0.09[a,b]	−35.0	−18.8	0.53 ± 0.07[a,b,c]	−47.0	−33.8	−18.5
HDL										
Cholesterol, mmol/L	1.06 ± 0.17	1.07 ± 0.2	+0.9	1.11 ± 0.24	+4.7	+3.7	1.11 ± 0.17	+4.7	+3.7	0
Triglycerides, mmol/L	0.17 ± 0.05	0.15 ± 0.03	−11.8	0.17 ± 0.04	0	+13.3	0.15 ± 0.04	−11.8	0	−11.8
Apolipoprotein AI, g/L	1.26 ± 0.15	1.19 ± 0.14	−5.6	1.23 ± 0.18	−2.4	+3.4	1.27 ± 0.16	+0.8	+6.7	+3.3

Lipid/lipoprotein profile after a six-week treatment with ezetimibe (10 mg/d) alone; simvastatin (40 mg/d) alone; ezetimibe (10 mg/d) plus simvastatin (40 mg/d); and placebo.

[a] $P < 0.05$ versus placebo.

[b] $P < 0.05$ versus ezetimibe.

[c] $P < 0.05$ versus simvastatin.

[d] %Δ represents the percentage of change between treatment.

TABLE 2. Kinetic parameters of ApoB-100 and ApoB-48 following treatment phases

	Placebo	Ezetimibe	%Δ Placebo[d]	Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	Ezetimibe + Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	%Δ Simvastatin[d]
TRL apoB-48										
PS (mg)	50.3 ± 31.7	33.7 ± 16.8[a]	−33.0	31.4 ± 17.1[a]	−37.6	−6.8	25.7 ± 14.6[a]	−48.9	−23.7	−18.2
FCR (pools/day)	7.3 ± 2.9	7.8 ± 2.4	+6.8	8.3 ± 2.8	+13.7	+6.4	8.7 ± 4.0	+19.2	+11.5	+4.8
PR (mg/kg/day)	3.9+2.1	3.0+1.5	−23.1	3.0+1.7	−23.1	0	2.4+1.4[a,b]	−38.0	−20.0	−20.0
VLDL apoB-100										
PS (mg)	451.0 ± 190.2	371.9 ± 137.8[a]	−17.5	355.2 ± 167.2[a]	−21.2	−4.4	299.8 ± 112.5[a,b]	−33.5	−19.4	−15.6
FCR (pools/day)	7.4 ± 2.3	9.1 ± 2.1[a]	+22.9	8.8 ± 2.6[a]	+18.9	−3.3	10.4 ± 2.7[a,b,c]	+40.5	+14.3	+18.1
PR (mg/kg/day)	36.2 ± 10.0	39.1 ± 12.0	+8.0	34.9 ± 11.1	−3.5	−10.7	35.6 ± 10.9	−1.7	−9.0	+2.0
Conversion to IDL (mg/kg/day)	8.4 ± 6.3	9.5 ± 3.7	+13.1	8.3 ± 3.6	−1.2	−12.6	12.0 ± 6.3	+42.9	+26.3	+44.6
Conversion to LDL (mg/kg/day)	4.8 ± 4.7	5.3 ± 3.9	+10.4	5.4 ± 5.2	+12.5	+1.9	5.0 ± 6.5	+4.2	−5.7	−7.4
IDL apoB-100										
PS(mg)	42.4 ± 21.2	45.0 ± 23.8	+6.1	35.0 ± 18.1	−17.5	−22.2	31.0 ± 13.6	−26.9	−31.1	−11.4
FCR (pools/day)	8.7 ± 2.0	9.4 ± 2.1	+8.0	10.8 ± 2.8	+24.1	+14.9	12.5 ± 4.3[a]	+43.7	+33.0	+15.7
PR (mg/kg/day)	4.2 ± 1.5	4.8 ± 2.1	+14.3	4.2 ± 1.6	0	−12.5	4.5 ± 2.2	+7.1	−6.3	+7.1
Conversion to LDL (mg/kg/day)	8.3 ± 6.4	6.9 ± 3.7	−16.9	8.1 ± 3.9	−2.4	17.4	11.1 ± 6.1	+33.7	+60.9	+37.0
LDL apoB-100										
PS(mg)	3817 ± 1581	2740 ± 1033[a]	−28.2	1921 ± 789[a,b]	−49.7	−29.9	1693 ± 791[a,b]	−55.6	−38.2	−11.9
FCR (pools/day)	0.34 ± 0.12	0.41 ± 0.14[a]	+20.6	0.58 ± 0.23[a,b]	+70.6	+41.5	0.81 ± 0.19[a,b,c]	+138.2	+97.6	+39.7
PR (mg/kg/day)	14.1 ± 5.5	13.0 ± 5.2	−7.8	12.3 ± 5.1	−12.8	−5.4	15.7 ± 4.9[c]	+11.3	+20.8	+27.6

