Effect of Atorvastatin on Apolipoprotein B100 Containing Lipoprotein Metabolism in Type-2 Diabetes

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ABSTRACT

Seven hypertriglyceridemic patients with type-2 diabetes were treated with atorvastatin (40 mg/day) for 2 months. Kinetics of apolipoprotein B100 (apoB100)-containing lipoproteins were determined before and after atorvastatin treatment and compared with data obtained in five normolipidemic volunteers. ApoB100 metabolism was studied using stable isotopes and multicompartmental modeling. Compared with normolipidemic obese subjects, type-2 diabetic patients had a higher apoB100 concentration in very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and low-density lipoproteins (LDL) (P < 0.005). Kinetic analysis showed an increase in the total apoB100 production rate (P < 0.005) related to VLDL apoB100 overproduction (P < 0.005). Patients were also characterized by a lower fractional catabolic rate (FCR) in VLDL (not significant) or IDL (P < 0.005) mainly related to a decrease in VLDL and IDL delipidation rate (P < 0.005). Catabolism of LDL was also lower in diabetic patients (P < 0.05). Atorvastatin treatment significantly decreased plasma triglycerides (P < 0.05), total and LDL cholesterol (P < 0.05), apoB100 in LDL, IDL, and VLDL (P < 0.05). Treatment significantly decreased total apoB100 production rate (P < 0.05), but only for VLDL (P < 0.05). Treatment normalized FCR in IDL and LDL (P < 0.05). We concluded that atorvastatin improved lipid abnormalities in type-2 diabetic patients not only by increasing the clearance of apoB100-containing lipoproteins but also by decreasing VLDL production.

Atherosclerosis is the leading cause of morbidity and mortality in type-2 diabetic patients. Hypertriglyceridemia is an important lipid disorder associated with type-2 diabetes and may contribute to the development of atherosclerosis (Goldberg, 2001). Results of studies showing possible disturbances in LDL metabolism are inconsistent (Howard, 1987). Previous studies have underlined that the insulin resistance observed in type-2 diabetes is associated with an overproduction of apoB100 containing lipoproteins and reduced LDL and VLDL catabolism (Howard et al., 1987).

Atorvastatin is a strong competitive inhibitor of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. In patients with familial hypercholesterolemia and combined hyperlipidemia, the cholesterol-lowering effects are related to reduction of LDL cholesterol and are explained either by an increase in LDL receptor activity or by reduction of LDL apoB100 production with atorvastatin, as observed with other statins (Aguilar-Salinas et al., 1998). In addition to its cholesterol-lowering effect, atorvastatin also shows significant triglyceride-lowering properties in hypertriglyceridemic subjects (Lea and McTavish, 1997), but conflicting results were reported concerning its effect on VLDL metabolism. Various effects have already been reported on VLDL production with atorvastatin: inhibiting effect in animal (Burnett et al., 1997) or cell models (Wilcox et al., 1999), stimulating effect (Forster et al., 1996), and no effect (Forster et al., 2002) in combined hyperlipidemic subjects, and no effect in insulin resistant patients with no diabetes mellitus (Chan et al., 2002). It appears, however, that statins could have an effect on VLDL production in situations with high VLDL production (Aguilar-Salinas et al., 1998).

The aim of the present study was to evaluate metabolic changes induced by atorvastatin in patients with type-2 diabetes and marked hypertriglyceridemia. This study focuses on the apoB100-containing lipoproteins (VLDL, IDL, and LDL) in diabetic patients with combined dyslipidemia. We have studied apoB100 metabolism using endogenous labeling with deuterated leucine and multicompartmental modeling.

ABBREVIATIONS: LDL, low-density lipoprotein; ApoB100, apolipoprotein B100; VLDL, very low-density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; IDL, intermediate density lipoprotein; HOMA, homeostasis model assessment; FCR, fractional catabolic rate; VLDLR, very low-density lipoprotein remnant; LPL, lipoprotein lipase.
to analyze lipoprotein kinetics before and after atorvastatin treatment.

**Materials and Methods**

**Subjects**

Seven patients with type-2 diabetes and dyslipidemia (Table 1) were included in the study. Insulin treatment was an exclusion criterion. Patients were treated with either sulfonylureas or biguanides for at least 3 months, and no treatment change was allowed for the duration of the study. The insulin resistance was assessed by insulin sensitivity index (homeostasis model assessment; HOMA) (Matthews et al., 1985). Five normolipidemic obese subjects matched for age and body mass index with the diabetic patients were also studied as controls. The study subjects were instructed by a dietician to follow a weight-maintenance diet composed of 45% carbohydrate, 35% fat, and 20% protein for at least 1 week before study commencement. The experimental protocol was approved by the ethical committee of Nantes University Hospital, and written informed consent was obtained before the study was started.

