Cerivastatin Improves Insulin Sensitivity and Insulin Secretion in Early-State Obese Type 2 Diabetes

J.A. Paniagua,[1] J. López-Miranda,[1] A. Escribano,[3] F.J. Berral,[3] C. Marín,[1] D. Bravo,[1] E. Paz-Rojas,[1] P. Gómez,[1] M. Barcos,[2] J.A. Moreno,[1] and F. Pérez-Jiménez[1]

In a double-blind, placebo-controlled, randomized crossover study, 15 stable mild hyperglycemic patients without treatment and with features of metabolic syndrome were treated with cerivastatin (0.4 mg/day) or placebo for 3 months. The insulin sensitivity index during the euglycemic-hyperinsulinemic clamp (EHC; 5.4 mmol/l; 80 mU · m^{-2} · min^{-1}) was increased by cerivastatin treatment (66.39 ± 3.9 nmol · lean body mass $[LBM]^{-1}$ · min^{-1} · $pmol^{-1}$ · l^{-1}) as compared with placebo (58.37 ± 3.69 nmol · LBM^{-1} · min^{-1} · $pmol^{-1}$ · l^{-1}; $P < 0.01$) by 13.7%. Glucose oxidation during EHC was significantly higher with statin treatment (16.1 ± 1.37 μmol · LBM^{-1} · min^{-1}) as compared with placebo (14.58 ± 1.48 μmol · LBM^{-1} · min^{-1}; $P < 0.05$). During hyperinsulinemia (~800 pmol/l) in EHC steady-state, lipid oxidation was significantly decreased and respiratory quotient was significantly increased with statin treatment (0.33 ± 0.05 mg · LBM^{-1} · min^{-1}, 0.94 ± 0.01) as compared with placebo (0.48 ± 0.06 mg · LBM^{-1} · min^{-1}, 0.91 ± 0.01; $P < 0.01$ and $P < 0.05$, respectively). During statin treatment, the first-phase insulin response increased from 2.07 ± 0.28 to 2.82 ± 0.38 pmol · l^{-1} · $pmol^{-1}$ ($P < 0.05$). The second phase of insulin responses examined by C-peptide and insulin levels averaged during the hyperglycemic clamp (20 mmol/l) was unchanged. In conclusion, this study demonstrates that 0.4 mg cerivastatin therapy improves first-phase insulin secretion and increases insulin-mediated glucose uptake and respiratory quotient in the early state of obese type 2 diabetes. *Diabetes* 51:2596–2603, 2002

Type 2 diabetes represents the final stage of a progressive syndrome characterized by target-tissue resistance to insulin that cannot be overcome by β-cell hypersecretion (1). In the initial period, some subjects accumulate a constellation of major risk factors such as central (intra-abdominal) obesity, hypertriglyceridemia, low HDL cholesterol, raised blood pressure, and insulin resistance associated with proinflammatory states (2). In this state, patients receive primary care from their physicians with a recommendation for lifestyle changes (3). However, most people do not adequately maintain weight loss after participating in weight-control programs (4), and some subjects develop late micro- and macrovascular complications of diabetes. Approaches to reduce coronary heart disease have been focused on statins therapy (5,6). Several pleiotropic effects of statins could represent a potential means for controlling multifactorial atherosclerosis observed in diabetes. A post hoc analysis in the West of Scotland Coronary Prevention Study database provided evidence for the protective treatment effect of Pravastatin on the development of diabetes (7). However, whether statin treatment is beneficial in glucose metabolism of diabetes still remains to be seen. Suppression with high doses of statins on VLDL production of patients with type 2 diabetic hypertriglyceridemia could effect the release of fatty acids (FAs) from the liver (8). It is now recognized that plasma FA concentrations have profound effects on insulin action and glucose metabolism (9,10). Finally, cholesterol is a key component in the regulation of signal transduction through membrane lipid-ordered microdomains and in the regulation of gene expression through cholesterol-activated transcription factors (11). Intracellular cholesterol might serve as a link between fat cell size, glucose metabolism, and adipocyte metabolic activity (12). Sterol regulatory element-binding protein is implicated as a major mediator of insulin action (13). The major aim of the present study was to determine the effects of statin treatment on the action and secretion ability of insulin in patients in the initial state of mild hyperglycemic obese type 2 diabetes.

From the [1]Lipid and Atherosclerosis Unit, University Hospital Reina Sofía, Cordoba, Spain; the [2]Biochemical Laboratory, University Hospital Reina Sofía, Cordoba, Spain; and the [3]School of Medicine, University of Córdoba, Córdoba, Spain.

Address correspondence and reprint requests to Francisco Pérez-Jiménez, Unidad de Lípidos y Arteriosclerosis, Hospital Universitario Reina Sofía, Avda Menéndez Pidal, s/n 14004, Córdoba, Spain. E-mail: fperez@sofia.hrs.sas.cica.es.

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CRP, C-reactive protein; EHC, euglycemic-hyperinsulinemic clamp; FA, fatty acid; FFA, free fatty acid; HC, hyperglycemic clamp; IL, interleukin; IVGTT, intravenous glucose tolerance test; LBM, lean body mass; MCR, metabolic clearance rate; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; Rq, respiratory quotient; TG, triglyceride; TNF-α, tumor necrosis factor α.