ApoB-100 in VLDL, IDL, and LDL, and ApoB-48 in TRL following treatment phases with ezetimibe (10 mg) alone; simvastatin (40 mg) alone; ezetimibe (10 mg) plus simvastatin (40 mg); and placebo. FCR, fractional catabolic rate; PR, production rate; PS, pool size; TRL, triglyceride-rich lipoproteins.

[a] $P < 0.05$ versus placebo.

[b] $P < 0.05$ versus ezetimibe.

[c] $P < 0.05$ versus simvastatin.

[d] %Δ represents the percentage of change between treatment.

TRL apoB-48 FCR between treatments. Compared with placebo, combination therapy was associated with a significant decrease in TRL apoB-48 PR (−38.0%, $P = 0.003$). Monotherapy with ezetimibe and simvastatin both reduced TRL apoB-48 PR (−23.1%), but these differences did not reach statistical significance ($P = 0.2$ and $P = 0.1$, respectively). As shown in **Fig. 1**, a positive correlation has been observed between the combination therapy–induced percentage changes in TRL apoB-48 PS and percentage changes in TRL apoB-48 PR ($r = 0.85$; $P < 0.0001$). There was no such correlation with TRL apoB-48 FCR ($r = 0.10$; $P = 0.72$).

Kinetics of VLDL, IDL, and LDL apoB-100

As shown in Table 2, monotherapy with ezetimibe and simvastatin, as well as the combination therapy, significantly decreased VLDL apoB-100 PS (−17.5%, $P = 0.005$; −21.2%, $P = 0.0005$; and −33.5%, $P < 0.0001$, respectively), compared with placebo. These reductions were accompanied by significant increases in VLDL apoB-100 FCR (ezetimibe: +22.9%, $P = 0.03$; simvastatin: +18.9%, $P = 0.05$; combination: +40.5%, $P < 0.0001$). The combination therapy led to a significantly greater increase in VLDL apoB-100 FCR, compared with monotherapy with either ezetimibe (+14.3%, $P = 0.02$) or simvastatin (+18.1%, $P = 0.05$). No significant difference between the various treatments was observed in VLDL apoB-100 PR. Compared with placebo, LDL apoB-100 PS was significantly reduced with ezetimibe (−28.2%, $P < 0.0001$), with simvastatin (−49.7%, $P < 0.0001$), and with the combination therapy (−55.6%, $P < 0.0001$). These reductions were mainly attributed to significant increases in LDL apoB-100 FCR following each treatment (ezetimibe: +20.6%, $P = 0.0001$; simvastatin: +70.6%, $P < 0.0001$; combination: +138.2%, $P < 0.0001$). Simvastatin exerted a greater effect on LDL apoB-100 FCR compared with ezetimibe treatment (+41.5%, $P = 0.004$) while combination therapy increased LDL apoB-100 FCR by +97.6% ($P < 0.0001$) and +39.7% ($P = 0.03$) compared with monotherapy with ezetimibe and simvastatin, respectively. None of the treatments had significant impact on LDL apoB-100 PR. Finally, a significant inverse correlation was observed between percentage changes in LDL apoB-100 PS and percentage changes in LDL apoB-100 FCR ($r = −0.60$; $P = 0.01$) following combination therapy versus placebo (**Fig. 2**).

