Diabetologia (2008) 51:1382–1390
DOI 10.1007/s00125-008-1046-4

ARTICLE

Effects of 20 mg rosuvastatin on VLDL1-, VLDL2-, IDL- and LDL-ApoB kinetics in type 2 diabetes

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Received: 13 September 2007 / Accepted: 25 March 2008 / Published online: 5 June 2008
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Abstract
Aims/hypothesis In addition to its efficacy in reducing LDL-cholesterol, rosuvastatin has been shown to significantly decrease plasma triacylglycerol. The use of rosuvastatin may be beneficial in patients with type 2 diabetes, who usually have increased triacylglycerol levels. However, its effects on the metabolism of triacylglycerol-rich lipoproteins in type 2 diabetic patients remains unknown.
Methods We performed a randomised double-blind cross-over trial of 6-week treatment with placebo or rosuvastatin 20 mg in eight patients with type 2 diabetes who were being treated with oral glucose-lowering agents. In each patient, an in vivo kinetic study of apolipoprotein B (ApoB)-containing lipoproteins with [^{13}C]leucine was performed at the end of each treatment period. A central randomisation centre used computer-generated tables to allocate treatments. Participants, caregivers and those assessing the outcomes were blinded to group assignment.
Results Rosuvastatin 20 mg significantly reduced plasma LDL-cholesterol, triacylglycerol and total ApoB. It also significantly reduced ApoB pool sizes of larger triacylglycerol-rich VLDL particles (VLDL1; p=0.011), smaller VLDL particles (VLDL2; p=0.011), intermediate density lipoprotein (IDL; p=0.011) and LDL (p=0.011). This reduction was associated with a significant increase in the total fractional catabolic rate of VLDL1-ApoB (6.70±3.24 vs 4.52±2.34 pool/day, p=0.049), VLDL2-ApoB (8.72±3.37 vs 5.36±2.64, p=0.011), IDL-ApoB (7.06±1.68 vs 4.21±1.51, p=0.011) and LDL-ApoB (1.02±0.27 vs 0.59±0.13, p=0.011). Rosuvastatin did not change the production rates of VLDL2-, IDL- or LDL-, but did reduce VLDL1-ApoB production rate (12.4±4.5 vs 19.5±8.4 mg kg^{-1} day^{-1}, p=0.035). No side effects of rosuvastatin were observed during the study.
Conclusions/interpretation In type 2 diabetic patients rosuvastatin 20 mg not only induces a significant increase of LDL-ApoB catabolism (73%), but also has favourable effects on the catabolism of triacylglycerol-rich lipoproteins, e.g. a significant increase in the catabolism of VLDL1-ApoB (48%), VLDL2-ApoB (63%) and IDL-ApoB (68%), and a reduction in the production rate of VLDL1-ApoB (−36%). The effects of rosuvastatin on the metabolism of triacylglycerol-rich lipoproteins may be beneficial for prevention of atherosclerosis in type 2 diabetic patients.

Trial registration: ClinicalTrials.gov NCT00658463
Funding: This study was supported by a grant from AstraZeneca.

Keywords ApoB · Apolipoprotein B · Diabetes · Kinetic · LDL-cholesterol · Rosuvastatin · Statin · Triacylglycerol

Abbreviations

ApoA1	apolipoprotein A1
ApoB	apolipoprotein B
ApoC-III	apolipoprotein C-III
CETP	cholesteryl ester transfer protein
CVD	cardiovascular disease
HOMA-IR	homeostasis model assessment index of insulin resistance

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IDL	intermediate density lipoprotein
FCR	fractional catabolic rate
MTP	microsomal triacylglycerol transfer protein
VLDL1	larger triacylglycerol-rich VLDL particles
VLDL2	smaller VLDL particles

Introduction

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in patients with type 2 diabetes. CVD risk is increased two- to fourfold over non-diabetic patients [1–4]. Abnormalities of lipid metabolism, observed in type 2 diabetes, are one of the major factors contributing to vascular risk [1, 5]. Diabetic dyslipidaemia includes abnormal metabolism of apolipoprotein B (ApoB)-containing lipoproteins [6, 7]. Indeed, patients with type 2 diabetes show increased plasma triacylglycerol due to increased hepatic production of VLDL particles and decreased catabolism of triacylglycerol-rich lipoproteins (VLDL, intermediate density lipoprotein [IDL]) [6–10]. Moreover, overproduction of VLDL particles has been shown to be mainly represented by the formation of larger triacylglycerol-rich VLDL particles (VLDL1), known to be more atherogenic than the smaller VLDL particles (VLDL2) [11, 12]. Although the LDL cholesterol concentration is generally normal or only moderately raised in type 2 diabetic patients, LDL-ApoB metabolism is impaired. Indeed, a significant reduction of LDL catabolism associated in parallel with a diminution of LDL production is observed in patients with type 2 diabetes [10]. The direct consequence of reduced turn-over of LDL particles is an increase in LDL plasma residence time, which is potentially harmful for the vascular wall. Thus abnormal metabolism of ApoB-containing lipoproteins is an important feature of diabetic dyslipidaemia and should be considered as a therapeutic target in patients with type 2 diabetes.

