Influence of Atorvastatin on Apolipoprotein E and Al Kinetics in Patients with Type 2 Diabetes

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ABSTRACT
Atorvastatin reduces both plasma cholesterol and triglyceride concentrations in patients with type 2 diabetes, but mechanisms underlying triglyceride decrease and the effect of atorvastatin on high density lipoprotein (HDL) still remain unclear. Apolipoprotein (apo) E plays a crucial role in modulating production and clearance of triglyceride-rich very low density lipoprotein (VLDL). The main effect of apoAI is to modulate HDL metabolism. The aim of this work was to study the influence of atorvastatin on apoAI and apoE kinetics and to determine whether its hypocholesterolemic and hypotriglyceridemic effects could be related to changes in this apolipoprotein metabolism. Plasma VLDL-apoE, HDL-apoE, and HDL-apoAI were studied in seven patients with diabetes with mixed hyperlipidemia using a stable isotope labeling technique ([2H3]leucine-primed constant infusion) and monocompartmental model before and after 2 months of treatment with 40 mg/day of atorvastatin. Plasma apoE concentration was significantly reduced (44.1 ± 19.1 versus 32 ± 11.6 mg/l, p < 0.05) after treatment. This decrease was associated with a diminution of HDL-apoE concentration (17.46 ± 6.71 versus 13.37 ± 6.05 mg/l, p < 0.05) and production rate (0.202 ± 0.085 versus 0.119 ± 0.047 mg/kg/day, p < 0.05), whereas an increase in VLDL-apoE concentration (6.44 ± 2.16 before versus 9.23 ± 4.02 mg/l after, p < 0.05) and production rate (0.827 ± 0.367 versus 1.524 ± 0.664 mg/kg/day, p < 0.05) was observed. No significant difference was observed after treatment for apoAI parameters. We conclude that atorvastatin treatment promotes different apoE distribution between HDL and VLDL, favoring VLDL apoE content. The increased number of apoE per VLDL particle suggests that atorvastatin could enhance the direct catabolism of triglyceride-rich VLDL through apoE receptor pathways.

Atorvastatin, inhibitor of hydroxy-methyl-glutaryl coenzyme A reductase, is an efficient drug to reduce plasma total and low density lipoprotein (LDL) cholesterol concentrations (Alaupovic et al., 1997; Le et al., 2000) by increasing the uptake by LDL receptor (LDL-R) but also by inhibiting in vitro hepatic cholesterol synthesis up to 96% (Wileox et al., 1999). Besides its cholesterol-lowering effect, atorvastatin can also reduce triglyceride (TG) concentration up to 45% (Bakker-Arkema et al., 1996). This decrease was not only found in very low density lipoprotein (VLDL) but also in high density lipoprotein (HDL) fraction (Alaupovic et al., 1997). In regard to its important effect on both cholesterol and TG concentrations, atorvastatin is proposed not only for the treatment of primary hypercholesterolemia but also for combined dyslipidemias (Bakker-Arkema et al., 1996; Robins, 2003).

Although the lowering effect of atorvastatin on cholesterol is clearly understood, little is known about its role on TG. Some studies have hypothesized that atorvastatin could increase TG-rich VLDL uptake by the liver (Burnett et al., 1998; Le et al., 2000). Accordingly, some authors have shown that atorvastatin increases the VLDL-apolipoprotein (apo) B fractional catabolic rate (FCR) (Chan et al., 2002; Watts et al., 2003) but has no effect on its production. The apoE is present in both VLDL and HDL and plays a key role in their metabolism. Thus, apoE mediates the direct hepatic clearance of VLDL and their remnants via hepatic receptors (e.g., LDL-R, LDL-related receptor, and glycosami-
noglycans) (Mahley and Ji, 1999) and could be implicated in direct HDL hepatic catabolism through specific receptors (Mahley, 1988; Ji et al., 1997). Furthermore, in vitro experiments have shown that apoE is highly exchangeable between lipoproteins, being first transferred from HDL to TG-rich lipoproteins and again transferred to HDL after lipolysis by lipoprotein lipase and hepatic lipase (Gibson and Brown, 1988). Relative distribution of apoE between VLDL and HDL, related to lipolytic cascade, may then influence cholesterol and TG clearance.