Plasma surrogates of cholesterol absorption and synthesis

The impact of each treatment on plasma surrogates of cholesterol absorption (campesterol, β-sitosterol) and synthesis (lathosterol) is shown in **Table 3**. Compared with placebo, monotherapy with ezetimibe significantly decreased plasma levels of campesterol (−35.8%, $P = 0.0006$) and β-sitosterol (−39.6%, $P = <0.0001$) and was associated with a significant increase in lathosterol levels (+36.4%, $P = 0.0002$). Treatment with simvastatin significantly reduced plasma levels of lathosterol (−54.3%, $P < 0.0001$) but significantly increased campesterol (+45.3%, $P = 0.001$) and β-sitosterol levels (+35.1%, $P = 0.001$). Finally, the combination therapy significantly decreased lathosterol concentrations (−36.6%, $P = 0.0003$) and β-sitosterol (−26.1%, $P = 0.0004$). The lathosterol-campesterol and lathosterol-β-sitosterol ratios, representing an index of cholesterol homeostasis, were significantly increased following monotherapy with ezetimibe but were significantly reduced by monotherapy with simvastatin and the combination therapy.

DISCUSSION

Coadministration of ezetimibe and simvastatin have been reported to reduce plasma cholesterol 34–42% and LDL-C 46–59%, depending on the dose of simvastatin

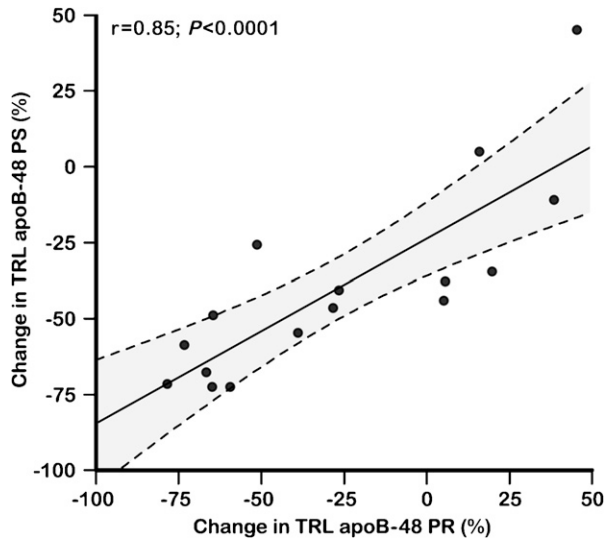

Fig. 1. Correlation between percentage changes in TRL apoB-48 pool size and percentage changes in TRL apoB-48 production rate following combination therapy with ezetimibe (10 mg/day) and simvastatin (40 mg/day) versus placebo.

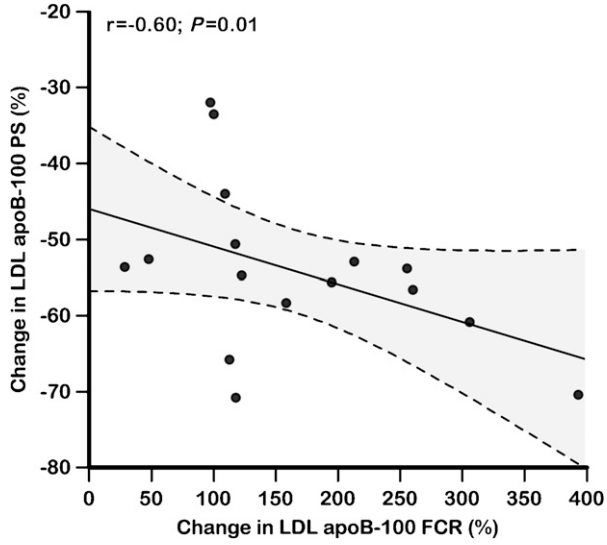

Fig. 2. Correlation between percentage changes in LDL apoB-100 pool size and percentage changes in LDL apoB-100 fractional catabolic rate following combination therapy with ezetimibe (10 mg/day) and simvastatin (40 mg/day) versus placebo.