Statins have been shown to significantly reduce the risk of cardiovascular events in patients with type 2 diabetes and statin therapy is highly recommended in this high cardiovascular risk population. However, a residual cardiovascular risk is observed in patients with type 2 diabetes who are being treated by statins. This may be due to the fact that statins do not correct all lipid abnormalities associated with diabetic dyslipidaemia, such as hypertriacylglycerol [13].

Rosuvastatin is a statin which has been shown to reduce LDL-cholesterol more than the other statins at an equivalent dose [14]. In addition, rosuvastatin significantly decreases plasma triacylglycerol levels with greater effect than pravastatin or simvastatin [14]. Interestingly, reduction of plasma triacylglycerol with rosuvastatin has been shown to be more pronounced in patients with the metabolic syndrome [15, 16]. Animal studies indicate that rosuvastatin may reduce VLDL-ApoB production [17, 18]. All these data suggest that rosuvastatin could have specific benefits in improving the metabolism of ApoB-containing lipoproteins, including VLDL, in patients with type 2 diabetes.

However, the effects of rosuvastatin on the metabolism of triacylglycerol-rich lipoproteins remain unknown in such patients. This prompted us to perform an in vivo kinetic study of ApoB-containing lipoproteins with [^{13}C]leucine in patients with type 2 diabetes. In the present study we aimed to assess the effect of 20 mg/day rosuvastatin on the metabolism of all ApoB-containing lipoproteins including VLDL1, VLDL2, IDL and LDL.

Methods

Participants We recruited eight patients with type 2 diabetes and typical diabetic dyslipidaemia defined by triacylglycerol > 1.70 mmol/l and HDL-cholesterol < 1.03 mmol/l in men and <1.29 mmol/l in women. These patients had been treated with oral glucose lowering agents (metformin alone in four patients, metformin + sulfonylureas or glinides in the other four) for at least 6 months and had stable HbA_{1c} over the last 6 months. Patients with HbA_{1c} > 9%, LDL-C > 4.90 mmol/l, CVD, renal impairment (creatinine clearance < 0.5 ml/s), abnormal liver or muscle enzymes, history of alcohol and/or drug abuse, hyper- or hypothyroidism, insulin treatment and use of drugs known to affect lipid metabolism (corticoids, retinoids, antiproteases, oestrogens, cyclosporin, glitazones, statins other than rosuvastatin, fibrates, cholestyramine, ezetimibe, nicotinic acid, *n*-3 fatty acids or phytosterols) were excluded. The protocol was approved by the Dijon University Hospital Ethics Committee and written informed consent was obtained from each participant before the study.

Study design This was a randomised, double-blinded, placebo-controlled crossover trial. Eligible patients entered a 4-week placebo maintenance (weight, glucose, lipids) lead-in period followed by randomisation to a 6-week treatment period with rosuvastatin 20 mg or placebo taken orally once daily with crossover to a further 6-week treatment period. Patients were advised to continue on isoenergetic diets and to keep their levels of physical activity constant. Compliance was assessed by tablet count.

Two kinetic studies were performed in each type 2 diabetic patient. The first one was at the end of the first treatment period and the second at the end of the second treatment period.

The day before the kinetic study, each patient was admitted to the diabetology ward in the morning after a 12 h fast for physical examination and blood sampling. The following day, the kinetic study was performed in the fed state. Food intake, with a leucine-poor diet (7,116 kJ/day,

55% carbohydrate, 39% fat, 7% protein), was fractionated in small portions, which were provided every 2 h starting 6 h prior to the tracer infusion up to the end of the study in order to avoid large variations in apolipoprotein plasma concentration, as previously performed by our group [19, 20] and others [21]. The endogenous labelling of ApoB was carried out by administration of L-[1–^{13}C]leucine (99 at.% [wt/vol.]; Eurisotop, Saint Aubin, France), dissolved in 0.9% (wt/vol.) NaCl solution. At 08:00 hours, each participant received, intravenously, a primed infusion of 0.7 mg/kg of tracer, immediately followed by a 16 h constant infusion of 0.7 mg kg^{-1} h^{-1}. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15 and 16 h after the primed infusion. Serum was separated by centrifugation for 10 min at 4°C and 3,000×*g*. In order to avoid the influence of acute exercise on lipid metabolism, all studied participants were instructed to refrain from strenuous exercise 3 days prior to the kinetic study.