RESEARCH DESIGN AND METHODS

Patients. The study included 15 patients with type 2 diabetes diagnosed by repeated fasting glucose at time of presentation according to new World Health Organization criteria (i.e., a fasting plasma glucose concentration >7.0 mmol/l) (14). All patients had received standard treatment in an established primary diabetes care program and were on diets, and none of them had received any medication known to affect glucose metabolism. The purpose and risks of the study were carefully explained before patients gave informed consent to participate. The study protocol was reviewed and approved by the Ethics Committee of the Reina Sofía University Hospital of Cordoba. Inclusion criteria were as follows: all subjects were >35 and <70 years of age, had a history of 1–3 years of moderate hyperglycemia with fasting glycemia >110 mg/dl and <140 mg/dl and HbA_{1c} <7% without treatment, had a BMI (calculated as weight [in kilograms] divided by height [in square meters]) >27 kg/m^2 (15) (this index was not modified for at least 6 months before the study), and had a waist circumference of ≥102 cm and ≥88 cm in men and

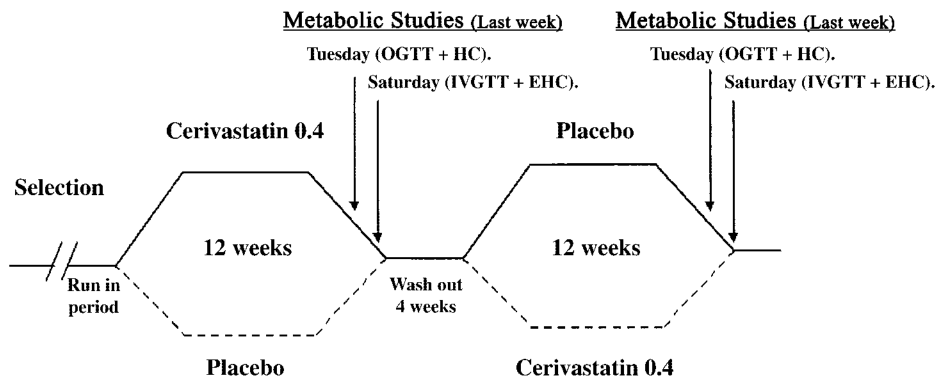

FIG. 1. Each subject was followed for a run-in period of 4 weeks before being chosen at random for daily administration of either cerivastatin (0.4 mg/d) or a placebo with the appearance of an alternative drug. Each treatment period lasted 3 months. There was a washout period of 4 weeks between placebo and cerivastatin. In the last week of each treatment period, on Tuesday an OGTT + HC was performed, and on Saturday an IVGTT + EHC was performed.

women, respectively (16,17). All patients presented with serum triglyceride (TG) levels of >150 mg/dl and <350 mg/dl. Exclusion criteria were as follows: signs of diabetic retinopathy, nephropathy, neuropathy, or macrovascular complications; cigarette smoking and alcohol consumption; and use of diuretics, steroids, and β-blockers. All subjects had normal results on screening blood tests of hepatic, renal, thyroid, and adrenal function. Upon evaluation, two female patients presented with elevated levels in fasting (8:00 A.M.) plasma cortisol. After a low-dose dexamethasone suppression test, both patients presented fasting (8:00 A.M.) plasma cortisol values of <140 nmol/l. Hypertension in five patients was treated with Enalapril 10 (3) or 20 mg/day (2).

Study design. The trial was performed as a single-center, double-blind, randomized crossover, placebo-controlled study (Fig. 1). Each subject was followed for a run-in period of 4 weeks before being chosen at random for daily administration of either cerivastatin (0.4 mg/d) or a placebo with the appearance of an alternative drug. Both active tablets and placebo tablets were supplied by Bayer (Leverkusen, Germany). Each treatment period lasted 3 months. There was a washout period of 4 weeks between placebo and cerivastatin. In the last week of each treatment period, on Tuesday an oral glucose tolerance test (OGTT) + hyperglycemic clamp (HC) was performed, and on Saturday an intravenous glucose tolerance test (IVGTT) + euglycemic-hyperinsulinemic clamp (EHC) was performed.

Metabolic studies. The subjects reported to the metabolic unit at 08:00 A.M. On Tuesday, after an overnight fast of 12 h, an OGTT with 75 g of glucose dissolved in 300 ml of water was performed. Samples were obtained at 0, 15, 30, 45, 60, 90, and 120 min. An HC was started immediately afterward. Glucose was elevated and clamped to 360 mg/dl glucose (20 mmol/l), with adjustment of the rate of infusion of a 20% glucose solution at 10-min intervals with a precision syringe pump (LifeCare Pump Model 4; Abbot Laboratories, Chicago, IL). Samples were obtained at 150, 165, and 180 min (30, 45, and 60 min of infusion). In both tests, plasma insulin, C-peptide, and glucose concentrations were determined. On Saturday, after an overnight fast of 12 h, an IVGTT was performed (18). A 300-mg/kg body wt glucose load (maximum 25 g) was briefly given intravenously over 2 min as a 50% solution. Blood samples for glucose and insulin assay were collected at −5, 0, 2, 4, 6, 8, and 10 min. For measuring the action of insulin, an EHC was performed immediately (19). A priming dose of insulin (0.1 units/kg) was followed by constant intravenous insulin infusion at a rate of 80 mU $\cdot$ m^{-2} $\cdot$ min^{-1} using a precision syringe LifeCare pump and continued throughout the 180-min study. Glucose was determined at 5-min intervals. Plasma glucose was clamped to 100 mg/dl glucose (5.6 mmol/l), with adjustment of the rate of infusion of the 20% glucose solution at 10-min intervals with a precision syringe LifeCare pump. The desired plasma glucose level was attained within 60 min. Blood samples for glucose, insulin, C-peptide, plasma lactate, and serum free fatty acid (FFA) were collected at fasting (−5) and 120, 140, 160, and 180 min. The average value for the period from 140 to 180 was used to calculate the rates of whole-body glucose uptake.

Anthropometry and body composition. Stature and weight were measured. The waist circumference of each subject was measured at the umbilicus, and the widest hip circumference was measured at the gluteal fold. Bioelectrical impedance was measured with a multifrequency bioelectrical impedance analyzer in fasting state with empty bladders. This method estimates lean body mass (LBM) in kilograms. Body fat mass was determined by subtracting LBM from body weight (20).

Indirect calorimetry. The energy production and respiratory quotient (Rq) were measured by continuous indirect calorimetry using a computerized, flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland). Energy expenditure was measured after an equilibration period of 10 min. The gas-exchange rate was recorded for 30 min in the fasting state and during the last 30 min of the euglycemic clamp, between 150 and 180 min. From knowledge of the V_{O_2} and VCO_2 (l/min) and the urinary nitrogen (g/min), glucose, lipid, and protein disappearance rates (g/min) can be calculated according to calorimetric formulas (21). The protein oxidation rate was estimated from urinary nitrogen excretion (1 g of nitrogen = 6.25 g of protein) in 24-h urine collections from the same day and over the clamp studies (22). The energy expenditure data in minutes were adjusted for differences by LBM. Nonoxidative glucose metabolism was determined by subtracting the glucose disappearance (oxidation) rate, measured by indirect calorimetry, from the whole insulin-mediated glucose intake during the EHC.