TABLE 3. Effect of treatment on markers of cholesterol synthesis and intestinal absorption

Marker	Placebo	Ezetimibe	%Δ Placebo[d]	Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	Ezetimibe + Simvastatin	%Δ Placebo[d]	%Δ Ezetimibe[d]	%Δ Simvastatin[d]
Lathosterol	207.6 ± 87.9	283.1 ± 97.0[a]	+36.4	94.9 ± 53.2[a,b]	−54.3	−66.5	131.6 ± 48.3[a,b,c]	−36.6	−53.5	+38.7
Campesterol	155.4 ± 61.6	99.8 ± 54.9[a]	−35.8	225.8 ± 82.3[a,b]	+45.3	+126.3	156.7 ± 89.1[b,c]	+0.8	+57.0	−30.6
β-sitosterol	183.1 ± 80.9	110.6 ± 48.7[a]	−39.6	247.4 ± 100.0[a,b]	+35.1	+123.7	135.3 ± 46.3[a,b,c]	−26.1	+22.3	−45.3
Lathosterol/campesterol ratio	1.59 ± 0.84	3.66 ± 2.18[a]	+130.2	0.49 ± 0.36[a,b]	−69.2	−86.6	1.09 ± 0.64[a,b,c]	−31.4	−70.2	+122.4
Lathosterol/β-sitosterol ratio	1.41 ± 0.83	3.27 ± 2.03[a]	+131.9	0.46 ± 0.33[a,b]	−67.4	−85.9	1.10 ± 0.54[b,c]	−22.0	−66.3	+139.1

Ezetimibe (10 mg/d), simvastatin (40 mg/d), and ezetimibe (10 mg/d) plus simvastatin (40 mg/d).
[a] $P < 0.05$ versus placebo.
[b] $P < 0.05$ versus ezetimibe.
[c] $P < 0.05$ versus simvastatin.
[d] %Δ represents the percentage of change between treatment.

(10–80 mg) (20, 39, 40). In the present study, monotherapy with ezetimibe 10 mg/d and simvastatin 40 mg/d resulted in a 21.4% and 39.0% reduction in LDL-C concentrations, and the combination of both medications showed a greater reduction (−53.6%). TRL apoB-48 PS was significantly decreased with ezetimibe (−33.0%) and with simvastatin (−37.6%), but monotherapies had no significant impact on either TRL apoB-48 FCR or TRL apoB-48 PR. On the other hand, combination therapy was associated with a significant reduction in TRL apoB-48 PS (−48.9%) due mainly to a significant reduction in TRL apoB-48 PR (−38.0%). All treatment modalities significantly reduced VLDL and LDL apoB-100 PS mainly through a significant elevation of apoB-100 FCR in these fractions.

Ezetimibe selectively inhibits cholesterol absorption by preventing its uptake at the level of the intestine wall (14, 15). Recent studies have shown that Niemann-Pick C1 Like 1 plays a critical role in the absorption of intestinal cholesterol and has been established as the direct target of ezetimibe action (16, 17). In agreement with a previous report from our group (41), the present study showed that ezetimibe significantly decreased VLDL and LDL apoB-100 PS by increasing apoB-100 FCRs in these fractions. This outcome is likely mediated by the inhibitory effect of ezetimibe on cholesterol absorption and the ensuing reduction in the delivery of cholesterol from the intestine to the liver, which then mediates an increase in LDL receptor-mediated uptake of apoB-100–containing lipoproteins (42–44).