Isolation of ApoB VLDL1, VLDL2, IDL and LDL were isolated from plasma by gradient ultracentrifugation, using a SW 41 rotor in a L90 apparatus (Beckman Instruments, Palo Alto, CA, USA) as previously reported [22]. VLDL1 fractions were dialysed against a 10 mmol/l ammonium bicarbonate buffer pH 8.2 containing 0.01% (wt/vol.) EDTA and 0.013% (wt/vol.) sodium azide, then delipidated for 1 h at −20°C using ten volumes of diethylether–ethanol 3:1. ApoB-100 from VLDL1 was isolated by preparative SDS-PAGE: the delipidated ApoB-100-containing material was solubilised in 0.05 mol/l Tris buffer pH8.6, containing 3% (wt/vol.) SDS, 3% (vol./vol.) mercaptoethanol and 10% (vol./vol.) glycerol and applied to a 3 mm thick vertical slab gel (3% [wt/vol.] acrylamide). After staining with Coomassie Blue R-250, ApoB-100 was cut from the gel and hydrolysed in HCl 6 mol/l for 16 h at −110°C under nitrogen vacuum. Samples were then centrifuged (2,000×*g* for 10 min) to remove polyacrylamide. Supernatant fractions were lyophilised in a Speed Vac (Savant Instrument, Farmingdale, NY, USA). Lyophilised samples were dissolved in 50% (vol./vol.) acetic acid, applied to an AG-50W-X8 200–400 mesh cation exchange column (Bio-Rad, Richmond, CA, USA), and amino acids were recovered by elution with NH4OH 4 mol/l and lyophilised.

VLDL2-, IDL- and LDL-ApoB-100 was isolated by selective precipitation with butanol-isopropyl ether as previously described [23].

Determination of leucine enrichment by gas chromatograph/combustion/isotope ratio mass spectrometry Amino acids were converted to *N*-acetyl *O*-propyl amino acid esters and analysed with an isotope ratio mass spectrometer (Delta C; Finnigan Mat, Bremen, Germany) coupled to a HP 5890 series II gas chromatograph (Hewlett Packard, Waldbronn, Germany), as previously described [23]. [^{13}C]Leucine enrichment was initially expressed in delta ‰ and converted to tracer:tracee ratio prior to modelling [24–26].

Modelling The data were analysed with the Simulation Analysis and Modeling II program (SAAM Institute, Seattle, WA, USA) by using the multicompartmental model shown in Fig. 1. This model has already been used by others for ApoB kinetic studies performed with stable isotope constant infusion [26]. A forcing function, corresponding to VLDL1-ApoB-100 plateau enrichment, was used to drive the appearance of leucine tracer into the different lipoprotein fractions [26, 27]. The delay compartment accounted for the time required for the synthesis and secretion of ApoB-100 into the plasma. Compartments 1 and 2 represent plasma VLDL1-ApoB-100 and VLDL1 remnants-ApoB-100 respectively. Compartments 11 and 12 represent plasma VLDL2-ApoB-100 and VLDL2 remnants-ApoB-100 respectively. Compartments 21 and 22 represent plasma IDL-ApoB-100 and IDL remnants-ApoB-100 respectively. Compartment 31 represents plasma LDL-ApoB-100.

In stable isotope kinetic studies, the tracee is in steady state while the tracer is in a dynamic condition. It is generally

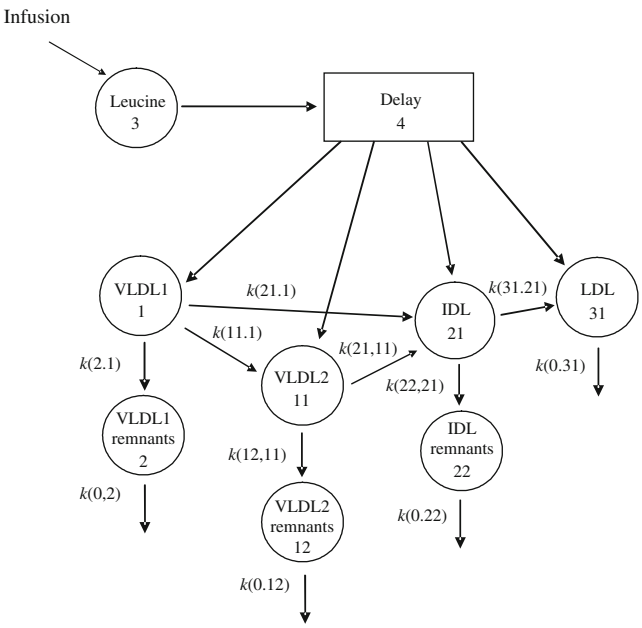

Fig. 1 Multicompartmental model for kinetic analysis of ApoB-100 metabolism. Compartment 3 represents plasma leucine into which the leucine tracer was injected. Compartment 4 is the delay compartment, representing the time required for the synthesis and secretion of ApoB-100 into the plasma. Compartments 1 and 2 represent plasma VLDL1-ApoB-100 and VLDL1 remnants-ApoB100 respectively. Compartments 11 and 12 represent plasma VLDL2-ApoB-100 and VLDL2 remnants-ApoB100 respectively. Compartments 21 and 22 represent plasma IDL-ApoB100 and IDL remnants-ApoB100 respectively. Compartment 31 represents plasma LDL-ApoB-100. *k*(y,x) represents the fractional transfer coefficient from compartment x to compartment y (arrows)

