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ORIGINAL ARTICLE

Rosuvastatin Enhances the Catabolism of LDL apoB-100 in Subjects with Combined Hyperlipidemia in a Dose Dependent Manner

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Abstract Dose-associated effects of rosuvastatin on the metabolism of apolipoprotein (apo) B-100 in triacylglycerol rich lipoprotein (TRL, $d < 1.019$ g/ml) and low density lipoprotein (LDL) and of apoA-I in high density lipoprotein (HDL) were assessed in subjects with combined hyperlipidemia. Our primary hypothesis was that maximal dose rosuvastatin would decrease the apoB-100 production rate (PR), as well as increase apoB-100 fractional catabolic rate (FCR). Eight subjects received placebo, rosuvastatin 5 mg/day, and rosuvastatin 40 mg/day for 8 weeks each in sequential order. The kinetics of apoB-100 in TRL and LDL and apoA-I in HDL were determined at the end of each phase using stable isotope methodology, gas chromatography-mass spectrometry, and multicompartmental modeling. Rosuvastatin at 5 and 40 mg/day decreased LDL cholesterol by 44 and 54 % (both $P < 0.0001$), triacylglycerol by 14 % (ns) and 35 % ($P < 0.01$), apoB by 30 and 36 % (both $P < 0.0001$), respectively, and had no significant effects on HDL cholesterol or apoA-I levels. Significant decreases in plasma markers of cholesterol synthesis and increases in cholesterol absorption markers were observed. Rosuvastatin 5 and 40 mg/day increased TRL apoB-100 FCR by 36 and 46 % (both ns) and LDL apoB-100 by 63 and 102 % (both $P < 0.05$), respectively. HDL apoA-I PR increased with low dose rosuvastatin (12 %, $P < 0.05$) but not with maximal dose rosuvastatin. Neither rosuvastatin dose altered apoB-100 PR or HDL apoA-I FCR. Our data indicate that maximal dose rosuvastatin treatment in subjects with combined hyperlipidemia resulted in significant increases in the catabolism of LDL apoB-100, with no significant effects on apoB-100 production or HDL apoA-I kinetics.

Keywords Lipoprotein kinetics · Statins · Metabolism · Plasma sterols · HDL subpopulations

Clinical Trial Registration Number: none assigned (study prior to 2005).

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Abbreviations

ABCA1	ATP-binding cassette A1
Apo	Apolipoprotein
BMI	Body mass index
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
d	Density
EDTA	Ethylenediaminetetraacetic acid
FCR	Fractional catabolic rate
GC–MS	Gas chromatography-mass spectrometry
HMG-CoA	3-Hydroxy-3-methylglutaryl-CoA
IDL	Intermediate density lipoprotein

PR	Production rate
PS	Pool size
R5	Rosuvastatin low dose (5 mg/day)
R40	Rosuvastatin maximal dose (40 mg/day)
TC	Total cholesterol
TAG	Triacylglycerol
TRL	Triacylglycerol rich lipoprotein
VLDL	Very low density lipoprotein

Introduction

Statins are a well-tolerated and highly efficacious means for reducing both LDL cholesterol (LDL-C) and coronary heart disease (CHD) risk [1–3]. Recently new guidelines have been released recommending that patients with established CHD should receive high intensity statin therapy, defined as either atorvastatin 40 or 80 mg/day or rosuvastatin 20 or 40 mg/day, and ideally attain a >50 % reduction in LDL-C levels [4]. In the Treating to New Targets Trial, atorvastatin 80 mg/day was found to be significantly more effective in reducing CHD risk than atorvastatin 10 mg/day [5]. It has also been reported that treatment with either atorvastatin 80 mg/day or rosuvastatin 40 mg/day resulted in coronary atheroma regression in more than 60 % of CHD patients over a 2 year period [6].

Statins inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis and, thereby, significantly decrease the hepatic cholesterol pool, resulting in an upregulation of LDL receptor activity and a reduction in plasma concentrations of apolipoprotein (apo) B-containing lipoproteins. Rosuvastatin has been shown to be the most effective LDL-lowering statin currently available, as well as being the most efficacious in raising HDL cholesterol (HDL-C) levels [7, 8]. In previous studies, we documented that the maximal doses of both atorvastatin and rosuvastatin significantly lowered the levels of small dense LDL-C [9] and raised the levels of apoA-I in large α-1 HDL [10].

There have been three prior studies examining the mechanisms whereby maximal doses of these statins alter lipoprotein metabolism. Bilz *et al*. [11] and Lamon-Fava *et al*. [12] have reported that treatment with atorvastatin 80 mg/day in subjects with combined hyperlipidemia enhanced the clearance of apoB-100-containing lipoproteins and had no significant effect on apoB-100 production or HDL apoA-I kinetic parameters. In contrast, Ooi *et al*. [13] found that in subjects with the metabolic syndrome rosuvastatin 40 mg/day significantly enhanced the clearance of all apoB-100-containing particles and decreased the production of LDL apoB-100. In these insulin resistant subjects, maximal dose rosuvastatin also significantly decreased both HDL apoA-I production and catabolism [14].

The present study was designed to assess the effects of two doses of rosuvastatin on the kinetic parameters of apoB-100 in triacylglycerol rich lipoproteins [TRL, density (d) < 1.019] and LDL and of apoA-I in HDL in subjects with combined hyperlipidemia. We hypothesized that maximal dose rosuvastatin would result in the greatest reduction in the apoB-100 and cholesterol content of LDL, due not only to enhanced clearance but also to decreased production of apoB-100. Moreover, our study was designed to assess the effects of rosuvastatin on plasma markers of cholesterol synthesis and absorption, as well as on the levels of apoA-I in HDL subpopulations, relative to the changes in apolipoprotein metabolism. Our data indicate that maximal dose rosuvastatin therapy in subjects with combined hyperlipidemia resulted in significant increases in the catabolism of LDL apoB-100, with no significant effects on apoB-100 production or HDL apoA-I kinetics, and, in turn, clearly delineate the major mechanism whereby rosuvastatin lowers LDL cholesterol.