The effects of statin treatment on the in vivo production and clearance rates of apoB-100 have been examined in both normolipidemic and hyperlipidemic patients. In normolipidemic subjects, Malmendier et al. (11) found no consistent effect of simvastatin on LDL apoB-100 PR but observed increased FCR via the LDL receptor-dependent pathway. More recently, atorvastatin was shown to increase the clearance of apoB-100 in VLDL, IDL, and LDL with no effect on the PR in these fractions, a finding consistent with upregulation of LDL receptors (13, 45). Similarly, in subjects with the metabolic syndrome, rosuvastatin significantly increased the catabolism of VLDL, IDL, and LDL-apoB-100 and decreased the corresponding pool sizes, with evidence of a dose-related effect (46). In that study, however, LDL apoB-100 PR fell significantly with rosuvastatin at maximal dosage, with no change in VLDL and IDL-apoB PR. In contrast, previous studies in patients with familial combined hyperlipidemia have shown that lovastatin had no detectable effect on FCR of LDL apoB-100, but it decreased apoB-100 PR in this fraction (8, 9). These results suggest that statins can lower apoB-100 levels by decreasing PR and increasing FCR, with somewhat variable effects that may depend on the patient population and statin regimen. In the present study, we found that simvastatin decreased the pool sizes of VLDL and LDL apoB-100 by increasing the apoB-100 FCRs in these fractions, an effect most likely mediated by the activation of the expression of the LDL receptor (47) and, alternatively, by statin-induced changes in the composition of apoB-containing lipoproteins increasing their affinity for the LDL receptor (48). Compared with

monotherapies, the combination of ezetimibe and simvastatin incrementally enhances the catabolism of VLDL and LDL apoB-100 with no significant effect on VLDL and LDL apoB-100 PR. Our results indicate that the combination therapy is more effective than monotherapy with ezetimibe and simvastatin to increase catabolism of atherogenic apoB-100–containing lipoproteins. These results are consistent with those reported in a study by Telford et al. (23), in which hepatic LDL receptor expression was measured.

Our study is the first to investigate the effects of the coadministration of ezetimibe and simvastatin on TRL apoB-48 kinetics. A novel finding in our study was the observation that combination therapy had a greater effect than monotherapies on the reduction of TRL apoB-48 PS and that this effect was associated with a significant reduction in TRL apoB-48 PR along with a nonsignificant increase in FCR. Compared with placebo, monotherapies with ezetimibe and simvastatin both increased FCR and decreased PR of TRL apoB-48, although these effects were not statistically significant. This lack of significance is most likely related to measurement variances and the relatively small sample size. Our results also showed that TRL apoB-48 PS was highly correlated with TRL apoB-48 PR in subjects receiving combination therapy, a finding supporting the concept that coadministration of ezetimibe and simvastatin decreases TRL apoB-48 PS mainly by lowering intestinal production of lipoproteins. Previous studies have emphasized the importance of lipid availability for the translocation of apoB across endoplasmic reticulum (49). Insufficient lipid is thought to result in enhanced apoB degradation due to inefficient transfer of apoB from the cytosolic side to the luminal side. One can speculate that lack of intracellular cholesterol availability, as a result of inhibition of HMG-CoA reductase by simvastatin and cholesterol absorption by ezetimibe, may reduce the translocation of apoB-48 across the endoplasmic reticulum leading to cytosolic degradation of apoB-48. Consistent with this hypothesis, earlier in vitro studies have shown that treatment of enterocytes with statins was able to reduce apoB-48 production (50) and secretion with a concomitant reduction in intracellular cholesterol levels (51). Interestingly, the inhibition of apoB-48 production and secretion in these cells was not observed in the basal state but only when exogenous sterols were present in the cell culture media, a finding suggesting that the effects of HMG-CoA reductase inhibitors on TRL apoB-48 production may become apparent only under sufficient stimulatory conditions. Finally, Phillips et al. (52) have recently shown that statin therapy was able to reduce the expression of microsomal triglyceride transfer protein (MTP), a key protein responsible for the assembly of TRL in the liver and the small intestine. If lipid availability and MTP expression were altered by statin plus ezetimibe, however, it would be anticipated that apoB-100 PR as well as apoB-48 PR would be affected, which was not observed. Further studies are required to clarify the impact of statins on the regulation of intestinal production of lipoproteins and to characterize the specific mechanisms underlying this effect. From a physiological point of view, it should be emphasized that our results do not preclude the hypothesis that combination therapy may also reduce TRL apoB-48 concentrations by enhancing LDL receptor-mediated clearance of these lipoproteins. In fact, our results showed that combination therapy was associated with a nonsignificant 19.2% increase in TRL apoB-48 FCR compared with placebo. These results are consistent with a previous study by Lamon-Fava et al. (45) showing that a treatment with atorvastatin 20 mg/d was associated with a nonsignificant 11% elevation in TRL apoB-48 FCR and a nonsignificant 9% reduction in PR. It can be hypothesized that the reduction in TRL apoB-48 concentrations following statin therapy is partly mediated by an increased clearance of these particles by LDL receptors, whose elevated expression also likely mediates the increased catabolism of apoB-100. Previous studies using oral fat load tests in hypertriglyceridemic subjects have shown a decrease in both triglycerides and apoB-48 in the chylomicron fraction following treatment with atorvastatin (53, 54), although no evidence for decreased production of chylomicrons was found. Differences in the characteristics of the subjects, study design, methodology, and kinetic model used most likely account for the differences in results among the studies. Therefore, our results and most previous reports (55–58) suggest that the reduction in intestinal lipoproteins following treatment with statins may be attributable, at least in part, to an increased uptake of these lipoproteins by LDL receptors.