assumed that the tracee steady state is not perturbed by the tracer injection [28, 29]. In stable isotope experiments two sets of differential equations are needed to describe the model because two state variables q (tracer) and Q (tracee) are present in the tracer:tracee ratio equation $z(t) = q(t)/Q(t)$. One set of differential equations describes the movement of tracer through the model and the other set describes the movement of tracee through the model [25, 28]. From the structure of the model, the SAAM software internally constructs the system of differential equations represented by the model. For instance, in our model, time changes of the amount of tracer in compartment 2 (q_2) equals the difference between the input from compartment 1 (represented as the product of the amount of tracer in this compartment [q_1] multiplied by the rate constant from 1 to 2) and the output from compartment 2 toward the environment. Expressed as a formula, this is: $dq_2/dt = k(2,1)q_1 - k(0,2)q_2$. In general, k(y,x) are the fractional transfer coefficients from compartment x to compartment y [28].

Because the experiment was performed in the steady state, the fractional synthetic rate equalled the fractional catabolic rate (FCR) [25, 28].

Direct FCRs of VLDL1-ApoB and FCRs from VLDL1 to VLDL2 or IDL (indirect FCR), expressed in pools per day, were calculated as follows:

$$\text{Direct FCR}_{\text{VLDL1}} = M_2 k(0,2)/(M_1 + M_2) \quad (1)$$

$$\text{Indirect FCR}_{\text{VLDL1}\rightarrow\text{VLDL2}\rightarrow\text{IDL}} = M_1[k(11,1) + k(21,1)/(M_1 + M_2)] \quad (2)$$

where M_j represents the ApoB mass (expressed as concentration per litre of plasma) of compartment j. Total FCR of ApoB VLDL1 is the sum of direct $\text{FCR}_{\text{VLDL1}}$ and indirect $\text{FCR}_{\text{VLDL1}\rightarrow\text{VLDL2}\rightarrow\text{IDL}}$.

Direct FCRs of VLDL2-ApoB and FCRs from VLDL2 to IDL (indirect FCR), expressed in pools per day, were calculated as follows:

$$\text{Direct FCR}_{\text{VLDL2}} = M_{12} k(0,12)/(M_{11} + M_{12}) \quad (3)$$

$$\text{Indirect FCR}_{\text{VLDL2}\rightarrow\text{LDL}} = M_{11} k(21,11)/(M_{11} + M_{12}) \quad (4)$$

Direct FCRs of IDL-ApoB and FCR from IDL to LDL (indirect FCR) were calculated as follows:

$$\text{Direct FCR}_{\text{IDL}} = M_{22} k(0,22)/(M_{21} + M_{22}) \quad (5)$$

$$\text{Indirect FCR}_{\text{IDL}\rightarrow\text{LDL}} = ML_{21} k(31,21)/M_{21} + M_{22} \quad (6)$$

The FCR of LDL-ApoB was calculated as follows:

$$\text{FCR}_{\text{LDL}} = k(0,31) \quad (7)$$

Production rates of ApoB-100 in each lipoprotein fraction were normalised to body weight and calculated as follows:

$$\text{PR} = \text{ApoB FCR(each lipoprotein)} \times \text{ApoB pool size/body weight} \quad (8)$$

where PR is the production rate and ApoB pool size is calculated by multiplying the ApoB concentration in the lipoprotein fraction (VLDL, IDL or LDL) by the estimated plasma volume (4.5% of body weight) [25]. In obese participants (BMI ≥ 30), a correction of plasma volume was performed as previously reported by many authors [30, 31]. The plasma volume was modified by multiplying by a correction factor to take into account the decrease in relative plasma volume associated with an increase in body weight [32]. Akaike's information criterion was used to compare different models and the model with the lowest Akaike's information criterion value was chosen. Moreover, the physiological plausibility of the model has been verified. The goodness of fit of the model was assessed by the analysis of the residuals with the runs test [28]. The model satisfied prior and posterior identifiability. The parameters were uniquely estimated for every single patient.

Biochemical analysis Plasma glucose concentrations were measured by an enzymatic method (glucose oxidase) on an analyser (Vitros 950; Ortho Clinical Diagnostics, Rochester, NY, USA). HbA_{1c} was measured with ion exchange HPLC (Bio-Rad Laboratories, Richmond, CA, USA). Total cholesterol, LDL- and HDL-cholesterol, triacylglycerol, ApoB and apolipoprotein A1 (ApoA1) concentrations were measured on a dimension analyser with dedicated reagents (Dade Behring, Newark, NE, USA). ApoB and ApoA1 were measured by immunoturbidimetry. The within-run coefficient of variation for that method was less than 5% at 0.02 g/l. Apolipoprotein C-III (ApoC-III) was measured by immunoturbidimetry on the dimension analyser with DIASYS reagents (DIASYS, Condom, France). Fructosamine was measured on the dimension analyser with ABX Diagnostics reagents (Montpellier, France). Plasma lathosterol measurement was performed by GC-MS analysis using a Hewlett Packard HP6890 Gas Chromatograph equipped with an HP7683 Injector and a HP5973 Mass Selective Detector. Plasma insulin was measured by radioimmunoassay (CIS Bio International, Gif sur Yvette, France). Insulin sensitivity was assessed using the homeostasis model assessment (HOMA-IR) described by Matthews et al. [33]

Statistical analysis Data are reported as mean ± SD. Statistical calculations were performed using the SPSS software package (Chicago, IL, USA). Comparisons of continuous variables on placebo versus on rosuvastatin

20 mg were performed using the nonparametric Wilcoxon matched-pair test. Correlation coefficients were calculated by the Spearman test. A two-tailed probability level of 0.05 was accepted as statistically significant.