Only one study has reported the effect of atorvastatin on apoE (Le et al., 2000), showing that simultaneously to TG reduction, apoE plasma concentrations were reduced by 37 or 49%, depending on the daily dose intake (20 mg or 80 mg/day, respectively). The decrease in apoE concentration was mainly related to a reduction of apoE in non-apoB-containing lipoproteins.

The influence of atorvastatin on apoAI and HDL fraction still remains controversial. Studies in HepG2 cells suggested that, as fibrates, atorvastatin could enhance transcription of apoAI mRNA by activating peroxisome proliferator-activated receptor-α (Martin et al., 2001). Some studies have shown increased plasma HDL-cholesterol concentration after atorvastatin treatment (Alapovice et al., 1997), whereas others have shown no effect (Bakker-Arkema et al., 1996; Le et al., 2000) or even a slight decrease with a high dose (Jones et al., 1998). Similarly, although some studies suggested a beneficial effect of atorvastatin on apoAI production (Schafer et al., 1999), enhancing transcription of apoAI mRNA by activating peroxisome proliferator-activated receptor-α (Martin et al., 2001), others suggested a dose-dependent effect on apoAI concentration (increase under low dose and decrease under higher dose) (Wierzbicki and Mikhailidisa, 2002). No effect on apoAI kinetics (Watts et al., 2003) and concentration (Asztalos et al., 2002), or even a decrease in apoAI concentration, associated with an enhanced catabolic rate in an animal model (Rashid et al., 2002), has been reported.

Because apoB, apoE, and apoAI play crucial roles on TG-rich lipoprotein metabolism, we conducted a kinetic study to analyze the effect of atorvastatin and to determine whether the hypocholesterolemic and the hypotriglyceridemic effect of this drug could be related to these apolipoprotein kinetics. This study was conducted in patients with type 2 diabetes because TG and apoAI are critical determinants of lipid abnormalities observed in these patients (Howard, 1994).

We have first shown that in patients with type 2 diabetes, although atorvastatin enhanced total apoB catabolic rate, no effect on VLDL apoB catabolic rate is obtained, whereas a decrease of its production rate (Ouguerram et al., 2003) is observed. Here, we present complementary and original results obtained on apoE and apoAI kinetics.

Materials and Methods

Subjects and Study Protocol

Kinetic studies of apoE and apoAI were performed in seven subjects with type 2 diabetes with mixed dyslipidemia (4 men, 3 women; age 47–65 years; mean, 57 years). Patients included in the protocol study had moderately elevated total plasma TG and cholesterol concentrations (169 mg/dl < TG < 670 mg/dl and 219 mg/dl < cholesterol < 321 mg/dl). None were engaged in any type of exercise program or were excessively sedentary. None were suffering from any infection as assessed by clinical examination, platelets, and leukocyte formulation or were taking any medication that could affect lipids for at least 2 months before the study. All the women were postmenopausal. None had proteinuria or hypothyroidism, and none were regularly cigarette smokers or alcohol consumers. They were not treated with insulin. A diettian instructed the study subjects to eat a weight-maintenance isocaloric diet composed of 50% of the usual daily caloric intake as carbohydrate, 35% as fat, and 15% as protein. During the inclusion period, patients received placebo and underwent two biological examinations. Patients underwent a basal kinetic study the day before the beginning of the treatment. They then received 40 mg of atorvastatin daily for 8 weeks. The second kinetic study was carried out at the end of treatment period. The experimental protocol was made according to the regulations of the U.S. Food and Drug Administration and was approved by the Ethical Committee of Nantes University Hospital (France). Informed consent was obtained before the study was started.

Kinetic studies of apoE (Bach-Ngouhou et al., 2002) and apoAI (Frénaisa et al., 1997) have been previously described. Briefly, endogenous labeling of these apolipoproteins was performed by administration of [1-5,5,5-2H3]leucine (99.8 atom % 2H3; Cambridge Isotope Laboratories, Andover, MA) dissolved in a 0.9% saline solution and tested for sterility and the absence of pyrogens before the study. All the subjects fasted overnight for 12 h before the study and remained fasting during the entire protocol. Each subject received i.v. a prime of 10 μmol/kg of tracer, immediately followed by a constant tracer infusion (10 μmol/kg/h) for 14 h. Venous blood samples were withdrawn in EDTA tubes (Venoject; Terumo Medical Corp., Somerset, NJ) at baseline, every 15 min during the first hour, every 30 min during the next 2 h, and then hourly until the end of the study. Plasma was immediately separated by centrifugation for 30 min at 4°C. Sodium azide, an inhibitor of bacterial growth, and Pefabloc SC (Roche Applied Science, Indianapolis, IN), a protease inhibitor, were added to blood samples at a final concentration of 1.5 and 0.5 mM, respectively.