Methods

Subjects

Eight subjects with combined hyperlipidemia, four men and four postmenopausal women, were enrolled in this study (mean age ± SEM: 62 ± 4 years; BMI: 25.7 ± 1.1 kg/m^2). Plasma lipid criteria for enrollment were as follows: triacylglycerol (TAG) concentrations ≥150 mg/dl, LDL-C ≥140 mg/dl, and HDL-C <50 mg/dl. Patients were chosen to demonstrate both moderate to high elevations in both TRL (d < 1.019 g/ml) TAG and LDL-C, therefore, representing combined elevations in these two lipoprotein species. Subjects on or off lipid-lowering or blood pressure medications were eligible to participate. Subjects on lipid-lowering medications at the time of enrollment were monitored during a 4–6 week period off such medications prior to starting the study. All subjects met the lipid inclusion criteria at the beginning of the study [total cholesterol (TC) 234 ± 14 mg/dl; TAG 184 ± 37 mg/dl; LDL-C 171 ± 19 mg/dl; HDL-C 43 ± 5 mg/dl]. Exclusion criteria included age <40 years, hormone replacement therapy, chronic kidney or liver disease, and a history of cancer. The study protocol was approved by the Human Institutional Review Board of Emory University, the Research and Development Committee at the Atlanta Veterans Affairs Medical Center, and the Human Institutional Review Board of Tufts University. Informed, written consent was obtained from each study subject. No serious adverse event and no drug-related adverse event were reported during the study.

The subjects were instructed to follow a National Cholesterol Education Program/American Heart Association

Step 1 diet (≤30 % of calories as total fat, <10 % as saturated fatty acids, and <300 mg/day cholesterol) [15] and to engage in no excessive physical activity. There was no change in individual body weights for the duration of the study (see Supplement Table 1).

Study Design

The study was designed as a placebo-controlled, dose titration, single-blind protocol. All subjects, independent of prior cholesterol-lowering medication, were followed for three 8-week phases in sequence: (1) placebo, (2) rosuvastatin 5 mg/day (low dose, R5), and (3) rosuvastatin 40 mg/day (maximal dose, R40). There was no washout period between the phases. The subjects were instructed to take two tablets every morning, i.e., two placebo tablets during the first phase, one rosuvastatin 5 mg tablet and one placebo tablet during the second phase, and two rosuvastatin 20 mg tablets during the third phase.

At the end of each 8-week treatment phase, following an overnight fast and the collection of a fasting blood sample (8 AM), the subjects received a bolus injection of [5,5,5-^{2}H]L-leucine (Cambridge Isotopes, Andover, MA), 60 μmol/kg body weight, to determine the kinetics of apoB-100 and apoA-I, as previously described [16]. Blood samples were collected in tubes containing EDTA (0.15 %) at baseline (0 h) and at 30 min, 1, 2, 4, 6, 8, 10, 12, 14, 21, 27, 33, 45, 57, and 69 h. TRL (d < 1.019 g/ml), LDL (d = 1.019-1.063 g/ml), and HDL (d = 1.063-1.21 g/ml) were isolated from 3 ml of fresh plasma by sequential density ultracentrifugation and stored at −80 °C until analysis. During the first 48 h of the metabolic study, subjects were given daily, five equal portions (2500 kcal/day) of a fat-free energy drink (240 g genisoy powder, 850 g strawberry sorbet, 58 g sugar per 1000 ml), at 10 AM, 1 PM, 4 PM, 7 PM, and 10 PM, in order to minimize the intermittent influx of chylomicrons that might interfere with the kinetics of apoB-100 [17, 18]. The feeding protocol began 2 h after the isotope was injected.

Plasma Lipid, Lipoprotein, and Sterol Analyses

The protocol for plasma lipid, apolipoprotein, and sterol quantification and lipoprotein particle characterization was performed as previously described [10, 12, 19–21]. In brief, non-fasting plasma and apolipoproteins values represent the average of five measurements obtained during the metabolic study (timepoints 2, 4, 6, 8, and 10 h). TRL cholesterol concentration was calculated as the difference between TC and the sum of LDL-C and HDL-C, measured directly with enzymatic assays. ApoB concentration within plasma and TRL was determined by an automated assay (Kamiya Diagnostics, Seattle, WA), and the concentration of LDL apoB was calculated as the difference between total plasma apoB and TRL apoB. No correction was made for apoB-48, determined in our previous studies to represent <5 % of the total apoB concentration in the d < 1.019 g/ml fraction when study subjects are in the fed state [12].

Biomarkers of cholesterol synthesis (lathosterol and desmosterol) and of fractional cholesterol absorption (campesterol, β-sitosterol, and cholestanol) were isolated from non-fasting plasma, and the concentrations were determined by gas chromatography-mass spectrometry (GC–MS) [19, 20]. Because the non-cholesterol sterols are transported in plasma by lipoproteins, the concentrations in the current study are expressed relative to the plasma concentration of total cholesterol, as well as in absolute terms, thereby, correcting for the different number of lipoprotein acceptor particles during the placebo and treatment phases.

The number and size of TRL and LDL particles were analyzed by NMR spectroscopy (LipoScience, Raleigh, NC) [21]. The values represent the mean of at least three analyses of fasting plasma obtained on the day of the metabolic study. The distribution of apoA-I-containing HDL subpopulations in non-fasting plasma obtained during the metabolic study was determined in six of the eight subjects using non-denaturing two-dimensional agarose-polyacrylamide gel electrophoresis [10]. With this method, the absolute concentrations (in milligrams of apoA-I per deciliter of plasma) of apoA-I-containing HDL subpopulations are calculated by multiplying the plasma total apoA-I concentration for a given subject (mg/dl) by the percentile value of each apoA-I-containing subpopulation.

Apolipoprotein Kinetic Analysis

To determine isotopic enrichment and apolipoprotein metabolism, TRL apoB-100, LDL apoB-100, and HDL apoA-I were separated by gradient SDS-PAGE and hydrolyzed as described previously [12]. Plasma free amino acids were isolated from the trichloroacetic acid extract of whole plasma by cation exchange chromatography. Amino acids were converted to n-propyl ester, heptafluorobutyramide derivatives and analyzed for isotopic enrichment by GC–MS [12]. Percentage deuterated leucine enrichment (D_3-leucine/[D_3-leucine + leucine]) for each sample was calculated from the area under the curve and corrected for the isotopic enrichment of the D_3-leucine tracer [22]. The isotopic enrichment of the tracer used in this study was 99.94 %, as analyzed by GC-MS.