Results

Glucose and lipid parameters Baseline characteristics in the form of glucose and plasma lipid parameters are shown in Table 1 for patients with type 2 diabetes on placebo and on rosuvastatin 20 mg/day. Treatment with rosuvastatin 20 mg/day was well tolerated in all patients. Body weight, HbA_{1c}, fasting glycaemia, fructosamine and HOMA-IR were similar in participants on placebo and on rosuvastatin. Treatment with rosuvastatin 20 mg induced a significant decrease of total cholesterol (−38%), LDL-cholesterol (−51%), triacylglycerol (−38%), plasma ApoB (−40%), ApoC-III (−30%) and lathosterol (−85%) (Table 1). HDL-cholesterol and plasma ApoA-I were not modified by rosuvastatin 20 mg/day. Treatment with rosuvastatin was accompanied with a reduction of esterified cholesterol in VLDL1, VLDL2, IDL and LDL and of triacylglycerol in VLDL1 and LDL (−31%; Table 1).

ApoB-100 kinetic parameters Table 2 gives the pool sizes and kinetics of VLDL1-, VLDL2-, IDL- and LDL-ApoB-100 for participants on placebo and on rosuvastatin 20 mg. Compared with placebo, rosuvastatin 20 mg significantly decreased the pool sizes of VLDL1- (−57%), VLDL2- (−42%), IDL- (−43%) and LDL-ApoB-100 (−44%) (Table 2). Compared with placebo, rosuvastatin 20 mg significantly increased the total FCRs of VLDL1- (48%), VLDL2- (63%), IDL- (68%) and LDL- ApoB-100 (73%). The increase in VLDL1-ApoB-100 total FCR was due to an increase in indirect VLDL1-ApoB-100 FCR (towards VLDL2 and IDL) (Table 2). Compared with placebo, rosuvastatin 20 mg significantly reduced the production rate of VLDL1-ApoB-100 (−36%; Table 2). The production rates of VLDL2-, IDL- and LDL-ApoB-100 were not modified by rosuvastatin.

The isotopic enrichment curves for VLDL1-, VLDL2-, IDL- and LDL-ApoB with [^{13}C]leucine in the eight patients are shown in Fig. 2 for participants treated with placebo (Fig. 2a) and those treated with rosuvastatin 20 mg (Fig. 2b). On rosuvastatin, the rate of appearance of tracer within VLDL1-, VLDL2-, IDL- and LDL-ApoB-100 fractions was increased, indicating an acceleration of the corresponding rates of catabolism.

The decrease in LDL-triacylglycerol that was induced by rosuvastatin 20 mg correlated with the increase in total FCR of VLDL1-ApoB-100 (r=0.73, p=0.05). The decrease in plasma ApoC-III correlated negatively with the increase in VLDL1-ApoB-100 FCR without reaching statistical significance (r=−0.52, p=0.18).

Discussion

Our present study provides new information on the effects of rosuvastatin on the metabolism of triacylglycerol-rich lipoproteins in patients with type 2 diabetes and diabetic dyslipidaemia. We showed that rosuvastatin significantly reduces the pool of all ApoB-containing lipoproteins:

Table 1 Baseline lipid and glycaemic parameters of patients on placebo and on 20 mg/day rosuvastatin