Analytical Methods

Measurement, Isolation, and Preparation of Apolipoproteins. VLDL (d < 1.006 g/ml) and LDL (1.020 < d < 1.063) were isolated from 3 ml of plasma by a sequential ultracentrifugation using an angle rotor (RPS 40T; Kontron Instruments, Watford, Herts, UK) at 40,000 rpm for 24 h at 10°C (Centrikon T-2070; Kontron Instruments). HDL (1.063 < d < 1.210 g/ml) was isolated by a density gradient ultracentrifugation modified method (Chapman et al., 1981) using a swinging bucket rotor (RPS 55T; Kontron Instruments) at 40,000 rpm for 24 h at 10°C (Centrikon T 2060; Kontron Instruments). Cholesterol in plasma and lipoprotein fractions and TG concentrations were measured using commercially available kits (Roche Diagnostica, Mannheim, Germany) at three different sampling times: 3, 8, and 12 h. The apoAI concentrations were measured in plasma by immunonephelometry (Dade Behring, Inc., Deerfield, IL) at the same sampling times. Serum insulin concentrations were measured using commercial radioimmunossay kits (Bi-Inulin IRMA; Cibio International, Bagnols-sur-Cèze, France), and serum glucose concentration was assayed enzymatically using a multiparametric analyzer (Hitachi 747; Roche Molecular Biochemicals, Meylan, France). Total serum apoE, VLDL, and HDL-apoE concentrations were measured by electroimmunossay (Sebia Electrophoresis, Norcross, GA). The apoB concentrations were obtained in lipoprotein fractions using the isopropanol precipitation method (Eguasa et al., 1983). Leucine content in precipitated apoB100 from VLDL was determined by the use of norleucine as the internal standard (Beghin et al., 2000). A constant amount of norleucine (6 μmol) was added to precipitate apoB100 in VLDL, and the same procedure for hydrolysis, cation exchange chromatography, and derivatization was undertaken. In mass spectrometry, the ratio of leucine ion abundance to norleucine ion abundance was used to determine the quantity of leucine in the sample from a standard curve containing different
amounts of leucine (0–200 μmol) and a constant amount of norleucine (6 μmol). The corresponding amount of apoB100 was then calculated according to the known number of leucine molecules per apoB100 molecules.

The apoE phenotyping was determined on plasma delipidated with ethanol/ether (3:1, v/v) by preparative isoelectric focusing on 7.5% polyacrylamide-urea (8 M) gels (pH gradient 4–7) (Menzel and Utermann, 1986). Five of the seven diabetic subjects were phenotyped as E3/E3, one as E4/E3, and one as E3/E2.

VLDL and HDL-apoE were isolated by preparative gradient (4–19%), SDS-polyacrylamide gel electrophoresis. Because this method allows for the separation of total apoE, kinetic results in subjects with a heterozygous apoE phenotype will represent the weighted average of the kinetic behavior of individual isoforms in lipoprotein fractions. The apoAI was isolated by discontinuous 4.5-10% SDS-polyacrylamide gel electrophoresis gradient. Apolipoprotein bands were identified by immunoblotting using specific polyclonal antibodies (SERLIA-INSERM UR545, Institut Pasteur, Lille, France) and by comparing migration distances with known molecular weight standards (Rainbow Markers; Bio-Rad, Hercules, CA; and electrophoresis calibration kit; GE Healthcare, Little Chalfont, Buckinghamshire, UK). Apolipoprotein bands were excised from polyacrylamide gels and hydrolyzed with 1 ml of 4 N HCl (Sigma-Aldrich, St. Louis, MO) at 110°C for 24 h. Hydrolysates were then evaporated to dryness, and the amino acids were purified by cation exchange chromatography using a Dowex 50WX8 ion exchange resin (Sigma-Aldrich). Plasma amino acids were esterified with propanol/acetyl chloride and further derivatized using heptafluorobutyric anhydride acid (Fluka, Buchs, Switzerland) before analysis.