Analytical procedures. Plasma glucose were measured using a Hitachi 917 analyzer by the glucose oxidase method (GOD-PAP; Boehringer, Mannheim, Germany). Plasma insulin concentrations were measured by microparticle enzyme immunoassay (Abbott Diagnostics, Matsudo-shi, Japan; lower limit 1 μU insulin/ml; total assay variation 6.1%) using an AxSYM system Analyzer (Abbott Laboratories, Dallas, TX). Plasma C-peptide content was measured by RIA (BYK Sangtec, Dietzenbach, Germany; lower limit 0.003 ng/ml). Total glycosylated hemoglobin was determined in fresh samples by high-performance liquid chromatography (Bio-Rad, Paris, France; normal range 4.5–6.4%; interassay coefficient of variation 3.92%) using the fully automated Glycosylated Hemoglobin Variant II Analyzer (Bio-Rad, Tokyo). A Hitachi P-800 analyzer (Roche Diagnostics, Tokyo, Japan) was used to measure fructosamine by an enzymatic colorimetric method (Roche, Mannheim, Germany; intra-assay coefficient of variation 4.3%). Automated methods were used for measurement of cholesterol and TGs (CHOD-PAP and GPO-PAP, respectively; Boehringer). Plasma HDL cholesterol was determined by a dextran sulfate-magnesium precipitation procedure. FFA (nonesterified fatty acids [NEFAs]) concentration was measured in triplicate in each sample using an enzymatic colorimetric assay (Roche Molecular Biochemical, Mannheim, Germany; total coefficient of variation 7.8%). An enzymatic colorimetric lactate oxidase method (Roche Diagnostics; normal range 0.5–2.2 mmol/l; interassay coefficient of variation 2.3%) was used to determine plasma lactate by a Cobas Integra 400 Analyzer. The high-sensitivity C-reactive protein (CRP) was measured using immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE). A Quantikine HS Immunoassay kit (R & D Systems, Minneapolis, MN; intra-assay coefficient of variation 14%) was used to determine human tumor necrosis factor α (TNF-α) in serum. An enzyme-linked immunosorbent assay was used for the quantitative measurement of leptin in serum (DRG Diagnostics, Germany; intra-assay coefficient of variation 4.28%).

Calculations and statistical analysis. The acute insulin response (first phase) to the glucose pulse was considered to be the mean of the postglucose insulin concentrations from 4, 6, 8, and 10 min (IVGTT). The insulin index was defined as the ratio between peak and basal plasma insulin values. The ratio $\Delta I_{30-0}/\Delta G_{30-0}$, increment of plasma insulin (pmol/l) to increment in glucose (mmol/l), was also used as a measure of early insulin secretion during OGTT. Maximal insulin and C-peptide secretion (second phase) was assessed as the average plasma insulin and C-peptide concentrations at 150, 165, and 180 min of the HC. The incremental areas under the curves for insulin and glucose during 2-h OGTT were calculated as the deviations from the basal value using a trapezoidal method. Insulin sensitivity was measured as an insulin sensitivity index, calculated by dividing the average glucose infusion rate (nanomoles per LBM per minute) by the average plasma insulin concentration (picomoles per liter) at 140, 160, and 180 min of the EHC. Kinetics of peripheral insulin metabolism were evaluated by measurement of metabolic clearance rate (MCR) of exogenously administered insulin during the EHC. MCR (ml $\cdot$ m^{-2} $\cdot$ min^{-1}) = IIR (μUI $\cdot$ m^{-2} $\cdot$ min^{-1})/[IC_s − IC_e (μU/ml)], where IIR = insulin infusion rate during the EHC; IC_s = steady state of mean insulin concentration during the EHC (140, 160, and 180 min); IC_e = endogenous steady-state insulin

TABLE 1
Clinical characteristics of subjects

	At evaluation	Placebo	Cerivastatin 0.4 mg	*P*
Sex	8 men, 7 women			
Age (years)	57 ± 7			
Duration of diabetes (months)	25 ± 12			
BMI (kg/m^2)	32.3 ± 1.1	31.8 ± 1	32.1 ± 1	NS
Treatment of hypertension	5 yes, 10 no			
Blood pressure (mmHg)				
Systolic	145 ± 5.8	146 ± 5.6	149 ± 5.8	NS
Diastolic	86 ± 1.8	83 ± 1.9	84 ± 3.7	NS
Mean*	105 ± 2.7	104 ± 2.9	105 ± 3.2	NS
Waist circumference (cm; men/women)	116.1/98.3	114.3/96.8	114.8/95.3	NS
Hip circumference (cm; men/women)	112.5/106.5	110.4/104.2	111.6/103.8	NS
Waist-to-hip ratio (men/women)	1.03/0.92	1.03/0.92	1.03/0.92	NS
Total fat mass (kg)		31.8 ± 1.66	32.1 ± 1.79	NS
LBM (kg)		50.4 ± 3	51.1 ± 2.63	NS

Data are mean ± SE. *Mean blood pressure = (systolic − diastolic)/3 + diastolic.

concentration assessed from the mean of two fasting insulin concentration per (steady-state C-peptide levels during the EHC/mean of two fasting C-peptide levels) (23). Plasma C-peptide/plasma insulin ratio was considered to be the index of hepatic insulin extraction in the fasting state and during the HC (mean values at 150, 165, and 180 min).

To compare the two study periods and to assess the effect of the sequence in which the patients received the placebo and cerivastatin treatment, we used repeated-measures ANOVA, followed by paired *t* test analyses (24). For variables with a skewed distribution, logarithmic transformation was performed. Results are presented as the mean ± SE. Covariance analysis with change in body weight as a covariant was performed to separate the effects of treatment from the effects of changes in body weight.