Parameter	Placebo	Rosuvastatin (20 mg/day)	p value
Age (years)	55.4±10.2		
Sex (male/female)	5/3		
BMI (kg/m^2)	34±4		
Body weight (kg)	97.2±19.9	97.2±20.1	NS
Waist circumference (cm)	113±16	113±15	NS
Fasting glycaemia (mmol/l)	8.58±2.9	8.96±4.3	NS
HbA_{1c} (%)	6.9±1.2	7.0±1.5	NS
Fructosamine (μmol/l)	375±63	412±41	NS
HOMA-IR	3.29±1.52	3.54±1.68	NS
Total cholesterol (mmol/l)	5.52±1.21	3.43±0.79	0.011
Triacylglycerol (mmol/l)	3.09±1.40	1.92±0.79	0.011
LDL-cholesterol (mmol/l)	3.14±0.82	1.55±0.46	0.011
HDL-cholesterol (mmol/l)	0.98±0.25	1.00±0.23	NS
ApoB (g/l)	1.12±0.16	0.67±0.12	0.011
ApoA-I (g/l)	1.29±0.22	1.28±0.20	NS
ApoC-III (g/l)	0.18±0.06	0.13±0.04	0.011
Lathosterol (mg/l)	4.72±2.47	0.74±0.31	0.011
VLDL1-FC (mmol/l)	0.60±0.24	0.50±0.21	0.017
VLDL1-EC (mmol/l)	0.54±0.30	0.34±0.22	0.011
VLDL1-triacylglycerol (mmol/l)	2.04±0.75	1.19±0.47	0.017
VLDL1-PL (mmol/l)	0.67±0.18	0.58±0.25	0.011
VLDL2-FC (mmol/l)	0.30±0.19	0.26±0.13	0.035
VLDL2-EC (mmol/l)	0.42±0.31	0.33±0.18	0.017
VLDL2-triacylglycerol (mmol/l)	0.64±0.45	0.42±0.20	NS
VLDL2-PL (mmol/l)	0.36±0.22	0.32±0.17	NS
IDL-FC (mmol/l)	0.17±0.05	0.17±0.07	NS
IDL-EC (mmol/l)	0.34±0.13	0.27±0.10	0.017
IDL-triacylglycerol (mmol/l)	0.21±0.08	0.17±0.09	NS
IDL-PL (mmol/l)	0.22±0.06	0.20±0.10	NS
LDL-FC (mmol/l)	0.77±0.23	0.39±0.10	0.011
LDL-EC (mmol/l)	2.37±0.86	1.15±0.44	0.011
LDL-triacylglycerol (mmol/l)	0.19±0.07	0.13±0.05	0.011
LDL-PL (mmol/l)	1.02±0.28	0.62±0.19	0.017

Data are means ± SD
EC, esterified cholesterol; FC, non-esterified cholesterol; PL, phospholipids

Table 2 VLDL1-, VLDL2-, IDL- and LDL-ApoB-100 kinetic parameters in eight type 2 diabetic participants on placebo and on 20 mg/day rosuvastatin

Parameter	Placebo	Rosuvastatin 20 mg/day	*p* value
VLDL1			
VLDL1-ApoB-100 pool size (mg)	474±212	205±92	0.011
VLDL1-ApoB-100 total FCR (pool/day)	4.52±2.34	6.70±3.24	0.049
VLDL1-ApoB-100 direct FCR (pool/day)	2.21±2.87	1.39±2.15	0.273
VLDL1-ApoB-100 indirect FCR (pool/day)	2.31±1.78	5.31±2.40	0.011
VLDL1-ApoB-100 PR (mg kg^{-1} day^{-1})	19.5±8.4	12.4±4.5	0.035
VLDL2			
VLDL2-ApoB-100 pool size (mg)	397±252	230±86	0.011
VLDL2-ApoB-100 total FCR (pool/day)	5.36±2.64	8.72±3.37	0.011
VLDL2-ApoB-100 direct FCR (pool/day)	0.89±1.86	2.47±4.02	0.273
VLDL2-ApoB-100 indirect FCR (pool/day)	4.47±2.83	6.25±1.64	0.100
VLDL2-ApoB-100 PR (mg kg^{-1} day^{-1})	19.10±7.60	19.13±4.17	0.674
Total VLDL			
Total VLDL-ApoB-100 PR (mg kg^{-1} day^{-1})	27.71±9.76	21.58±6.65	0.262
IDL			
IDL-ApoB-100 pool size (mg)	392±130	224±87	0.011
IDL-ApoB-100 total FCR (pool/day)	4.21±1.51	7.06±1.68	0.011
IDL-ApoB-100 direct FCR (pool/day)	0.41±0.86	1.10±1.54	0.465
IDL-ApoB-100 indirect FCR (pool/day)	3.80±1.94	5.96±2.22	0.093
IDL-ApoB-100 PR (mg kg^{-1} day^{-1})	16.59±5.08	16.41±5.66	0.779
LDL			
LDL-ApoB-100 pool size (mg)	2890±677	1626±477	0.011
LDL-ApoB-100 FCR (pool/day)	0.59±0.13	1.02±0.27	0.011
LDL-ApoB-100 PR (mg kg^{-1} day^{-1})	17.38±3.44	16.83±3.81	0.779
Direct input (hepatic synthesis)			
VLDL2-ApoB-100 (mg kg^{-1} day^{-1})	8.8±3.4	8.6±4.0	0.67
IDL-ApoB-100 (mg kg^{-1} day^{-1})	2.0±2.4	2.1±1.4	0.78
LDL-ApoB-100 (mg kg^{-1} day^{-1})	4.7±2.1	5.2±3.1	0.46

Data are means±SD
PR, production rate

VLDL1, VLDL2, IDL and LDL. The reduction of VLDL1-, VLDL2-, IDL- and LDL-ApoB pools was associated with a significant increase in the catabolism of all ApoB-containing lipoproteins (VLDL1, VLDL2, IDL and LDL) and a significant decrease in the VLDL1-ApoB production rate.