**Determination of Tracer-to-Tracee Ratios.** Chromatographic separations were carried out on a 30 m × 0.25 mm × 0.25 μm i.d. DB-5 capillary column (J & W Scientific, Folsom, CA). The column temperature program was as follows: initial temperature was held at 80°C, increased at 10°C/min to 170°C, and then increased at 40°C/min to a final temperature of 250°C. Electron-impact gas chromatography-mass spectrometry was performed on a 5890 series II gas chromatograph connected with a 5971A quadrupole mass spectrometer (Hewlett Packard, Palo Alto, CA). The isotopic ratio was determined by selected ion monitoring at m/z of 282 and 285. Calculations of apoE kinetic parameters were based on the tracer-to-tracee mass ratio (Foster et al., 1993).

**Modeling.** Kinetic analysis of tracer-to-tracee ratios was achieved by computer software for simulation, analysis, and modeling (SAAM II v 1.0.1; Resource Facility for Kinetic Analysis, Department of Bioengineering, SAAM Institute, Seattle, WA). VLDL-apoE, HDL-apoE, and HDL-apoAI data were kinetically analyzed using a monocompartmental approach (Frénais et al., 1997; Bach-Ngouhou et al., 2002): \( A(t) = \text{Ap}(1 - \exp(-kt - d)) \), where \( A(t) \) is the tracer-to-tracee ratio at time \( t \); Ap, the tracer-to-tracee ratio of the precursor pool of apoAI or apoE; \( d \), the delay between the beginning of the experiment and the appearance of tracer in the apolipoprotein; and \( k \), the fractional production rate (FPR) of the apolipoprotein (Fig. 1). We estimated the FPR, i.e., the proportion of apoE or apoAI entering the pool per unit time (day⁻¹), and the absolute production rate (APR), i.e., the amount of apoE entering the pool per unit time (mg/kg/day). APR was the product of FPR multiplied by the apoE mass, respectively in VLDL and in HDL or the apoAI mass in HDL.

The plateau enrichment of VLDL-apoE was used as its own precursor. For the estimation of HDL-apoE synthesis, we used the plateau of VLDL-apoE tracer-to-tracee ratio as precursor pool enrichment. The apoE pool was considered to be constant because no significant variation was observed between measurements made at three different infusion times (data not shown). Under these steady-state conditions, FPR equals FCR.

For apoAI, the VLDL-apoB100 tracer-to-tracee ratio at the end of the primed infusion, assumed to correspond to the tracer-to-tracee ratio of the leucine pool precursor, was used as an estimation of its precursor pool. This estimation was made on the assumption that the liver synthesizes apoB100 and the majority of apoAI (Ikewaki et al., 1993). This pool was considered to be constant, owing to the steady state observed on the plasma leucine tracer-to-tracee ratio (data not shown). Under these conditions, FPR equals FCR. VLDL-apoB100 and HDL-apoAI data were kinetically analyzed using a monoexponential function (Ikewaki et al., 1993): \( A(t) = \text{Ap}(1 - \exp(-kt - d)) \), where \( A(t) \) is the tracer-to-tracee ratio at time \( t \); Ap, the tracer-to-tracee ratio at the plateau of the VLDL apoB100 curve (representing the tracer-to-tracee ratio of the precursor pool); \( d \), the delay between the beginning of the experiment and the appearance of tracer in the apolipoprotein; and \( k \), the FPR of the apolipoprotein.

**Statistical Analysis**

Statistical analysis was performed with Statview F-4.5 (Abacus Concepts, Berkeley, CA). The Wilcoxon paired test was used to compare clinical and kinetic data in subjects with type 2 diabetes before and after atorvastatin treatment. Spearman rank correlation was done using Statview F-4.5 (Abacus Concepts) to look at relationships between variables. A two-tailed probability concentration of 0.05 was accepted as statistically significant.