RESULTS

Clinical and biochemical characteristics of the subjects. The mean (±SD) age of patients was 57 ± 7 years (range 38–68). The mean period observed for moderate fasting hyperglycemia was 25 ± 12 months (Table 1). At baseline, the patients presented with a mean BMI of 32.3 ± 1.1 kg/m^2 and a central body fat distribution. LBM and calculated body fat mass were similar during placebo (50.4 ± 3; 31.8 ± 1.7) as compared with cerivastatin treatment (51.1 ± 2.6; 32.1 ± 1.8), respectively. The weight, BMI, LBM, and waist-to-hip ratio did not change throughout the study. Mean arterial blood pressure remained unchanged during the study. The mean HbA_{1c} level was 6.4 ± 0.13% at randomization with no significant differences observed between placebo and cerivastatin treatment (6.7 ± 0.15% and 6.6 ± 0.2%; $P = 0.8$ [$r = 0.63$, $P = 0.012$]; Table 2). Plasma fructosamine concentrations measured in a single assay were also similar (271 ± 15 vs. 270 ± 14 μmol/l; $P = 0.9$ [$r = 0.88$, $P = 0.000$]). The mean serum cholesterol concentration at admission was 5.64 ± 0.2 mmol/l. Compared with the baseline and placebo period, cerivastatin treatment resulted in a decrease in fasting plasma total cholesterol concentration by 25 and 19.5% ($P < 0.001$), respectively. After cerivastatin treatment, cytokine TNF-α was significantly lowered as compared with placebo period (2.68 ± 0.3 vs. 2.16 ± 0.4 pg/ml; $P < 0.05$ [$r = 0.747$, $P = 0.001$]). High-sensitivity CRP also decreased with cerivastatin treatment (3.57 ± 0.6 vs. 2.23 ± 0.4 mg/dl; $P < 0.05$ [$r = 0.45$, $P = 0.08$]). Leptin levels did not change with cerivastatin treatment and were positively correlated with body fat mass ($r = 0.537$, $P = 0.062$; $r = 0.619$, $P = 0.032$, placebo and treatment period, respectively).

Effects on glucose tolerance and insulin secretion. The mean fasting blood glucose concentration at admission was 7.3 ± 0.3 mmol/l and remained unchanged during the study (placebo 6.8 ± 0.3 to cerivastatin 6.9 ± 0.3; $P = 0.9$ [$r = 0.66$, $P = 0.007$]; Table 2). Mean fasting plasma insulin levels presented a slight but nonsignificant increase after cerivastatin treatment (69 ± 6.8 to 76 ± 5.6 pmol/l; $P = 0.31$ [$r = 0.3$, $P = 0.3$]; Table 3). Neither difference was observed in mean fasting C-peptide levels (placebo 1.29 ± 0.34 to cerivastatin 1.14 ± 0.17 pg/ml; $P = 0.5$ [$r = 0.77$, $P = 0.001$]; Table 3). Fasting hepatic insulin extraction (pmol C-peptide/pmol insulin) was found to increase after

TABLE 2
Biochemical characteristics of subjects

	Baseline	Placebo	Cerivastatin 0.4 mg	*P*
FPG (mmol/l)*	7.3 ± 0.30	6.8 ± 0.3	6.9 ± 0.3	NS
HbA_{1c}(%)	6.4 ± 0.13	6.7 ± 0.15†	6.6 ± 0.2†	NS
Fructosamine		271 ± 15	270 ± 14	NS
Total cholesterol (mmol/l)*	5.64 ± 0.2	5.25 ± 0.23†	4.23 ± 0.13‡	0.000
TGs (mmol/l)*	1.80 ± 0.1	1.46 ± 0.06†	1.25 ± 0.06‡	0.03
TNF-α (pg/ml)	ND	2.68 ± 0.3	2.16 ± 0.35	0.03
PCR-h (mg/dl)	ND	3.57 ± 0.6	2.23 ± 0.4	0.02
Leptin (ng/ml)	ND	28.49 ± 4.1	30.66 ± 5.5	NS

FPG, fasting plasma glucose; PCR-h, high-sensitivity C-reactive protein; ND, not determined. *Mean of basal determinations obtained on two separate occasions. To convert values for glucose, cholesterol, and TGs to mg/dl, divide by 0.056, 0.026, and 0.011, respectively. Data are mean ± SE. *P* value, placebo versus cerivastatin period. †$P < 0.05$, ‡$P < 0.01$ versus baseline.

TABLE 3
Evaluation of insulin sensitivity and insulin response

	Placebo	Cerivastatin	Difference (95% CI)	P
Fasting plasma insulin (pmol/l)*	69 ± 6.8	76 ± 5.6	−7.17 (−23.5 to 8.5)	0.31
First-phase insulin response (IVGTT; pmol/l)	144 ± 25	210 ± 39	−66 (−105 to −26)	0.003
Insulin index ($pmol \cdot l^{-1} \cdot pmol^{-1}$)	2.07 ± 0.28	2.82 ± 0.38	−0.7 (−1.4 to −0.04)	0.039
$\Delta I_{30\text{-}0}/\Delta G_{30\text{-}0}$ (OGTT; pmol/mmol)	32.1 ± 7.0	46.5 ± 9.9	−14.41 (−28 to −0.63)	0.04
Maximum C-peptide secretion (HC; pmol/l)	4.34 ± 0.68	3.80 ± 0.58	0.55 (−0.90 to 2)	0.43
ISI (EC; $nmol \cdot LBM^{-1} \cdot min^{-1} \cdot pmol \cdot l^{-1}$)	58.37 ± 3.69	66.39 ± 3.9	−8.02 (−3.51 to −2.53)	0.007
MCR ($ml \cdot m^{-2} \cdot min^{-1}$)	713.7 ± 28.4	756.2 ± 19.2	−42.5 (−77.5 to −7.5)	0.021

Data are means ± SE. To convert values for insulin to μU/ml, divide by 6. To convert values for C-peptide to nanomoles per liter, multiply by 0.33. *Mean of basal determinations obtained on two separate occasions. ISI, insulin sensitivity index. MCR, metabolic clearance rate.

cerivastatin treatment as compared with placebo (14.87 ± 1.5 vs. 9.34 ± 1.2; $P = 0.02$).