**Experimental Protocol**

Diabetic patients were treated for 2 months with atorvastatin (40 mg/day). Kinetic studies of apoB100 metabolism were performed before and after treatment. The endogenous labeling of apoB100 was obtained before the study was started.

Each subject received intravenously a prime of 10 μmol·kg⁻¹·h⁻¹ of tracer immediately followed by a constant tracer infusion (10 μmol·kg⁻¹·h⁻¹) for 14 h. Venous blood samples were drawn into EDTA tubes (Venoject, Paris, France) at baseline and at 15, 30, 45 min, at 1, 1.5, 2, and 2.5 h, and then hourly until 14 h. Sodium azide, an inhibitor of bacterial growth, and Pefabloc SC (Interchim, Montluçon, France), a protease inhibitor, were added to blood samples at a final concentration of 1.5 and 0.5 mM, respectively.

**Analytical Procedures**

**Isolation and Measurement of Enrichment of Lipoprotein Containing apoB100.** VLDL1 (Svedbery flotation rate 400–600) and VLDL2 (Svedbery flotation rate 60–20) were separated by density gradient ultracentrifugation (Gaw et al., 1992) using a swinging bucket rotor at 40,000 revolutions/min for 24 h at 10°C (RPS 40T; Hitachi, Hialeah, FL). IDL (1.006 < density < 1.025 g/ml) and LDL (1.025 < density < 1.063 g/ml) were separated by standard sequential ultracentrifugation methods (Havel et al., 1955) using a fixed-angle rotor at 40,000 revolutions/min for 22 h at 10°C (CP70; Hitachi).

Isolation and measurement of leucine enrichment in apoB100 have been described previously (Maugéas et al., 2001). Briefly, apoB100 in lipoproteins was isolated by SDS-polyacrylamide gel electrophoresis. Apolipoprotein bands were dried under a vacuum and then hydrolyzed. The amino acids were purified by cation exchange chromatography, then esterified, and derivatized. Electron-impact gas chromatography-mass spectrometry was performed on a 5891A gas chromatograph connected with a 5971A quadrupole mass spectrometer (Hewlett-Packard Co., Palo Alto, CA). The isotopic ratio was determined by selected ion monitoring at m/z of 282 and 285. Calculations of apoB100 kinetic parameters were based on the tracer-to-tracee mass ratio (Cobelli et al., 1992).

**Measurements of Lipids and apoB100.** Cholesterol and triglyceride levels were measured using commercially available enzymatic kits (Boehringer Mannheim GmbH, Mannheim, Germany) at three different sampling times. The apolipoprotein AI concentration in plasma was measured by immuno nephelometry (Behring, Rueil Malmaison, France) at the same sampling times. ApoB100 concentrations were obtained in lipoprotein fractions by combining selective precipitation and mass spectrometry (Beghin et al., 2000). The percentage recovery of cholesterol, triglycerides, and apolipoprotein B100 after centrifugation was higher than 85%.

**Modeling.** Kinetic analysis of tracer-to-tracee ratios was achieved using computer software for simulation, analysis and modeling (Bar-
Statistical Analysis

Results are reported as the mean ± standard deviation. The Mann-Whitney test, performed with Statview F-4.5 (Abacus Concept, Berkeley, CA) was used. A two-tailed probability level of 0.05 or less was accepted as statistically significant.

Results

Lipoproteins, Lipids, and Apolipoproteins. Concentrations of lipids and apoB100 in plasma and in lipoprotein fractions are shown in Tables 1 and 2 for control subjects and diabetic patients before and after atorvastatin treatment. Patients before treatment compared with controls showed hypertriglyceridemia (340 ± 167 mg/dl, P < 0.05) and hypercholesterolemia (269.7 ± 31 mg/dl, P < 0.05). These diabetic patients showed higher total apoB100 concentration than controls (P < 0.005). This difference was explained by a higher concentration in the three studied lipoproteins. In VLDL, higher (P < 0.005) apoB100 content in diabetic patients was related to an increased content in both VLDL1 (P < 0.05) and VLDL2 (P < 0.05) apoB100 of the different lipoprotein fractions (Maugeais et al., 2001).

For comparison between the three groups (controls and diabetics subjects before and after treatment) the VLDL1, VLDL2, and VLDLR data were presented as VLDL dilipidation rate and VLDL fractional catabolic rate (FCR), which represents the sum of dilipidation and direct removal rate. The VLDL dilipidation rate was calculated as VLDL2 delipidation flux divided by total VLDL mass. The VLDL direct removal was calculated as flux of VLDL1, VLDL2, and VLDLR direct removal divided by total VLDL mass.