The kinetic parameters of apoB-100 and apoA-I were assessed using multicompartmental models (Fig. 1) and the SAAM II program (The Epsilon Group, Charlottesville, VA). Both the apoB-100 model (Fig. 1a) and the apoA-I model (Fig. 1b) include a four-compartment subsystem that describes plasma leucine kinetics and an

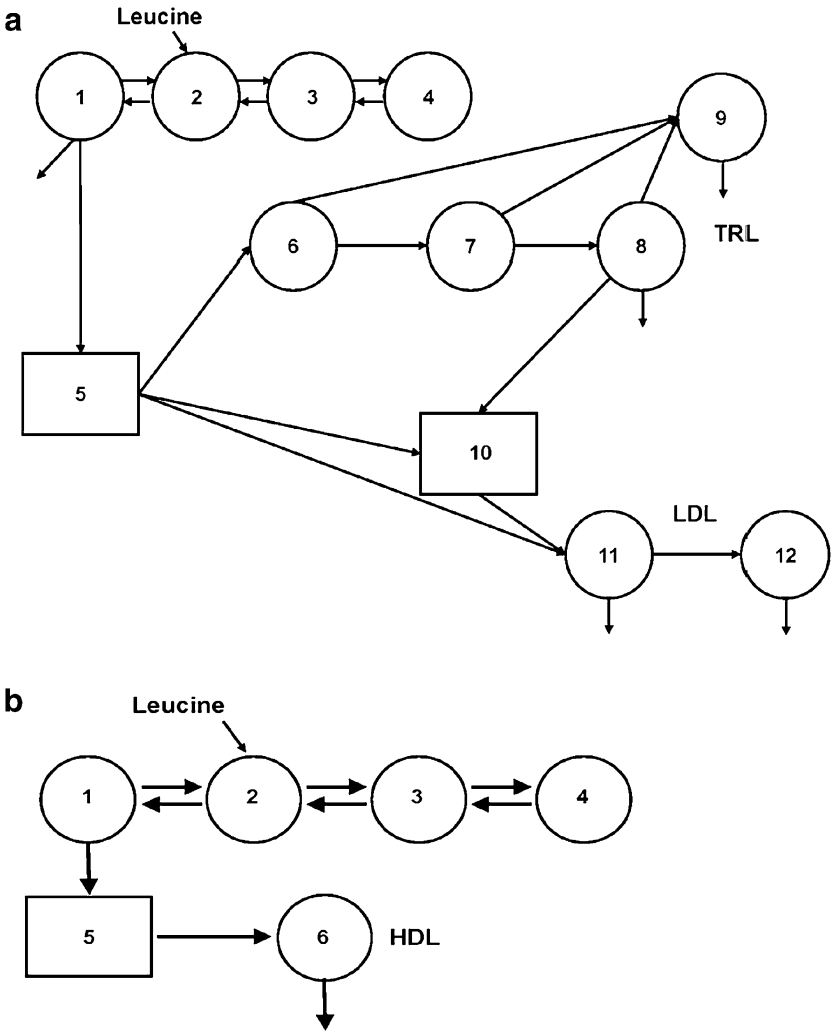

Fig. 1 Multicompartmental models describing kinetics of apoB-100 (**a**) and apoA-I (**b**). Each model includes a 4-compartment subsystem (compartments *1–4*) that describes plasma leucine kinetics. This subsystem is connected to an intra-hepatic delay compartment (compartment *5*), which accounts for the synthesis, assembly, and secretion of the apolipoprotein into plasma. **a** Compartments *6–9* describe the kinetics of apoB-100 in the TRL plasma fraction and allow for the delipidation cascade (compartments *6–8*) and a slowly turning over TRL pool (compartment *9*). LDL apoB-100 kinetics in plasma is described by compartments *11–12*. In order to fit the LDL apoB tracer data, a delay compartment (compartment *10*) between the TRL (compartment *8*) and LDL compartments (compartment *11*) is required. **b** A single plasma compartment (compartment *6*) describes the kinetics of HDL apoA-I

intra-hepatic delay compartment that accounts for the time required for the synthesis, assembly, and secretion of the apolipoprotein into plasma. In the case of apoB-100, the model provides for the direct secretion of apoB-100 into the TRL and LDL fractions, as well as the extra-hepatic delipidation of TRL to LDL. In order to fit the LDL apoB enrichment data, a second delay compartment was required. The presence of a delay between TRL and LDL apoB-100 has been reported previously [23], with studies suggesting that TRL may leave the plasma and reappear later in LDL [24]. The kinetics of HDL apoA-I (Fig. 1b) are described by a single plasma compartment. The fractional catabolic rate (FCR) of TRL apoB-100, LDL apoB-100, and HDL apoA-I were derived from the model parameters giving the best model fit to the enrichment data. Production rate (PR) was calculated as the product of the FCR and the pool size (PS), which equals the plasma concentration of the apolipoprotein multiplied by plasma volume. Plasma volume was estimated as 4.5 % of body weight in kilograms.

Due to technical difficulties, LDL apoB-100 kinetics could not be assessed in two of the subjects (one man and one woman). Data for these subjects are included in all of the other analyses described below, except the analysis of HDL subpopulations.

Statistical Analysis

The SAS System for Windows (release 9.2; SAS Institute) was used for statistical analysis. A logarithmic transformation was applied to the data not normally distributed before formal analysis. Significant differences in the means between placebo and treatment phases were assessed by paired *t* tests. The percentage change of rosuvastatin 5 and 40 mg/day relative to placebo and of rosuvastatin 40 mg/day relative to rosuvastatin 5 mg/day were calculated on an individual basis and summarized descriptively. All data in the text, tables, and graphs are presented in the original scale of measurement as means ± SEM. $P < 0.05$ was considered significant.

Table 1 Effects of rosuvastatin on non-fasting plasma lipid and apolipoprotein levels ($n = 8$)

	Placebo	Rosuvastatin (5 mg/day)	Rosuvastatin (40 mg/day)	Percentage change (R5 vs P)	Percentage change (R40 vs P)	Percentage change (R40 vs R5)
Cholesterol (mg/dl)						
Total	229 ± 14	162 ± 12§	140 ± 10§,††	−29 ± 2	−39 ± 2	−13 ± 3
TRL	37 ± 7	32 ± 8	25 ± 3*	−12 ± 10	−20 ± 12	−5 ± 14
LDL	147 ± 10	83 ± 9§	70 ± 10§,**	−44 ± 4	−54 ± 5	−17 ± 7
HDL	46 ± 5	47 ± 4	45 ± 4	6 ± 5	1 ± 5	−4 ± 4
Triacylglycerol (mg/dl)	188 ± 31	156 ± 30	113 ± 21†	−14 ± 9	−35 ± 8	−20 ± 10
ApoB (mg/dl)	98 ± 5	70 ± 8‡	63 ± 7‡,¶	−30 ± 5	−36 ± 5	−9 ± 6
ApoA-I (mg/dl)	109 ± 6	115 ± 6	113 ± 4	5 ± 3	4 ± 4	−1 ± 4