After OGTT, three patients presented with impaired glucose tolerance (2-h glucose >7.7 and <11.1 mmol/l) during the placebo period and two patients presented with impaired glucose tolerance during cerivastatin treatment (Fig. 2, top left). The rest of the patients were considered diabetic because of the oral glucose load. The incremental glucose area under the curve was not changed (placebo 21.25 ± 1.4 $mmol \cdot l^{-1} \cdot h^{-1}$, cerivastatin 21.47 ± 1.4 mmol/l; $P = 0.8$ [$r = 0.55$, $P = 0.03$]).

The incremental insulin area under the curve after oral glucose load showed no significant difference (placebo 512 ± 108 $pmol \cdot l^{-1} \cdot h^{-1}$, cerivastatin 622 ± 124 pmol/l; $P = 0.3$ [$r = 0.59$, $P = 0.02$]; Fig. 2, bottom left). The insulinogenic index ($\Delta I_{30-0}/\Delta G_{30-0}$) increased significantly with cerivastatin treatment (placebo 32.1 ± 7.0 pmol/mmol, cerivastatin 46.5 ± 9.9 pmol/mmol; $P < 0.05$ [$r = 0.76$, $P = 0.001$]; Table 3).

Mean values for glucose infusion rates during the HC studies increased slightly in patients on placebo compared with patients on cerivastatin treatment (952 ± 51 vs. 992 ± 59 $\mu mol \cdot LBM^{-1} \cdot min^{-1}$; $P = 0.059$ [$r = 0.8$, $P = 0.001$]; Fig. 2, right). The incremental insulin area under the curve increased slightly with placebo (25%) as compared with cerivastatin treatment (1,193.9 ± 251 vs. 1,492 ± 252; $P = 0.2$ [$r = 0.53$, $P = 0.04$]). Insulin sensitivity index increased after cerivastatin treatment as compared with placebo (209.6 ± 38 vs. 288 ± 44 $nmol \cdot LBM^{-1} \cdot min^{-}1 \cdot pmol^{-1} \cdot l^{-1}$; $P = 0.036$ [$r = 0.67$, $P = 0.006$]). The incremental C-peptide area under the curve during the HC was slightly but not significantly lower after cerivastatin (8,908 ± 1,488 vs. 7,460 ± 1,656 pmol/h; $P = 0.4$ [$r = 0.45$, $P = 0.1$]). Hepatic insulin extraction during the HC was not changed (placebo 9.38 ± 1.2 vs. cerivastatin 10.21 ± 1.4; $P = 0.6$).

After hyperglycemic stimulus, the average plasma glucose concentrations were similar for the two periods studied (placebo: 16.7 ± 0.6 vs. cerivastatin: 16.6 ± 0.6

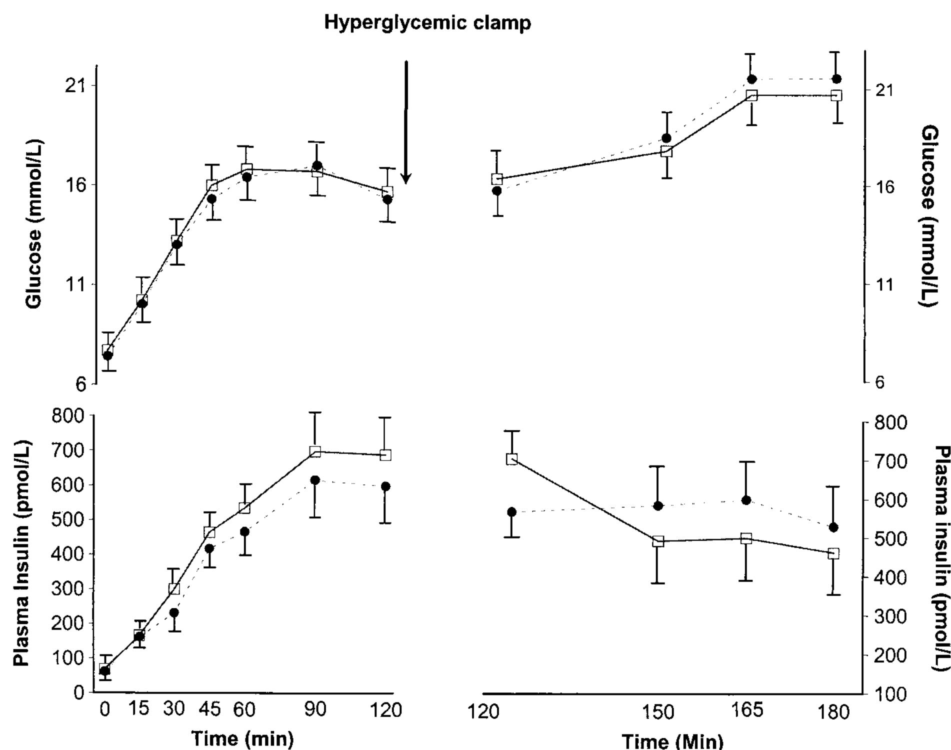

FIG. 2. Serum glucose concentration (top) and plasma insulin responses (bottom) during OGTT (left) and HC study (right). □, values after cerivastatin treatment; ●, values on placebo.