**Results**

**Characteristics of Subjects Studied.** Clinical and biological characteristics of the subjects studied are presented in Table 1. Patients were moderately hypercholesterolemic as assessed by total cholesterol concentration ranging from 219 to 321 mg/dl and moderately to severely hypertriglycerideremic as assessed by total TG concentrations ranging from 169 to 670 mg/dl. After 8 weeks of treatment with 40 mg/day of atorvastatin, plasma total- and LDL-cholesterol concentrations were significantly reduced by 30% (p < 0.05) and 34% (p < 0.05), respectively, and LDL- and VLDL-apoB concentrations were significantly reduced by 37% (p < 0.05) and 28% (p < 0.05), respectively. Total plasma TG was reduced significantly by 26% (p < 0.05). HDL-cholesterol (Table 1) and HDL-apoAI (Table 2) increased, although not significantly. Plasma total apoE decreased significantly by -35% after atorvastatin treatment (p < 0.05). This decrease was associated with a significant decrease in HDL-apoE concentration by -23% and increase in VLDL-apoE concentration by +43% (Table 3).

![Fig. 1. Monocompartmental model for kinetic analysis of VLDL-apoE, HDL-apoE, and HDL-apoAI achieved by a computer software for simulation, analysis, and modeling (SAAM II v 1.0.1; Resource Facility for Kinetic Analysis, Department of Bioengineering, SAAM Institute, Seattle, WA): A(t) = Ap(1 - exp(-kt - d)), where A(t) is the tracer-to-tracee ratio at time t; Ap, the tracer-to-tracee ratio of the precursor pool of apoAI or apoE; d, the delay between the beginning of the experiment and the appearance of tracer in the apolipoprotein; and k, the FPR of the apolipoprotein.](image-url)
VLDL-apoE Concentrations and Kinetic Parameters. Within 30 min of infusion, the tracer-to-tracee ratios of plasma-free leucine reached steady state in all the study subjects. This was sustained throughout the entire infusion period (data not shown). Mean of tracer-to-tracee ratio curves of VLDL-apoE is shown in Fig. 2A. Tracer-to-tracee curve of VLDL-apoE increased rapidly during the infusion period in all the subjects.

Table 3 shows apoE concentrations and kinetic parameters in VLDL and HDL fractions. Plasma concentration and production rate of VLDL-apoE were significantly higher after treatment, whereas no significant difference was observed for the FCR of VLDL-apoE. The E/B-VLDL, representing the proportion of apoE relative to apoB content of VLDL, was significantly increased after treatment (0.36 ± 0.08 versus 0.78 ± 0.32, p = 0.02).

HDL-apoE Concentrations and Kinetic Parameters. The mean of tracer-to-tracee ratio curves of HDL-apoE is shown in Fig. 2B. Contrasting with VLDL-apoE enrichment, the tracer-to-tracee curve of HDL-apoE enrichment increased slowly in all the subjects, and the tracer-to-tracee ratio of HDL-apoE increased slightly later than before treatment.

Plasma concentration and production rate of HDL-apoE were significantly reduced after treatment, whereas the FCR of HDL-apoE was unchanged (Table 3). The E/A ratio, representing the proportion of apoE relative to apoAI, was significantly reduced after atorvastatin treatment (14.97 ± 8.32 versus 10.98 ± 5.50, p = 0.02).

The apoAI Concentrations and Kinetic Parameters. The apoAI plasma concentration and kinetic parameters are described in Table 2. The apoAI concentrations were not significantly different before and after atorvastatin treatment. The apoAI APR and FCR tended to be decreased, but these results were not significant.

Discussion This study is the first kinetic analysis of the effect of atorvastatin on apoE metabolism in type 2 diabetes. We have found that atorvastatin treatment of patients with type 2 diabetes leads to a decreased plasma total apoE concentration associated with an enhanced production rate of VLDL-apoE, whereas the production rate of HDL-apoE was reduced. This distribution favoring VLDL enrichment with apoE may enhance direct hepatic captation of TG-rich VLDL containing apoE through apoE receptor. This effect is complementary to the decreased VLDL production that we have previously shown (Ouguerram et al., 2003), both explaining the atorvastatin hypotriglyceridemic effect. No significant change was observed in HDL and apoAI.