To date, very few studies have analysed the effects of statins on ApoB metabolism in patients with type 2 diabetes [34, 35]. In seven patients with type 2 diabetes and hypertriacylglycerolaemia, Ouguerram et al. found that atorvastatin 40 mg decreased plasma triacylglycerol and ApoB-100 in VLDL, IDL and LDL, this being associated with a significant reduction of IDL-and LDL-ApoB-100 FCRs and a significant decrease in VLDL-ApoB-100 production rate [34]. Myerson et al. showed in five patients with type 2 diabetes that simvastatin 80 mg increased IDL-ApoB-100 FCR and reduced LDL-ApoB-100 production rate [35]. A kinetic study with rosuvastatin 10 mg and 40 mg on ApoB metabolism in individuals with the metabolic syndrome has recently been reported [36]. However, patients with type 2 diabetes were excluded from that study. Because of the important role of diabetic dyslipidaemia in the promotion of CVD in patients with type 2 diabetes, and because statin therapy is highly recommended in patients with type 2 diabetes, it is essential to know the effects of rosuvastatin, a potent hydroxymethylglutaryl CoA reductase inhibitor, on the metabolism of ApoB-containing lipoproteins.

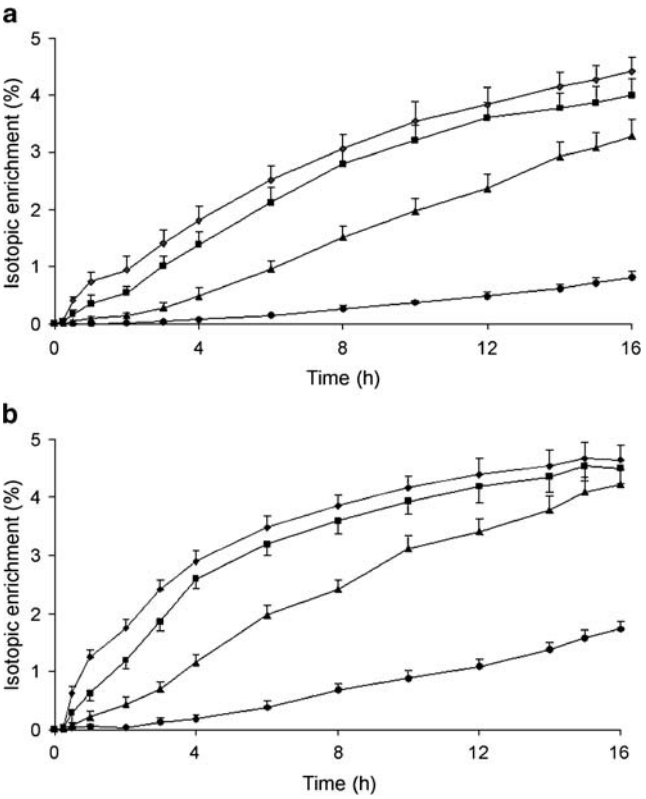

Fig. 2 Isotopic enrichment curves of VLDL1-ApoB100 (diamonds), VLDL2-ApoB100 (squares), IDL-ApoB100 (triangles) and LDL-ApoB-100 (circles) with [^{13}C]leucine in eight patients with type 2 diabetes on placebo (**a**) and on rosuvastatin 20 mg/day (**b**). Data are means±SEM

Diabetic dyslipidaemia includes abnormal metabolism of ApoB-containing lipoproteins with increased hepatic production of VLDL particles (mostly VLDL1) and decreased catabolism of VLDL, IDL and LDL [6–11]. In the present study, we showed that rosuvastatin 20 mg significantly increased the total catabolism of VLDL1-, VLDL2-, IDL- and LDL-ApoB-100. These results are consistent with those of Ooi et al. showing increased FCR of VLDL-, IDL- and LDL-ApoB-100 with rosuvastain in individuals with the metabolic syndrome, although a specific study of VLDL1 and VLDL2 kinetics was not performed in that study [36]. Our data indicate that the increased total catabolism of VLDL1-, VLDL2- and IDL-ApoB-100 that is induced by rosuvastatin is mainly due to an increase in indirect catabolism of those lipoproteins. This suggests that rosuvastatin could increase the clearance of VLDL1, VLDL2 and IDL mainly through the lipase-mediated catabolic pathway. Indeed, several statins have been shown to increase lipoprotein lipase expression in adipose tissue [37–39]. It has been demonstrated that atorvastatin 40 mg increases lipoprotein lipase activity by 25% in patients with type 2 diabetes [40]. Statins may increase lipoprotein lipase activity by different mechanisms. On the one hand, they have been shown to reduce mRNA as well as plasma levels of ApoC-III, the lipoprotein lipase inhibitor [37]; on the other hand, cholesterol depletion of the cell, induced by statins, may directly increase lipoprotein lipase activity via a functional responsive element for sterol regulatory binding proteins [39, 41] and via peroxisome proliferator-activated receptor γ in the lipoprotein lipase gene promoter [42]. In our study, we did not find a significant correlation between the fall in plasma ApoC-III concentration and the increase in VLDL1-ApoB-100 FCR, but only a trend. Due to the limited number of patients in our study, we cannot totally exclude a role of the decrease in plasma ApoC-III in the augmentation of VLDL1-ApoB-100 FCR. However, our data indicate that it is unlikely to be a major factor. Furthermore, we do not know whether this decrease in plasma ApoC-III is due to decreased hepatic synthesis and/or increased VLDL catabolism and transfer to other lipoproteins, especially HDL. The acceleration, induced by rosuvastatin, of the catabolism of all ApoB-containing lipoproteins directly reduces their plasma residence time, which may diminish their potential harm on the vascular wall.