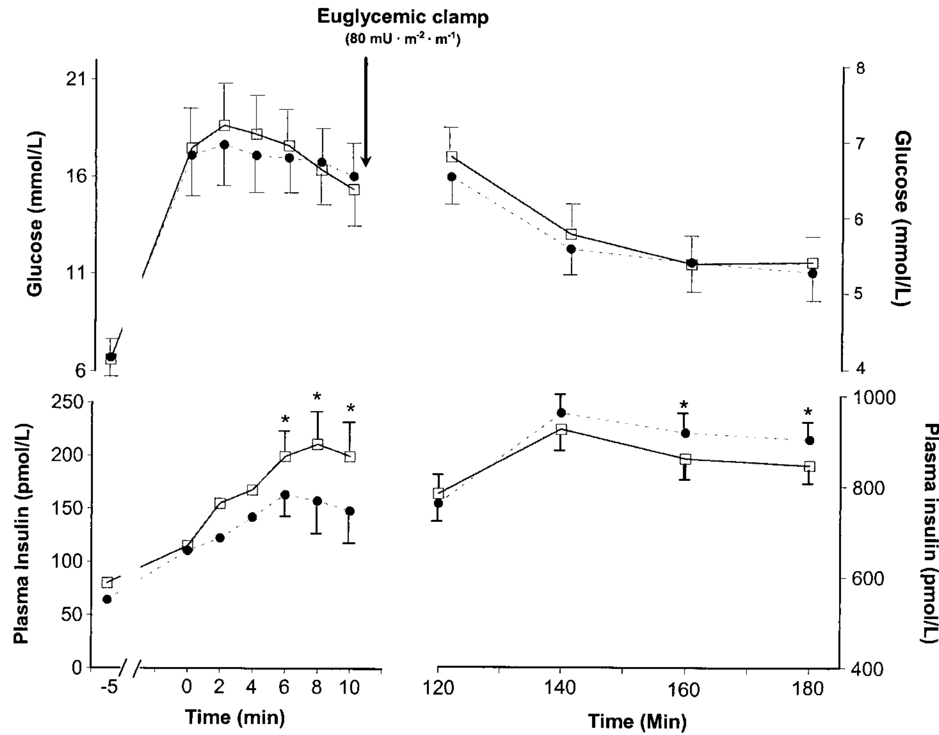

FIG. 3. Serum glucose concentration (top) and plasma insulin responses (bottom) during IGTT (left) and EHC study (right). □, values after cerivastatin treatment; ●, values during placebo period.

mmol/l, P = 0.9; [r = 0.8, P = 0.000]; Fig. 3, left). Acute insulin response was substantially higher after treatment with cerivastatin (144 ± 25 to 210 ± 39 pmol/l; P = 0.003 [r = 0.9, P = 0.000]). The insulin index was increased significantly with cerivastatin treatment by 36% (P = 0.039 [r = 0.56, P = 0.03]).

Effects on insulin sensitivity. The whole insulin-mediated glucose intake assessed during the EHC was significantly higher (6.5%) in patients after cerivastatin treatment ($P < 0.05$ [r = 0.87, P = 0.000]; Table 4). The steady-state insulin levels averaged during the EHC were higher in patients on placebo (817 ± 35 vs. 762 ± 20 pmol/l; $P < 0.05$ [r = 0.86, P = 0.000]). The mean insulin sensitivity index values during the EHC, a rate that corrects whole glucose uptake for average steady-state plasma insulin levels, was substantially higher (13.75%) after cerivastatin treatment (58.37 ± 3.7 vs. 66.39 ± 3.9 nmol · $LBM^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$; $P < 0.01$ [r = 0.77, P = 0.001]). The MCR of exogenously infused insulin during the EHC increased by 6% after treatment with cerivastatin ($P < 0.05$ [r = 0.83, P = 0.000]; Table 3).

Changes in substrate, glucose, and lipid oxidation and energy expenditure. In the fasting state, the energy expenditure and Rq were similar in both periods studied (0.65 ± 0.09 vs. 0.67 ± 0.07 kcal · $m^{-2} \cdot min^{-1}$ [P = 0.8] and 0.83 ± 0.01 vs. 0.83 ± 0.01 kcal · $m^{-2} \cdot min^{-1}$ [P = 0.9], placebo and cerivastatin, respectively; Table 4). During the steady state of the EHC, an increase in energy expenditure and Rq was observed with cerivastatin (1.69 ± 0.16 vs. 1.96 ± 0.15 kcal · $m^{-2} \cdot min^{-1}$, $P < 0.01$ [r = 0.87, P = 0.000]; and 0.91 ± 0.01 vs. 0.94 ± 0.01, $P < 0.05$, respectively; Table 4). The rate of glucose oxidation in the fasting state was similar. During EHC, glucose oxidation rates increased by 10.5% after treatment with cerivastatin, which represents 50% of whole-body glucose uptake as compared with placebo (16.1 ± 1.37 vs. 14.28 ± 1.48 μmol · $LBM^{-1} \cdot min^{-1}$; $P < 0.05$; Table 4).

Figure 4 shows profiles of TG, serum NEFA, and plasma

TABLE 4
Substrate disappearance and energy production rate in fasting and steady-state during euglycemic clamp: serum FFA and plasma lactate levels

	Fasting state			Steady-state euglycemic clamp		
	Placebo	Cerivastatin	P	Placebo	Cerivastatin	P
Insulin-mediated glucose intake (μmol · $LBM^{-1} \cdot min^{-1}$)				48.1 ± 2.2	51.1 ± 2.8	0.039
Glucose oxidation (μmol · $LBM^{-1} \cdot min^{-1}$)	4.44 ± 0.7	4.45 ± 0.65	0.92	14.58 ± 1.48	16.1 ± 1.37	0.029
Lipid oxidation (mg · $LBM^{-1} \cdot min^{-1}$)	0.76 ± 0.07	0.71 ± 0.06	0.54	0.48 ± 0.06	0.33 ± 0.05	0.001
Energy production rate (kcal · $m^{-2} \cdot min^{-1}$)	0.65 ± 0.09	0.67 ± 0.07	0.63	1.69 ± 0.16	1.96 ± 0.15	0.012
Rq	0.83 ± 0.01	0.83 ± 0.01	0.94	0.91 ± 0.01	0.94 ± 0.01	0.01
Serum FFAs (mmol/l)	0.41 ± 0.03	0.39 ± 0.04	0.28	0.09 ± 0.01	0.04 ± 0.01	0.005
Plasma lactate (mmol/l)	1.36 ± 0.12	1.32 ± 0.10	0.66	1.24 ± 0.02	1.42 ± 0.03	0.03

Data are mean ± SE.