As all our patients are obese, pools of apoB100 in plasma or in VLDL, IDL, and LDL were calculated by multiplying the apoB100 concentration by 0.037 (U/kg), assuming a plasma volume of 3.7% of body weight (Dagher et al., 1986). The apoB100 production rate in milligrams per kilogram per hour represents the product of FCR and pool size of apoB100 in lipoprotein fractions.

Lipoprotein apolipoprotein B100 concentration (milligrams per liter) in controls (C) and diabetics before (diabetics I) and after (diabetics II) atorvastatin treatment.

### TABLE 2

<table>
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<tr>
<th></th>
<th>B-VLDL</th>
<th>B-VLDL1</th>
<th>B-VLDL2</th>
<th>B-IDL</th>
<th>B-LDL</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>D1</td>
<td>243.2</td>
<td>145.1</td>
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<td>51.2</td>
<td>699.8</td>
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<tr>
<td>D2</td>
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<td>93.3</td>
<td>83.0</td>
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* Comparison between diabetics before and after treatment (*P < 0.05).

### Fig. 1

Model of apolipoprotein B100-containing lipoprotein metabolism. Details are described under Materials and Methods.
At baseline a dramatic increase of total apoB100 production was observed in type-2 diabetic patients compared with controls (240% of controls, \(P < 0.005\) (Table 3). This was related to a higher apoB100-VLDL production (\(P < 0.005\)), with no change for other lipoproteins. VLDL FCR was lower (67% of the controls but not significantly) related to decreased delipidation rate (one-third of the controls, \(P < 0.005\)). IDL FCR was also decreased (\(P < 0.005\)) related to a lower delipidation rate (\(P < 0.05\)). In contrast to low LDL apoB100 FCR (57% of the controls, \(P < 0.05\)), the diabetic patients had no consistent perturbations in LDL-apoB100 production compared with controls (increased in two subjects, with no change in two subjects, and decreased in three subjects).

Treatment with atorvastatin significantly reduced overall production of apoB100 by 20% (0.91 ± 0.41 versus 1.17 ± 0.59 mg/kg/h, \(P < 0.05\)) (Table 3). This decrease was related to VLDL-apoB100 production (\(P < 0.05\)), with no significant change in direct production for IDL and LDL. The most dramatic change induced by atorvastatin treatment was the normalization of IDL and LDL FCR (2-fold increase compared with baseline, \(P < 0.05\)) with no change of VLDL FCR or delipidation rate. The increase of IDL FCR was related to significant increase of delipidation rate to LDL (\(P < 0.05\)).

**Discussion**

This study is the first kinetic analysis of effect of atorvastatin on apoB100 metabolism in type-2 diabetes. Analyses were performed using stable isotopes taking into account heterogeneity of VLDL and comparisons were made to matched controls. The model used in this study is consistent as shown by a low coefficient of variation for most of the individual parameters. The principal conclusion of this study is that in type-2 diabetes atorvastatin lowered triglyceridemia by lowering apoB100 VLDL production and cholesterolemia essentially by stimulating LDL uptake.

**Effect of Diabetes on apoB100 Metabolism.** Studied type-2 diabetic patients showed hypertriglyceridemia related to an increased VLDL concentration (both VLDL1 and VLDL2). This was linked to a higher VLDL production rate in all the patients and lower delipidation rate of VLDL resulting in a low total FCR of VLDL in most of the subjects. Previous kinetic studies have shown increased VLDL production rate in type-2 diabetes (Kissebah et al., 1982; Cummings et al., 1995). In rat hepatocytes, insulin inhibits apoB100 secretion by stimulating degradation of newly synthesized apoB100 (Sparks and Sparks, 1990). Then, hepatic VLDL-apoB100 overproduction in type-2 diabetes could result from impaired action of insulin. In animal models, resistance to insulin action increased intracellular stability of nascent apoB100 and enhanced expression of microsomal triglyceride transfer protein, which facilitated the assembly and secretion of apoB100-containing lipoprotein particles (Taghibiglou et al., 2000).

The observed decrease in VLDL delipidation rate compared with controls results in normal (Cummings et al., 1995) or lower (Kissebah et al., 1982) FCR of VLDL. The decrease of VLDL delipidation rate reported in type-2 diabetics (Taskinen et al., 1990) is related to insulin resistance (Eckel, 1989). Another possible cause could be the physical changes of VLDL making these lipoproteins a poor substrate for LPL (lipoprotein lipase) (Sahedi et al., 1993).