To date, the effects of statins on VLDL1 and VLDL2 metabolism have never been studied. A new finding was that rosuvastatin 20 mg significantly decreased the production rate of VLDL1-ApoB-100. Augmented production of VLDL1 is an important hallmark of diabetic dyslipidaemia and it is interesting to observe that treatment with rosuvastatin may reduce such abnormality. Animal studies have shown that rosuvastatin may reduce VLDL-ApoB production [17, 18]. Our results are in accordance with in vitro studies that have shown that rosuvastatin decreases activity of the hepatic diacylglycerol:acyl transferase, the last enzyme of the triacylglycerol synthesis pathway [17], and the levels of hepatic microsomal triacylglycerol transfer protein (MTP) [18]. A similar reduction of levels of hepatic MTP has also been reported with atorvastatin [43]. Both atorvastatin and rosuvastatin have been shown to stimulate ApoB degradation in hepatocytes, leading to a reduction in assembly and secretion of VLDL particles [18, 43, 44].

The effects of rosuvastatin on the kinetics of ApoB-containing lipoproteins in patients with type 2 diabetes may be considered to be positive. Indeed, we have shown in our study that rosuvastatin reverses the metabolic abnormalities of the VLDL–IDL–LDL cascade that are typical of diabetic dyslipidaemia (increased VLDL1 production and decreased catabolism of VLDL, IDL and LDL). As a consequence of the rosuvastatin-induced reduction of VLDL1-ApoB-100 production and the rosuvastatin-induced increase in the catabolism of VLDL1, VLDL2-, IDL- and LDL-ApoB-100, we observed a significant reduction of the pools of all ApoB-containing lipoproteins, which are known to be atherogenic. This reduction may potentially reduce cardiovascular risk. Furthermore, we observed that rosuvastatin not only reduces plasma triacylglycerol, but also LDL-triacylglycerol. The reduction in LDL-triacylglycerol was found to be correlated with the increase in the VLDL1 catabolic rate. It has been proposed that the large, triacylglycerol-rich VLDL1 particles promote the cholesteryl ester transfer protein (CETP)-mediated transfer of triacylglycerol to LDL particles, generating small dense triacylglycerol-rich LDL particles, which are potentially atherogenic [45]. Our data indicate that the increase in VLDL1 catabolism induced by rosuvastatin leads to reduced triacylglycerol transfer from VLDL1 to LDL, possibly by reducing both the VLDL1 pool and VLDL1 plasma residence time. Moreover, the decrease in plasma triacylglycerol, which is secondary to rosuvastatin-induced changes in triacylglycerol-rich lipoprotein kinetics, may also influence, via CETP, the remodelling of HDL particles. It remains to be examined whether this could modify HDL metabolism.

Our study has some limitations. Measurement of lipoprotein lipase and hepatic lipase activities might have shed more light on the mechanisms for increased catabolism of triacylglycerol-rich lipoproteins with rosuvastatin. Although the number of patients in our study was limited, we were able, using a crossover trial design in well selected patients featuring typical diabetic dyslipidaemia, to show significant modification of VLDL1, VLDL2, IDL and LDL kinetics with 20 mg rosuvastatin.

Based on current clinical evidence, statins are the first-choice pharmacological therapy to address diabetic dyslipidaemia, due to their potent LDL-cholesterol-reducing properties [46–49]. However, patients with type 2 diabetes show ‘residual’ cardiovascular risk even when treated with statins [13, 46, 47, 48, 49]. This ‘residual’ cardiovascular

risk may be due to the fact that statins do not correct all lipid abnormalities associated with diabetic dyslipidaemia, such as hypertriacylglycerolaemia. We showed, in the present study, that rosuvastatin not only increases the catabolism of LDL, but also increases the catabolism of all triacylglycerol-rich lipoproteins (VLDL1, VLDL2 and IDL) and reduces the production of VLDL1. Conceivably, these additive effects of rosuvastatin on the metabolism of triacylglycerol-rich lipoproteins may be beneficial for the reduction of cardiovascular risk in patients with type 2 diabetes. A recent intravascular ultrasound trial (ASTEROID), performed in coronary patients including some with type 2 diabetes, has shown a significant regression of atherosclerosis in individuals treated with rosuvastatin [50] in accordance with the beneficial modification of the metabolism of ApoB-containing lipoproteins observed in our study.

Acknowledgements This study was supported by a grant from AstraZeneca. We are indebted to V. Jost for the preparation of [^{13}C] leucine, to C. Gibassier for dietary assistance, and to E. Niot and L. Princep for invaluable technical assistance. We thank R. Chadarevian for her help during the preparation of the study.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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