Data are expressed as means ± SEM. Significance for comparison of absolute values with the placebo phase and between treatment phases was determined using a paired *t* test, with triacylglycerol being log-transformed before statistical analysis

P placebo, *R5* rosuvastatin 5 mg/day, *R40* rosuvastatin 40 mg/day

* $P = 0.07$, † $P < 0.01$, ‡ $P < 0.001$, § $P < 0.0001$ for comparison with placebo phase

¶ $P = 0.07$, ** $P < 0.05$, †† $P < 0.01$ for comparison with rosuvastatin 5 mg/day phase

Results

As shown in Table 1, treatment with rosuvastatin 5 mg/day lowered non-fasting plasma concentrations of TC, LDL-C, and total apoB significantly ($P < 0.001$ *vs* placebo) in this study. Increasing the treatment dose to 40 mg/day caused further marked reductions in these parameters, compared with both placebo ($P < 0.001$) and rosuvastatin 5 mg/day (TC: $P < 0.01$; LDL-C: $P < 0.05$; apoB: $P = 0.07$). Plasma TAG levels were also decreased by the higher dose ($P < 0.01$ vs placebo). Neither treatment dose had any significant effect on HDL-C or apoA-I concentrations.

Figure 2 indicates that low dose rosuvastatin treatment resulted in significant ($P < 0.05$) decreases in the number of LDL particles, as assessed in fasting plasma by NMR spectroscopy, compared with placebo. Decreases in both TRL and LDL particles were even more pronounced during the rosuvastatin 40 mg/day phase (TRL: $P < 0.008$; LDL: $P < 0.03$). A similar, dose-related pattern was observed in the plasma concentrations of TRL apoB-100 (R5: $P = 0.05$; R40: $P < 0.002$) and LDL apoB-100 (both $P < 0.0002$), compared with placebo.

The effects of rosuvastatin on non-cholesterol sterol concentrations in non-fasting plasma, expressed in absolute concentrations and relative to plasma TC concentrations, are presented in Table 2. As predicted by the inhibitory effect of statins on cholesterol biosynthesis, rosuvastatin treatment markedly decreased the plasma concentrations of lathosterol (R5: $P < 0.001$; R40: $P < 0.001$) and desmosterol (R5: $P < 0.01$; R40: $P < 0.001$); and the dose-dependent reductions were observed whether the markers were expressed in absolute or relative terms. The absolute concentrations of the absorption markers campesterol and β-sitosterol were not changed significantly by rosuvastatin treatment, while cholestanol levels were decreased (R5: $P < 0.05$; R40: $P \leq 0.001$). In relative terms (the ratio of non-cholesterol sterol to TC), however, the levels of all three absorption markers increased markedly with both rosuvastatin 5 mg/day (campesterol: 28 ± 7 %, $P < 0.01$; β-sitosterol: 32 ± 9 %, $P < 0.05$; cholestanol: 21 ± 6 %, $P < 0.05$) and rosuvastatin 40 mg/day treatment (campesterol: 55 ± 14 %, $P < 0.05$; β-sitosterol: 70 ± 17 %, $P = 0.09$; cholestanol: 32 ± 5 %, $P < 0.001$). In addition, both doses of rosuvastatin had a pronounced effect on the lathosterol/campesterol ratio, an indicator of cholesterol homeostasis, decreasing the ratio significantly compared with the placebo (R5: −48 ± 3 %; R40: −67 ± 5 %; both $P < 0.01$) and in a dose-dependent manner ($P < 0.05$). Similar results were observed with the lathosterol/β-sitosterol, desmosterol/campesterol, and desmosterol/β-sitosterol ratios (data not shown).

Figure 3 illustrates the observed deuterated leucine enrichment and the model-derived values for TRL apoB-100 (Fig. 3a) and LDL apoB-100 (Fig. 3b) during the placebo and rosuvastatin phases. On rosuvastatin, the levels of enrichment in the TRL and LDL fractions were greater and the rate of appearance of the tracer was increased, pointing to a decrease in apoB-100 concentration and an acceleration of the corresponding rates of catabolism.

The metabolic parameters of apoB-100 derived from the isotopic enrichment curves are provided in Table 3. With rosuvastatin, TRL apoB-100 PS decreased significantly (R5: −18 ± 11 %, $P < 0.05$; R40: −32 ± 5 %, $P < 0.01$). Overall, this decrease was associated with a non-significant increase in TRL apoB-100 FCR (R5: $P = 0.48$; R40: $P = 0.11$), with no change in PR, perhaps due to the marked individual variability in statin response (see Supplement Fig. 1). In the case of LDL, rosuvastatin 5 mg/day decreased