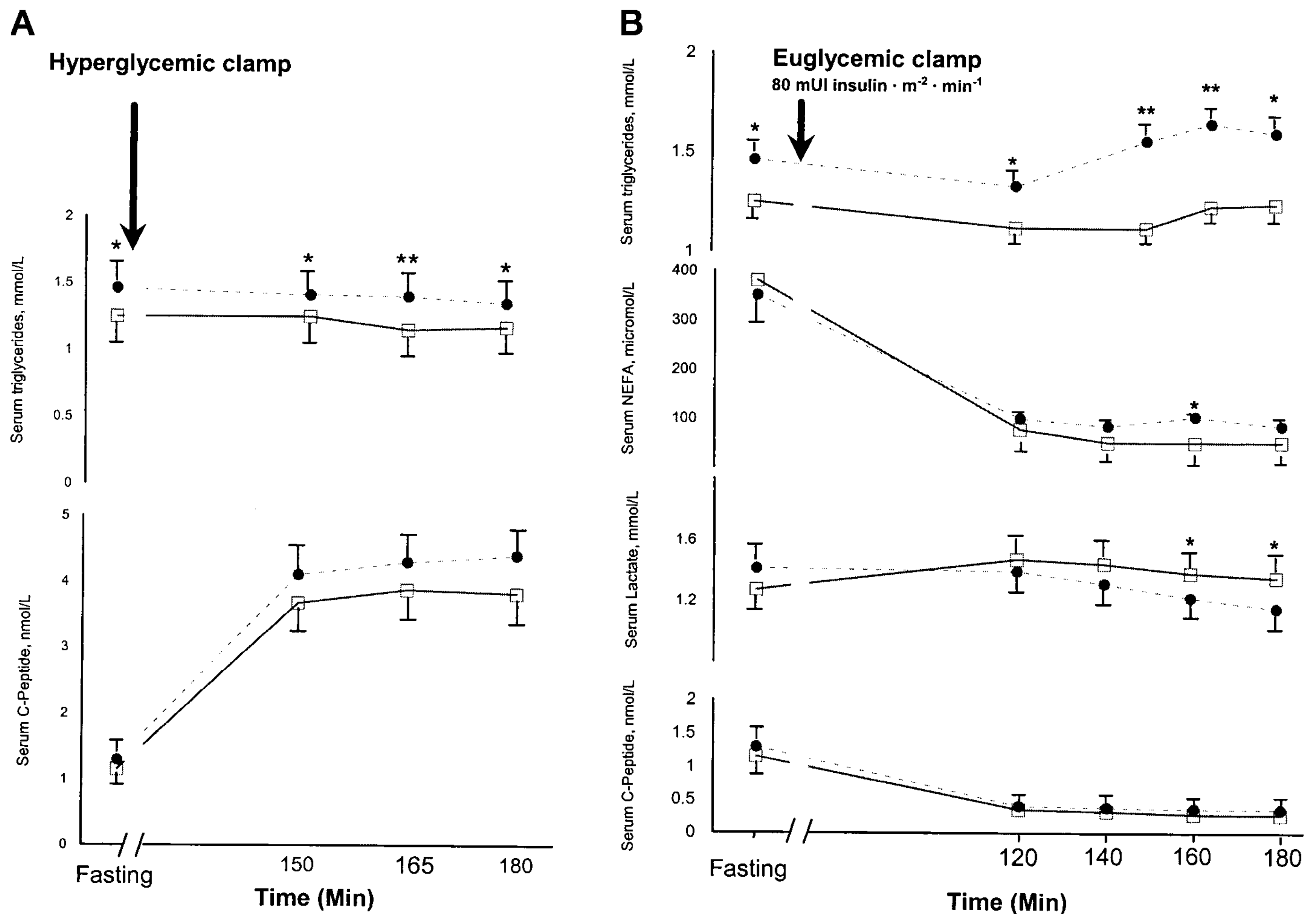

FIG. 4. ***A*****: Serum TG concentrations and plasma C-peptide responses during the HC study.** ***B*****: Serum TG, plasma NEFA, plasma lactate concentrations, and C-peptide responses during the EHC study. □, values after cerivastatin treatment; ●, values through placebo period.**

lactate levels during the HC and EHC. The fasting serum TG concentration after 0.4 mg cerivastatin treatment was reduced by 14% (1.25 ± 0.06 vs. 1.46 ± 0.06 mmol/l; $P < 0.05$ [$r = 0.51$, $P = 0.06$]). Average serum TG levels during the HC also decreased by 14% with cerivastatin treatment (1.16 ± 0.1 vs. 1.36 ± 0.1 mmol/l; $P < 0.01$ [$r = 0.8$, $P = 0.000$]). Average serum TG concentrations during the EHC showed a greater decrease (27%) after cerivastatin therapy (1.17 ± 0.1 vs. 1.61 ± 0.1 mmol/l; $P < 0.00$ [$r = 0.67$, $P = 0.006$]. Serum FFA concentrations and lipid oxidation in the fasting state were similar in both periods (0.41 ± 0.03 vs. 0.39 ± 0.04 mmol/l, $P = 0.2$ [$r = 0.62$, $P = 0.014$]; and 0.76 ± 0.07 vs. 0.71 ± 0.06 $mg \cdot LBM^{-1} \cdot min^{-1}$, $P = 0.5$ [$r = 0.51$, $P = 0.052$], placebo and cerivastatin, respectively; Table 4). Average FFA concentrations and lipid oxidation during the EHC were significantly higher in patients on placebo as compared with patients on cerivastatin (0.090 ± 0.016 vs. 0.042 ± 0.007 mmol/l, $P < 0.01$ [$r = 0.50$, $P = 0.058$]; and 0.48 ± 0.06 vs. 0.33 ± 0.05 $mg \cdot LBM^{-1} \cdot min^{-1}$, $P < 0.01$ [$r = 0.61$, $P = 0.015$], respectively; Fig. 3). Mean plasma lactate levels in the fasting state were similar for both treatment periods (Table 4). During the EHC, an increase was observed in average serum lactate levels in patients on cerivastatin treatment (1.24 ± 0.02 vs. 1.42 ± 0.03 mmol/l; $P < 0.05$ [$r = 0.56$, $P = 0.054$]; Fig. 3).

Adverse events. Overall, treatment with 0.4 mg cerivastatin was well tolerated. The following blood variables were analyzed: hemoglobin, erythrocytes, white blood cells, platelets, serum urea, creatinine, calcium, phosphorus, magnesium, sodium, chloride, potassium, creatine phosphokinase, lactate dehydrogenase, alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutaryl transferase, bilirubin, and β_2-microglobulin. The most common adverse effects were mild and transient headache (one patient) and gastrointestinal (two patients) side effects, which did not require the discontinuation of therapy in any of the patients. No adverse events in the present study were indicative of muscular, renal, or hepatocellular toxicity.