We have found that atorvastatin treatment leads to reduced total apoE concentration. This decrease concerns mainly HDL-apoE, whereas VLDL-apoE is increased. Accordingly, Le et al. (2000) have also found that reduced apoE concentration under atorvastatin treatment was mainly because of apoE content of non-apoB-containing lipoproteins (HDL), whereas apoE concentration of apoB-containing lipoproteins was increased. Our study examined more deeply these data by analyzing kinetic apoE metabolism and showed that the increased plasma VLDL-apoE concentration was because of an enhancement of its production rate with no significant change of its catabolic rate.
The interpretation of these results is relatively difficult. One of the explanations could be that glycemic control and insulin resistance would be improved during our study. An improvement in carbohydrate metabolism could lead to changes in lipoprotein concentration and kinetics. As a matter of fact, results from nondiabetic subjects (Bach-Ngohou et al., 2002) indicated that the APR of VLDL-apoE was lower ($0.130 \pm 0.100$ mg/kg/day) in nondiabetic subjects than in our subjects with diabetes ($0.714 \pm 0.343$ mg/kg/day) before atorvastatin therapy. Moreover, homeostasis model assessment tended to be correlated with APR of VLDL-apoE ($r = 0.58, p = 0.06$) and is significantly correlated with FCR of VLDL-apoE ($r = 0.64, p < 0.05$). This argues in favor of a deleterious effect of insulin resistance on apoE kinetics. However, no modification in glycemic control (as assessed by glycated hemoglobin) or insulin resistance (as assessed by homeostasis model assessment and insulin concentration) was observed before and after atorvastatin treatment (data not shown). Therefore, it is unlikely that glycemic control and insulin resistance improvement explain our data.

Interestingly, for the same subjects, although we found that plasma VLDL-apoE concentration is increased associated with an enhancement of its production rate, we have previously shown that atorvastatin decreased VLDL-apoB concentration, associated with a decrease of its production rate (Ouguerram et al., 2003). These results look conflicting because apoE and apoB both reflect VLDL metabolism: for apoE, we found an enhanced apolipoprotein production rate when for apoB it was reduced. In fact, VLDL lipoproteins are heterogenous particles, each containing one apoB100 molecule but various other apolipoproteins (Kane et al., 1975). Among these apolipoproteins, apoE is associated with TG (Mahley, 1988) and is highly exchangeable between lipoproteins (Blum, 1982). Thus, studying VLDL-apoE and VLDL-apoB kinetics does not give the same information. VLDL-apoB reflects total VLDL particle kinetics, and VLDL-apoE reflects TG-rich VLDL particles because apoE is associated with TG metabolism. Therefore, whereas the decreased VLDL-apoB production, which we have previously shown, reflected faithfully a VLDL particle decrease (Ouguerram et al., 2003), the increased VLDL-apoE production described here reflects the enrichment of the subpopulation of VLDL particles containing apoE. Accordingly, we found a significant increase in the E/B-VLDL ratio after treatment.

### TABLE 3

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total apoE</th>
<th>VLDL-apoE</th>
<th>HDL-apoE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/l</td>
<td>APR mg/kg/day</td>
<td>FCR d^-1</td>
</tr>
<tr>
<td>Before treatment</td>
<td>79.0</td>
<td>6.27</td>
<td>0.450</td>
</tr>
<tr>
<td>2</td>
<td>52.0</td>
<td>5.17</td>
<td>0.890</td>
</tr>
<tr>
<td>3</td>
<td>33.0</td>
<td>9.00</td>
<td>0.640</td>
</tr>
<tr>
<td>4</td>
<td>28.0</td>
<td>4.24</td>
<td>0.680</td>
</tr>
<tr>
<td>5</td>
<td>37.0</td>
<td>4.95</td>
<td>0.437</td>
</tr>
<tr>
<td>6</td>
<td>55.0</td>
<td>9.81</td>
<td>1.23</td>
</tr>
<tr>
<td>7</td>
<td>25.0</td>
<td>5.92</td>
<td>1.400</td>
</tr>
<tr>
<td>After treatment</td>
<td>48.0</td>
<td>12.56</td>
<td>1.98</td>
</tr>
<tr>
<td>2</td>
<td>32.0</td>
<td>6.40</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>29.0</td>
<td>10.63</td>
<td>1.24</td>
</tr>
<tr>
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<td>4.29</td>
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</tr>
<tr>
<td>5</td>
<td>37.0</td>
<td>11.25</td>
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</tr>
<tr>
<td>6</td>
<td>42.0</td>
<td>12.10</td>
<td>2.62</td>
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<tr>
<td>7</td>
<td>14.0</td>
<td>13.96</td>
<td>1.70</td>
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</table>