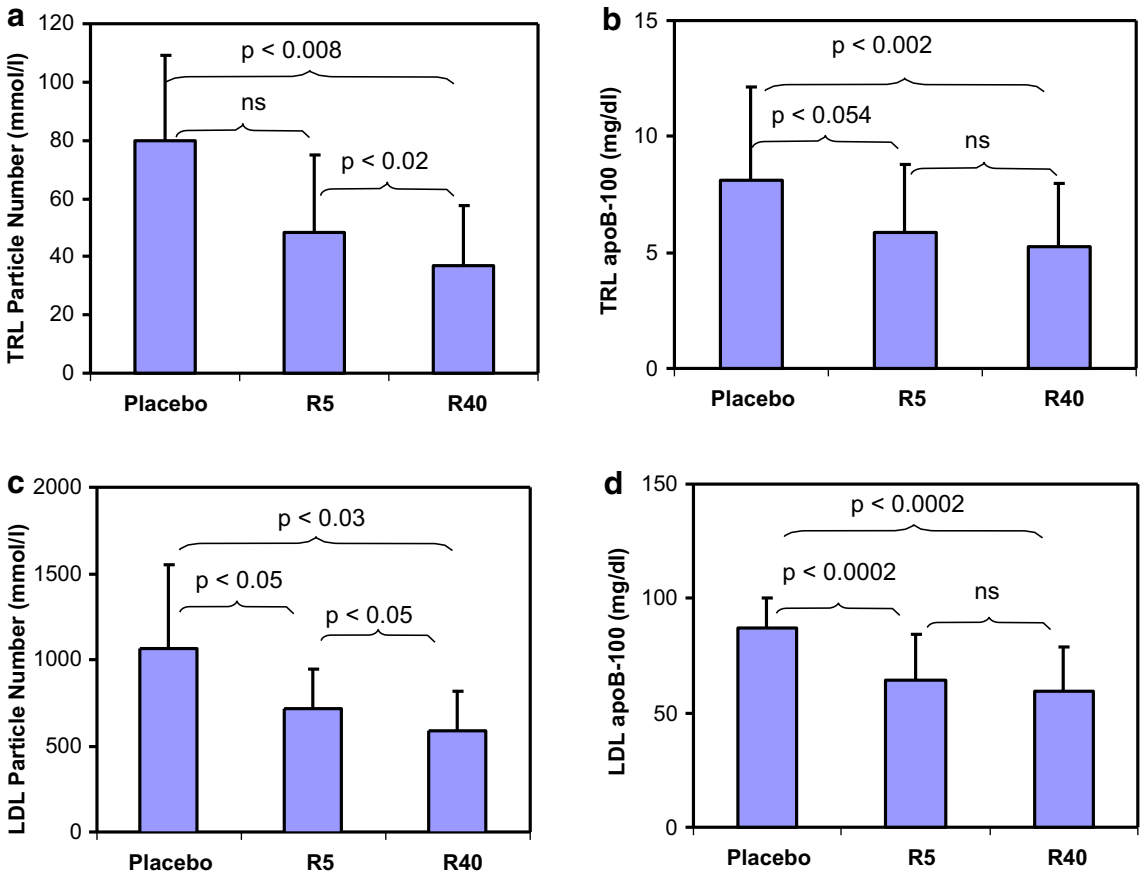

Fig. 2 Dose-dependent effects of rosuvastatin on the number of VLDL and LDL particles as assessed by NMR spectroscopy (**a** and **c**, respectively) and apoB-100 concentration (**b** and **d**, respectively)

LDL apoB-100 PS by 31 ± 4 % ($P < 0.001$) and increased LDL apoB-100 FCR by 63 ± 20 % ($P < 0.05$) relative to placebo. Rosuvastatin 40 mg/day decreased LDL apoB-100 PS by 42 ± 4 % ($P < 0.001$) and increased LDL apoB-100 FCR by 102 ± 22 % ($P < 0.01$). Rosuvastatin 40 mg/day changed LDL apoB-100 PS significantly more than rosuvastatin 5 mg/day ($P = 0.04$), but not LDL apoB-100 FCR ($P = 0.15$). No significant differences in LDL apoB-100 PR were observed. Based on the multicompartmental model used in this study (Fig. 1a), LDL apoB-100 was derived via two pathways: the extra-hepatic conversion of TRL to LDL due to lipolysis and the *de novo* secretion of LDL apoB-100 by the liver. The percentage of TRL apoB-100 converted to LDL did not differ significantly during the placebo, rosuvastatin 5 mg/day, and rosuvastatin 40 mg/day phases (64 ± 6, 69 ± 10, and 78 ± 13 %, respectively; R5: $P = 0.7$, R40: $P = 0.16$ *vs* placebo). The proportion of total apoB-100 that was secreted by the liver and entered the LDL pool via the TRL pool was at least four times greater than the amount secreted directly into LDL, and remained constant with increasing treatment dose (placebo: 82 ± 7 %; R5: 82 ± 3 %; R40: 79 ± 6 %, $P = 0.3$ *vs* placebo).

As depicted by the apoA-I isotopic enrichment curve (Fig. 3c), the effect of rosuvastatin on apoA-I kinetic parameters was modest (Table 3) and the total pool size did not increase significantly. During the rosuvastatin 5 mg/day phase, apoA-I PR increased (12 ± 5 %, $P = 0.04$), compared with placebo. Maximal dose rosuvastatin, however, had no significant effect on apoA-I kinetic parameters. Analysis of the HDL subpopulation profile (Table 4) demonstrated a pronounced shift towards large HDL particles during the rosuvastatin 40 mg/day phase. The percentage of apoA-I-containing α-1 particles increased significantly, compared with placebo (36 ± 12 %, $P < 0.05$), with the effect being dose dependent ($P < 0.01$); and a non-significant reduction in the percentage of apoA-I in preβ-1 HDL particles (−13 ± 6 %, $P = 0.10$) was observed.

Discussion

Treatment with the HMG-CoA reductase inhibitor rosuvastatin lowered the elevated plasma concentrations of TC, TAG, LDL-C, and apoB markedly and in a dose-associated

Table 2 Effects of rosuvastatin on non-fasting plasma sterols ($n = 8$)

	Placebo	Rosuvastatin (5 mg/day)	Rosuvastatin (40 mg/day)	Percentage change (R5 vs P)	Percentage change (R40 vs P)	Percentage change (R40 vs R5)
Concentration (μmol/l)						
Lathosterol	1.9 ± 0.3	0.8 ± 0.2[‡]	0.4 ± 0.2[‡,¶]	−61 ± 7	−79 ± 6	−34 ± 19
Desmosterol	1.3 ± 0.2	0.8 ± 0.2[†]	0.4 ± 0.1[‡,¶]	−43 ± 9	−67 ± 6	−29 ± 18
Campesterol	3.7 ± 0.8	3.1 ± 0.5	3.4 ± 0.9	−11 ± 7	−7 ± 8	7 ± 10
β-sitosterol	2.4 ± 0.6	2.1 ± 0.5	2.7 ± 1.0	−9 ± 7	2 ± 12	15 ± 13
Cholestanol	3.2 ± 0.4	2.7 ± 0.4*	2.6 ± 0.3[‡]	−17 ± 4	−21 ± 3	−4 ± 4
Relative to cholesterol[a]						
Lathosterol/TC	80.2 ± 10.5	52.9 ± 6.6[†]	38.9 ± 7.7[†,§]	−33 ± 5	−50 ± 9	−26 ± 9
Desmosterol/TC	58.6 ± 5.1	53.2 ± 5.8	40.3 ± 3.0[†,¶]	−8 ± 10	−29 ± 5	−18 ± 9
Campesterol/TC	154.1 ± 34.0	194.2 ± 39.9[†]	228.7 ± 50.2*	28 ± 7	55 ± 14	22 ± 10
β-sitosterol/TC	97.3 ± 22.7	131.7 ± 35.2*	173.8 ± 58.6	32 ± 9	70 ± 17	30 ± 12
Cholestanol/TC	140.2 ± 18.5	173.4 ± 28.6*	182.2 ± 22.4[‡]	21 ± 6	32 ± 5	10 ± 6
Lathosterol/campesterol[b]	0.73 ± 0.16	0.39 ± 0.09[†]	0.22 ± 0.05[†,¶]	−48 ± 3	−67 ± 5	−38 ± 8