Hypercholesterolemia was related to a high concentration of LDL in all the patients. This high concentration of LDL apoB100 was induced by a low FCR of LDL in most of the patients compared with controls. This decrease of LDL FCR already reported (Howard et al., 1987) could be explained by an insulin stimulating effect on LDL receptor activity (Taskinen et al., 1990) and a glycosylation of LDL particles that can alter binding to these receptors (Hiramatsu et al., 1985).

In type-2 diabetic patients, compared with controls, VLDL was less channeled toward conversion into LDL than direct removal (data not shown), as previously reported in other studies (Howard et al., 1987; Taskinen et al., 1990). This is related to the decreased VLDL delipidation rate discussed above. Such a situation could explain the lack of high concentrations of LDL frequently observed (Howard et al., 1987) in spite of low LDL catabolism. Both perturbations with contradictory effects on LDL concentrations could explain the inconsistent increase of LDL amount reported in type-2 diabetes.
Effect of Atorvastatin on apoB100 Metabolism in Diabetic Subjects. Atorvastatin treatment lowered VLDL total concentration by lowering VLDL production rate with no change in FCR. The low VLDL delipidation rate did not increase with treatment. This process, catalyzed by LPL (Olivecrona and Olivecrona, 1995), appears to be associated to insulin resistance, which was not improved by the treatment as seen by the HOMA index, which did not change after treatment.

Atorvastatin decreased the production rate of VLDL apoB100 in spite of insulin resistance. The production rate of VLDL is controlled by liver cholesterol availability and particularly from de novo synthesis (Watts et al., 1995) through possible effect on translocation, intracellular apoB100 degradation, ACAT activity, and microsomal triglyceride transfer protein activity (Huff and Burnett, 1997). Statins through their effects on HMG-CoA reductase inhibition decrease the intrahepatic free cholesterol concentration. It has been previously reported in cellular models that atorvastatin, but not simvastatin, decreased the production of VLDL apoB100 (Wilcox et al., 1999). This effect could be only observed for the most potent statins because it was shown in animals that the magnitude of decrease in hepatic VLDL apoB100 secretion is determined by the extent of HMG-CoA reductase inhibition (Burnett et al., 1999). The mechanisms possibly involved a defect of translocation of apoB100 into the lumen of the endoplasmic reticulum, increasing by this way the amount of apoB100 degraded within the cell (Mohammadi et al., 1998). Atorvastatin could decrease cholesterol synthesis and availability of cholesterol and cholesterol ester for the normal assembly of apoB100 containing lipoprotein particles. Using lovastatin (Arad et al., 1990; Cuchel et al., 1997) and pravastatin (Vega et al., 1990), it was shown a decrease in VLDL production. Nevertheless, it has recently been reported no atorvastatin effect on VLDL production rate in moderate combined hyperlipidemic subjects (Forster et al., 2002) and insulin resistant patients with no diabetes and less marked VLDL overproduction (Chan et al., 2002). These apparently conflicting results could come from studying patients with different metabolic profiles and specially different level of VLDL production (Illingworth, 1991; Aguilar-Salinas et al., 1997).

Opposite results were found for effect of treatment on delipidation rate of IDL and VLDL. Delipidation process of IDL is catalyzed by hepatic lipase (Olivecrona and Olivecrona, 1995) and was normalized by treatment. As discussed above, activity of lipoprotein lipase appears to be modulated by insulin resistance activity but hepatic lipase could be independent of insulin action and essentially modulated by changes of IDL composition. Whatever the mechanisms of the contradictory effects of atorvastatin on delipidation process, our results suggest that delipidation rates of VLDL and IDL are not regulated by the similar pathways, as previously reported (Peinado-Onsurbe et al., 1992).

The main change induced by the treatment concerned LDL. Treatment with a statin is reported to decrease LDL production rate and/or to increase LDL catabolism. For example, in combined hyperlipidemia (Cuchel et al., 1997), lovastatin decreased LDL production rate without change of catabolism. In another form of familial combined hyperlipidemia (Aguilar-Salinas et al., 1997), pravastatin increased LDL FCR with no change of production rate. The same result
was reported in normolipidemic volunteers during simvastatin treatment (Malmendier et al., 1989). This inconsistency could result from the dose used (and potency of the statin used) and from the studied pathological situation (Aguilar-Salinas et al., 1998). In the present study, atorvastatin normalized LDL concentration by normalizing FCR through stimulating LDL receptor activity, as previously demonstrated with simvastatin (Malmendier et al., 1989).

In conclusion, our kinetic data demonstrated the various effects of atorvastatin on dyslipidemia in type-2 diabetes and underlined the usefulness of this treatment even in case of increase of triglycerides and moderate increase of LDL cholesterol.

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