Monotherapy with ezetimibe caused an elevation in lathosterol, a plasma surrogate of cholesterol synthesis, and a reduction in campesterol and β-sitosterol, two markers of intestinal cholesterol absorption (23, 59). In contrast, monotherapy with simvastatin decreased plasma levels of lathosterol, and increased plasma levels of campesterol and β-sitosterol (60), whereas the combination therapy provided significant reductions in plasma levels of both lathosterol and β-sitosterol (23). The lathosterol-campesterol and lathosterol-β-sitosterol ratios, used as an indicator of cholesterol homeostasis, were increased by ezetimibe and reduced by simvastatin and the combination therapy compared with the placebo. The mechanism by which a compensatory elevation in cholesterol synthesis or absorption occurs upon abatement of absorption or synthesis may be explained by the plasma cholesterol production pathway. Indeed, HMG-CoA reductase, the rate-limiting enzyme of this pathway, is regulated by intracellular cholesterol through negative feedback. More specifically, intracellular cholesterol regulates HMG-CoA reductase concentrations through its ability to influence transcription, mRNA translation, and degradation of the enzyme. Suppression in cholesterol absorption by ezetimibe leads to a lower circulating cholesterol concentration. As a result, negative feedback of cholesterol on HMG-CoA reductase is inhibited, leading to increases in cholesterol synthesis. The reciprocal effect occurs when cholesterol synthesis is inhibited by the action of simvastatin, leading to increases in intestinal cholesterol absorption (61). In agreement with a previous study by Telford et al. (23), our results suggest that the specific effect on cholesterol homeostasis of monotherapy with ezetimibe and simvastatin could partially override the inhibitory effect

of these drugs on cholesterol levels and that combination therapy which simultaneously affect both the intestinal cholesterol absorption and biosynthesis is in fact more effective.

In conclusion, our study shows that treatment with simvastatin plus ezetimibe is effective to reduce plasma TRL apoB-48 levels, an effect mainly mediated by a reduction in the intestinal secretion of TRL apoB-48. Our results also indicate that the reduction in LDL-C following coadministration of simvastatin and ezetimibe, as well as monotherapy with simvastatin and ezetimibe, is associated with an increase in the FCR of apoB-100–containing lipoproteins. These data confirm that combination therapy inhibiting simultaneously both the cholesterol biosynthesis and intestinal cholesterol absorption represents the most effective cholesterol-lowering intervention.

The authors are grateful to the subjects for their excellent collaboration and to the dedicated work of Danielle Aubin, Johanne Marin, Pascal Dubé, and the staff of the Lipid Research Center.

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