Data are expressed as means ± SEM. Significance for comparison of absolute values with placebo phase and between treatment phases was determined using a paired *t* test

TC total cholesterol, *P* placebo, *R5* rosuvastatin 5 mg/day, *R40* rosuvastatin 40 mg/day

* $P < 0.05$, † $P < 0.01$, ‡ $P \leq 0.001$ for comparison with the placebo phase

§ $P = 0.06$, ¶ $P < 0.05$ for comparison with rosuvastatin 5 mg/d phase

[a] Ratio of plasma sterol to total cholesterol, 10^2 μmol/mmol

[b] Ratio of lathosterol/TC (μmol/mmol) to campesterol/TC (μmol/mmol)

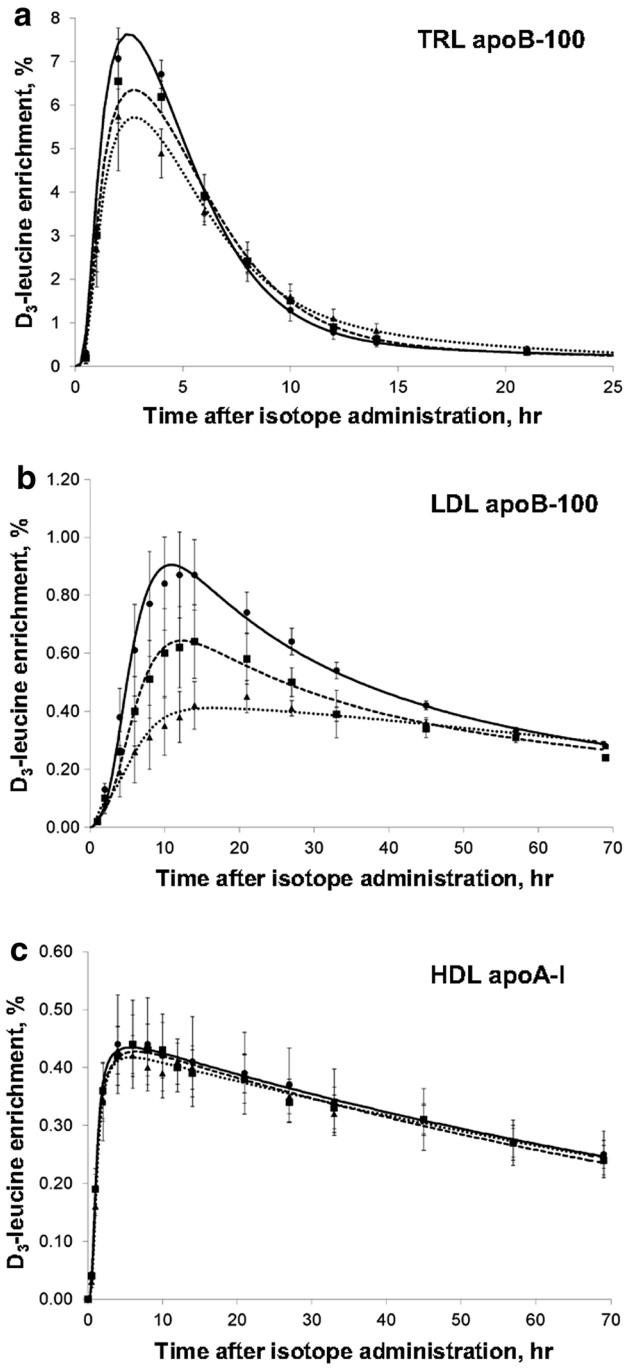

Fig. 3 D_3-leucine enrichment (%, mean ± SEM) of TRL apoB-100 (**a**) and LDL apoB-100 (**b**) and of HDL apoA-I (**c**) during the placebo (*triangles*), rosuvastatin 5 mg/day (*squares*), and rosuvastatin 40 mg/day (*circles*) phases. The *lines* represent the model-predicted values (placebo, *dotted line*; rosuvastatin 5 mg/day, *dashed line*; rosuvastatin 40 mg/day, *solid line*)

manner in this study of subjects with combined hyperlipidemia. These changes were accompanied by significant reductions in the plasma levels of cholesterol synthesis markers, notable increases in the fractional but not absolute concentrations of cholesterol absorption markers, and a shift toward large, more atheroprotective HDL particles. Analysis of the kinetic data revealed that the decrease in TRL apoB-100 and LDL apoB-100 PS was attributable to a notable increase in apoB-100 FCR. There was no change in the mean production rate of apoB-100 into TRL or LDL at either treatment dose relative to placebo. These findings fail to substantiate our hypothesis that rosuvastatin decreases the production of apoB-100 into either TRL or LDL.

Our finding that the maximal dose of rosuvastatin increases apoB-100 catabolism and, thereby, modulates plasma LDL-C, TAG, and apoB levels is consistent with the results of a previous study of rosuvastatin [13] (see Table 5). This study, however, differs from Ooi *et al*. [13] in an important aspect, that is, that the production rate of LDL apoB-100 did not fall during treatment with rosuvastatin 40 mg/day. The criterion for entry into the earlier rosuvastatin study was men who were diagnosed as having the metabolic syndrome. In the current study of subjects with combined hyperlipidemia, neither low or maximal dose rosuvastatin altered apoB-100 PR significantly, a finding also reported for atorvastatin treatment in combined hyperlipidemic subjects [11, 12; Table 5]. Such differences in results may relate to the underlying metabolic disorder being treated, as well as to the study design and the duration of the therapy. It is well recognized that the individual LDL-C response to statin therapy, as well as the statin-mediated effect on apoB metabolism (see Supplement Fig. 1), is quite variable. Genome-wide association studies attempting to identify the genetic contributors to this variability underscore the importance of genes associated with statin pharmacokinetics (*ABCG2*) and lipoprotein transport and uptake (*APOE, LDLR,* and *PCSK9*) in determining individual reductions in LDL-C [25] and, by inference, alterations in apoB metabolism.