DISCUSSION

The present data demonstrated that treatment with 0.4 mg cerivastatin increases insulin-mediated glucose uptake and improves first-phase insulin secretion in mild hyperglycemic patients in early state obese type 2 diabetes without pharmacological intervention. Type 2 diabetes involves a combination of peripheral tissue insulin resistance, excessive hepatic glucose production, and impaired insulin secretion (25). Various mechanisms of action of statins may involve glucose metabolism and insulin resistance. First, at mild to high doses, statins decrease plasma

TGs, reducing the production of VLDL. This effect was observed in our study. Plasma TG levels averaged during EHC decreased by 27%, whereas serum FFA decreased by 50% (from 90 to 40 μmol/l/l) with cerivastatin treatment. During the fasting state and the HC, serum TGs were lowered by 14%. Finally, whole insulin-mediated glucose uptake during euglycemic clamp was seen to increase significantly (6.5%). In addition, in our study, the weight and mean HbA_{1c} level was not changed throughout the study, whereas fasting plasma total cholesterol decreased by 19.5%. In contrast, in type 2 diabetes, an increase in glucose disposal rates by 13% with metformin therapy and by 54% with troglitazone therapy has been shown (26). However, weight loss or decreases in hyperglycemia induced with these treatments could partially have an effect on insulin sensitivity (27). In humans, during infusion of a TG emulsion, inhibitory effects have also been associated with whole glucose uptake and glucose storage after 3–4 h (28). The classic mechanism of FFA-induced peripheral insulin resistance was proposed by Randle and colleagues (29,30) through the inhibition of pyruvate dehydrogenase, phosphofructokinase, and hexokinase, which affect glucose transport (GLUT4) activity. Recent data suggest an early direct effect of intracellular FFA content on insulin stimulation GLUT4 transporter activity (31,32). Contrasting results in a few experimental studies regarding the effect of statins in glucose metabolism of patients with diabetes have been published (33–35). The nonhomogeneous selection of patients together with the low doses of statins and short times of EHC used could have limited some of these results. In addition, cerivastatin could have effects beyond the pharmacological class effect.

Second, after 3 months of statin treatment, a significantly higher rate of glucose oxidation was observed and represents ~50% of the glucose uptake observed during the EHC. This was accompanied by an increase in the Rq and a decrease in FFA oxidation. In addition, glucose oxidation during the steady state in EHC was negatively correlated with average FFA concentrations during the placebo period ($r = -0.452$, $P = 0.08$) and during cerivastatin treatment ($r = -0.475$, $P = 0.059$). It has recently been demonstrated that changes throughout the physiological range in plasma FFA concentrations (from ~50 to ~800 μmol/l) exert an inhibitory effect on insulin-stimulated glucose oxidation and are evident in the first hours (10). It is of note that part of the remaining ~50% of glycolytic nonoxidative glucose metabolism was due to an increase in glycolytic lactate observed during the euglycemic clamp with cerivastatin treatment. It has been previously shown that statins therapy may affect mitochondrial respiration and may be associated with a high blood lactate/pyruvate ratio (36). Whether 0.4 mg cerivastatin is related to mitochondrial dysfunction is unknown; the biochemical causes and precise functional significance of these defects remain to be seen.

First-phase insulin response increased substantially after cerivastatin treatment. Moreover, fasting insulin levels were slightly higher and hepatic insulin extraction increased after cerivastatin treatment in postabsorptive basal conditions. Direct effects on β-cell function of statins are little known. It has been shown that simvastatin inhibits glucose-induced insulin secretion in rat islet β-cells, whereas pravastatin had no effects (37). The lipid-lowering effects observed after 0.4 mg cerivastatin treatment, mainly on VLDL production with lower average plasma TG levels, could have an indirect effect in β-cell function increasing basal insulin secretor rates and first-phase insulin secretion (38). The continuous effect of 0.4 mg cerivastatin on VLDL production with lower averaged plasma TG levels could be related to higher basal insulin secretor rates and increased first-phase insulin secretion. Accordingly, in Zucker diabetic fatty rats, overaccumulation of triacylglycerol, the loss of function, and the loss of β-cells can be prevented by treatment with a thiazolidinedione if begun in the prediabetic stage (39). However, it is well established that the regulation of hepatic glucose production by insulin is a major determinant of blood glucose concentrations. In insulin-resistant states, chronic hyperinsulinemia suppresses the mRNA for insulin receptor substrate-2, a component of the insulin-signaling pathway in the liver, contributing to hepatic insulin resistance and inadequate gluconeogenesis (40). In our study, an approach to hepatic glucose uptake can be observed from OGTT results. Because the $\Delta I_{30-0}/\Delta G_{30-0}$ was increased and the incremental glucose area under the curve remained unchanged after cerivastatin treatment, it is possible that defects in liver insulin action on glucose metabolism remain unaltered after treatment with statins.

Last, during statin treatment, a significant reduction in plasma TNF-α and CRP concentrations was found. Recent data suggest that inflammation is involved in the early state of insulin resistance syndrome and predicted atherosclerotic disease (41,42). TNF-α is produced by adipocytes with a decrease of TNF-α mRNA expression observed after weight loss (43). Moreover, TNF-α impairs insulin action by effects on the insulin receptor and its substrate (44). In addition, TNF-α induces interleukin-6 (IL-6) production (45), and CRP synthesis in the liver is stimulated by IL-6 (46). In our study, CRP levels were associated with body fat mass in both periods studied. However, as patient weight was not modified in the study, it is possible that reductions in CRP and proinflammatory cytokine levels with cerivastatin are a direct effect of the statins (47). In summary, our results show that 0.4 mg cerivastatin therapy increases whole insulin-mediated glucose uptake during the euglycemic clamp and improves β-cell function measured as first-phase insulin secretion in early-state obese type 2 diabetes without previous treatment and with clinical features of metabolic syndrome. Increased insulin action was associated with lowered plasma TG and FFA concentrations and an increase in the Rq. Moreover, there is evidence that cerivastatin treatment improved the proinflammatory state of these patients.

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