Before treatment Mean 44.1* 5.74* 0.714* 3.436 17.81* 0.197* 0.302
S.D. 19.1 1.60 0.343 1.643 6.67 0.087 0.124
After treatment Mean 32.0 9.10 1.25 3.99 14.65 0.114 0.219
S.D. 11.6 3.93 0.52 1.64 5.90 0.048 0.089

* p < 0.05 was considered significant (Wilcoxon paired test).

![Fig. 2.](image-url) Experimental values of the tracer-to-tracee ratio for VLDL (A) and HDL (B) apoE in type 2 diabetes before (empty square) and after (full square) atorvastatin treatment. Fits (lines) were calculated using monocompartmental analysis during a prime constant infusion of deuterium-labeled leucine. Data are shown as mean ± S.E.M.
Enrichment with apoE may have two consequences for apoE-containing VLDL particles: 1) reduced lipolysis because of the inhibiting effect of apoE on LPL (Rensen and Van Berkel, 1996; Lambert et al., 2000; Tomiyasu et al., 2001), and 2) accelerated hepatic clearance probably through apoE receptor pathways (De Sylva et al., 1994; Tomiyasu et al., 2001). Thus, atorvastatin may induce both reduced lipolysis and enhanced direct hepatic catabolism of VLDL-apoE, leading finally to unchanged global VLDL-apoE FCR. According to our hypothesis, a reduced conversion of VLDL toward intermediate-density lipoprotein (Berglund et al., 1994) and an enhanced VLDL hepatic catabolism (Magot et al., 1991; Medhi et al., 1996) have also been observed after lovastatin and simvastatin treatment. Collectively, our results show that, for patients with type 2 diabetes, in addition to the reduced VLDL production shown previously (Ouguerram et al., 2003), atorvastatin could reduce plasma TG concentration by increasing direct hepatic uptake of TG-containing apoE-enriched VLDL particles.

We have also found that atorvastatin decreased HDL-apoE concentration and production rate, leading to a lower apoE content per HDL particle. This impoverishment, confirmed by the significantly reduced E/AI-HDL ratio, is consistent with the simultaneous enrichment of VLDL. As discussed previously, apoE VLDL enrichment may reduce VLDL lipolysis, decreasing then the lipolysis-associated apoE transfer to HDL and, thus, the HDL-apoE concentration. Furthermore, some recent studies have reported a significant reduction of cholesteryl ester transfer protein activity under atorvastatin treatment (Guerin et al., 2000; Le et al., 2000; Watts et al., 2003). Because this protein allows exchanges of TG from VLDL to HDL against cholesteryl ester, reduced cholesteryl ester transfer protein activity would lead to reduced transfer of TG and probably also of its associated apolipoprotein (apoE) from VLDL to HDL, explaining then partly the HDL-apoE decrease.

Concerning apoAI, contrasting with clinical trials suggesting a positive (Alaupovic et al., 1997) or a negative (Jones et al., 1998) effect of atorvastatin on apoAI concentration, we did not find any significant difference for apoAI concentration and kinetic parameters after atorvastatin treatment. Our results are consistent with those previously published by Watts et al. (2003), showing no effect of atorvastatin on apoAI kinetic parameters in mixed hyperlipidemic diabetic patients. However, Asztalos et al. (2002) have recently suggested, that even if concentration of total apoAI is not significantly increased, those of large HDL-rich cholesterol and large lipoprotein, responsible for the reverse transport of cholesterol toward the liver, are higher under atorvastatin treatment.

In summary, we have shown that atorvastatin treatment of patients with mixed dyslipidemic type 2 diabetes leads to a decreased plasma total apoE concentration associated with an enhanced VLDL-apoE production rate and a reduced HDL-apoE production rate. This distribution, favoring VLDL enrichment with apoE, suggests that in addition to the reduced VLDL production shown previously (Ouguerram et al., 2003), atorvastatin could also reduce plasma TG concentration by enhancing direct hepatic capture of TG-rich, VLDL-containing apoE.

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