The increase in apoB-100 FCR observed in this study was likely mediated by the inhibitory effect of rosuvastatin on cholesterol synthesis and the ensuing activation of the cholesterol-sensing transcription factor sterol-regulatory element binding protein 2 pathway, which then triggered an increase in the expression of the LDL receptor, one of the target genes of the resulting transcription factor [26]. We found that the plasma concentrations of the cholesterol synthesis biomarkers lathosterol and desmosterol were reduced in response to rosuvastatin, as anticipated [19], and that the decrease in TC was inversely associated with the increase in LDL apoB-100 FCR (data not shown). Our data indicate that the subjects who experienced the greatest reductions in cholesterol biosynthesis experienced the largest increases in LDL apoB-100 FCR. In addition, the relationship between the marked fall in plasma TAG and subsequent reduction in TRL particles was attributable in this study to increased TRL apoB-100 FCR. Such observations

Table 3 Effects of rosuvastatin on the kinetic parameters of TRL apoB-100, LDL apoB-100, and HDL apoA-I ($n = 8$)

	Placebo	Rosuvastatin (5 mg/day)	Rosuvastatin (40 mg/day)	Percentage change (R5 vs P)	Percentage change (R40 vs P)	Percentage change (R40 vs R5)
TRL apoB-100						
Pool size (mg)	287 ± 48	213 ± 42*	185 ± 32†	−18 ± 11	−32 ± 5	−7 ± 11
FCR (pools/day)	4.6 ± 1.1	5.2 ± 0.7	5.8 ± 0.7	36 ± 20	46 ± 15	13 ± 7
PR (mg/kg/day)	12.9 ± 1.1	13.0 ± 1.1	13.4 ± 1.7	1 ± 5	1 ± 6	2 ± 9
LDL apoB-100[a]						
Pool size (mg)	3132 ± 295	2183 ± 269‡	1848 ± 255‡,§	−31 ± 4	−42 ± 4	−15 ± 5
FCR (pools/day)	0.28 ± 0.05	0.43 ± 0.06*	0.55 ± 0.10†	63 ± 20	102 ± 22	33 ± 19
PR (mg/kg/day)	11.0 ± 1.5	11.6 ± 1.2	12.3 ± 1.2	11 ± 16	16 ± 11	14 ± 19
TRL converted to LDL (%)	64.4 ± 5.7	68.6 ± 10.0	78.3 ± 12.8	10 ± 16	18 ± 13	33 ± 37
HDL apoA-I						
Pool size (mg)	3825 ± 228	4055 ± 303	3976 ± 233	6 ± 3	5 ± 4	−1 ± 4
FCR (pools/day)	0.30 ± 0.02	0.32 ± 0.03	0.29 ± 0.02	7 ± 4	0 ± 8	−7 ± 6
PR (mg/kg/day)	14.3 ± 0.7	16.1 ± 1.0*	14.7 ± 1.0	12 ± 5	5 ± 10	−7 ± 7

Data are expressed as means ± SEM. Significance for comparison of absolute values with the placebo phase and between treatment phases was determined using a paired *t* test

FCR fractional catabolic rate, *PR* production rate, *P* placebo, *R5* rosuvastatin 5 mg/day, *R40* rosuvastatin 40 mg/day

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ for comparison with placebo phase

§ $P = 0.04$ for comparison with rosuvastatin 5 mg/day phase

[a] LDL apoB-100 kinetic parameters were assessed in 6 of the 8 subjects, due to technical difficulties

Table 4 Effects of rosuvastatin on apoA-I concentration in HDL subpopulations ($n = 6$)

	Placebo	Rosuvastatin (5 mg/day)	Rosuvastatin (40 mg/day)	Percentage change (R5 vs P)	Percentage change (R40 vs P)	Percentage change (R40 vs R5)
Concentration (mg/dl)						
Preβ-1	17.8 ± 2.5	18.4 ± 2.8	15.4 ± 2.0	4 ± 10	−10 ± 9	−7 ± 16
α-1	19.5 ± 4.5	21.4 ± 4.6	24.3 ± 4.2*,‡	16 ± 11	39 ± 16	19 ± 6
α-2	39.6 ± 3.0	39.9 ± 3.1	39.2 ± 1.0	2 ± 7	2 ± 8	1 ± 8
α-3	14.7 ± 2.5	17.0 ± 1.8	14.6 ± 1.6‡	25 ± 14	10 ± 17	−14 ± 5
α-4	8.6 ± 1.0	7.4 ± 0.9	8.2 ± 1.1	−11 ± 10	−3 ± 10	17 ± 20
Distribution (% of total)						
Preβ-1	15.4 ± 1.6	15.4 ± 1.8	13.1 ± 1.2	1 ± 9	−13 ± 6	−8 ± 14
α-1	16.1 ± 3.0	17.4 ± 3.1	20.4 ± 3.0†,§	14 ± 12	36 ± 12	21 ± 7
α-2	34.5 ± 1.1	34.0 ± 2.2	34.4 ± 1.8	−2 ± 5	0 ± 6	2 ± 5
α-3	13.6 ± 3.1	15.2 ± 2.8	12.9 ± 1.8	23 ± 16	11 ± 20	−13 ± 5
α-4	7.8 ± 1.3	6.6 ± 1.3	7.1 ± 1.0	−13 ± 10	−4 ± 10	18 ± 19

Data are expressed as means ± SEM. Significance for comparison of absolute values with the placebo phase and between treatment phases was determined using a paired *t* test

P placebo, *R5* rosuvastatin 5 mg/day, *R40* rosuvastatin 40 mg/day

* $P = 0.08$, † $P < 0.05$ for comparison with placebo phase

‡ $P < 0.05$, § $P < 0.01$ for comparison with rosuvastatin 5 mg/day phase

do not accord with our general hypothesis of a significant reduction of apoB-100 production at the maximal dose of a highly effective statin. However, combined hyperlipidemia is heterogeneous in terms of the underlying genetic and metabolic mechanisms. It remains possible that maximal dose rosuvastatin may suppress apoB-100 synthesis and secretion in some subjects (see Supplement Fig. 1).

The absence of notable changes in plasma HDL cholesterol or apoA-I concentrations notwithstanding, treatment with rosuvastatin 5 mg/day did cause a modest, but

Table 5 Effect of maximal dose atorvastatin and rosuvastatin on apoB-100 and HDL apoA-I metabolism in hyperlipidemic subjects

Reference	Statin (dose/day)	Subjects	Effect on apoB-100 and apoA-I metabolism
Bilz *et al.* [11]	Atorvastatin 80 mg	8 men Combined hyperlipidemia (LDL-C > 90, TAG > 300 mg/dl)	↓V_1 apoB PS due to ↑transfer to V_2 ↓V_2 apoB PS due to ↑FCR ↓IDL apoB PS due to ↑FCR (ns) ↓LDL apoB PS due to ↓PR (ns) and ↑FCR (ns) apoA-I: no significant effect
Lamon-Fava *et al.* [12]	Atorvastatin 20 mg and 80 mg	5 men and 4 women Combined dyslipidemia (TAG > 150, LDL-C > 160, HDL-C < 40 and <50 mg/dl[a])	↓VLDL PS due to ↑FCR ↓IDL apoB PS due to ↑FCR ↓LDL apoB PS due to ↑FCR apoA-I: no significant effect
Ooi *et al.* [13, 14]	Rosuvastatin 10 mg and 40 mg	12 men Metabolic syndrome (TAG < 400, LDL-C < 230, HDL-C < 46 mg/dl)	↓VLDL apoB PS due to ↑FCR ↓IDL apoB PS due to ↑FCR ↓LDL apoB PS due to ↑FCR and (40 mg) ↓PR ↑apoA-I PS (ns) due to ↓FCR and ↓PR
Present study	Rosuvastatin 5 mg and 40 mg	4 men and 4 women Combined hyperlipidemia (TAG > 150, LDL-C > 140, HDL-C < 50 mg/dl)	↓TRL apoB PS due to ↑FCR (ns) ↓LDL apoB PS due to ↑FCR ↑apoA-I PS (ns) due to ↑PR (5 mg)

FCR fractional catabolic rate, *HDL-C* HDL cholesterol, *IDL* intermediate density lipoprotein ($d = 1.006$–1.019 g/ml), *LDL-C* LDL cholesterol, *ns* not significant ($P > 0.05$), *PS* pool size, *PR* production rate, *TAG* triacylglycerol, *TRL* triacylglycerol rich lipoprotein ($d < 1.019$ g/ml), *VLDL* very low density lipoprotein ($d < 1.006$ g/ml); V_1 very low density lipoprotein S_f 60–400; V_2 very low density lipoprotein S_f 20–60

[a] HDL-C < 40 mg/dl for men, <50 mg/dl for women

statistically significant increase in apoA-I PR. Maximal dose rosuvastatin had no effect on apoA-I production. While this finding is consistent with the effects of maximal dose atorvastatin on apoA-I kinetics [11, 12], it does contrast with the reduction in apoA-I production observed in insulin resistant subjects treated with maximal dose rosuvastatin [14]. The difference may be a consequence of the extent of the HMG-CoA reductase inhibition. There is evidence that the cholesterol content of the liver is an important determinant of the amount of apoA-I synthesized, as well as the amount of cholesterol placed on newly synthesized preβ-1 HDL particles through the action of the ATP-binding cassette A1 (ABCA1) membrane transporter. In previous studies we observed that hepatic apoA-I gene expression in non-human primates was upregulated following cholesterol feeding [27, 28] and that HDL apoA-I production in humans was decreased when dietary cholesterol and saturated fat were restricted [23].

Rosuvastatin 40 mg/day treatment caused a significant change in the HDL subpopulation distribution, with apoA-I concentration shifting from the smaller preβ-1 to the larger α-1 subpopulation, a finding consistent with our previous report [10]. We have documented that the very small preβ-1 HDL particles serve as acceptors of cellular free cholesterol via ABCA1, while the large α-1 HDL particles serve as donors of cholesteryl ester to the liver via scavenger receptor-BI [29]. These observations suggest that highly intensive statin therapy may not only upregulate liver LDL receptor activity, but may also enhance the efficiency of reverse cholesterol transport.

It could be argued that statins also alter HDL metabolism by decreasing activity of the cholesteryl ester transfer protein (CETP). In their insulin resistant subjects, Ooi *et al.* [14] observed that the significant reduction in apoA-I clearance achieved with maximal dose rosuvastatin was accompanied by a significant decrease in CETP mass and activity and an increase in estimated HDL particle size. The authors attributed these metabolic changes to the statin-induced decrease in plasma TAG levels and, hence, in the number of CETP acceptor particles. In our hyperlipidemic subjects, no significant effect on apoA-I FCR was observed with either rosuvastatin dose; and the level of CETP and hepatic lipase activity and the rate of cholesterol esterification, which might explain the absence of a significant decrease in apoA-I FCR, were not assessed. We also found no associations between the redistribution of HDL subpopulations and apoA-I kinetic parameters. Nevertheless, it is well documented that intensive statin therapy decreases CETP activity, probably due to decreases in the number of TRL acceptor particles. Further studies examining the effects of statin therapy on the metabolism of apoA-I in HDL subpopulations would be of interest.

In this study, the intermediate density apoB-100-containing particles (IDL, $d = 1.006$–1.019 g/ml) were not separated from the very low density (VLDL) apoB-100-containing particles ($d < 1.006$), resulting in a less complex multicompartmental model for fitting the enrichment data. Nor were the kinetics of the several subspecies of VLDL and LDL analyzed. A focus on the two largest

pools of apoB-containing lipoproteins should provide the most accurate answer to the hypothesis that the maximal dose of rosuvastatin would suppress the production rate of apoB-100. In support of this, treatment with rosuvastatin 20 mg/day was found to lower $VLDL_1$ (S_f 60–400) apoB-100 PR significantly ($P = 0.035$) in eight subjects with type 2 diabetes [30].

It is possible that the small number of subjects, underscoring the well-established individual variability in statin response, may have limited our findings in this study (see Supplement Fig. 1). Ooi *et al.* [13] reported that maximal dose rosuvastatin caused a very small change in LDL apoB-100 PR in 12 subjects with metabolic syndrome and no change in the production of apoB in VLDL or IDL. Given the smaller number of subjects in the present study, we estimate that a 36 % change in LDL apoB-100 PR would be required to observe statistical significance. The fixed-sequence protocol, with no washout period between the two treatment phases, might suggest that the dose-associated effects we observed were due to the compounded intervention periods rather than the increased dose of rosuvastatin. However, metabolic sterol steady state has been shown to be achieved within 4 weeks; and studies in which a randomized, crossover study design was used to assess the effects of different doses of statins on lipoprotein metabolism argue that our observations were related to the treatment dose [12–14].

Our data indicate, overall, that in subjects with combined hyperlipidemia, maximal dose rosuvastatin enhances the catabolism of apoB-containing lipoproteins and has no effect on their production or on HDL apoA-I kinetics. From this and other investigations, it is clear that statins not only affect LDL levels, but also alter the entire plasma lipoprotein profile.

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