

# Effects of High-Dose Simvastatin Therapy on Glucose Metabolism and Ectopic Lipid Deposition in Nonobese Type 2 Diabetic Patients

JULIA SZENDROEDI, MD, PHD<sup>1,2,3</sup>  
CHRISTIAN ANDERWALD, MAG, MD<sup>1</sup>  
MARTIN KRSSAK, PHD<sup>4</sup>  
MICHAELA BAYERLE-EDER, MD<sup>1</sup>  
HARALD ESTERBAUER, MD, PHD<sup>5</sup>  
GEORG PFEILER, MD<sup>6</sup>

ATTILA BREHM, MD<sup>1</sup>  
PETER NOWOTNY<sup>1</sup>  
ASTRID HOFER<sup>1</sup>  
WERNER WALDHÄUSL, MD<sup>1</sup>  
MICHAEL RODEN, MD<sup>2,3</sup>

**OBJECTIVE** — Statins may exert pleiotropic effects on insulin action that are still controversial. We assessed effects of high-dose simvastatin therapy on peripheral and hepatic insulin sensitivity, as well as on ectopic lipid deposition in patients with hypercholesterolemia and type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — We performed a randomized, double-blind, placebo-controlled, single-center study. Twenty patients with type 2 diabetes received 80 mg simvastatin (BMI  $29 \pm 4$  kg/m<sup>2</sup>, age  $55 \pm 6$  years) or placebo (BMI  $27 \pm 4$  kg/m<sup>2</sup>, age  $58 \pm 8$  years) daily for 8 weeks and were compared with 10 healthy humans (control subjects; BMI  $27 \pm 4$  kg/m<sup>2</sup>, age  $55 \pm 7$  years). Euglycemic-hyperinsulinemic clamp tests combined with D-[6,6-d<sub>2</sub>]glucose infusion were used to assess insulin sensitivity (*M*) and endogenous glucose production (EGP). <sup>1</sup>H magnetic resonance spectroscopy was used to quantify intramyocellular and hepatocellular lipids.

**RESULTS** — High-dose simvastatin treatment lowered plasma total and LDL cholesterol levels by  $\sim 33$  and  $\sim 48\%$  ( $P < 0.005$ ) but did not affect *M*, intracellular lipid deposition in soleus and tibialis anterior muscles and liver, or basal and insulin-suppressed EGP. In simvastatin-treated patients, changes in LDL cholesterol related negatively to changes in *M* ( $r = -0.796$ ,  $P < 0.01$ ). Changes in fasting free fatty acids (FFAs) related negatively to changes in *M* ( $r = -0.840$ ,  $P < 0.01$ ) and positively to plasma retinol-binding protein-4 ( $r = 0.782$ ,  $P = 0.008$ ).

**CONCLUSIONS** — High-dose simvastatin treatment has no direct effects on whole-body or tissue-specific insulin action and ectopic lipid deposition. A reduction in plasma FFAs probably mediates alterations in insulin sensitivity in vivo.

*Diabetes Care* 32:209–214, 2009

Type 2 diabetes is commonly associated with dyslipidemia, which represents a synergistic risk factor for cardiovascular disease (1). High-circulating lipids (free fatty acids [FFAs]) induce insulin resistance because of impaired muscle glucose transport/phosphorylation, and intracellular lipids

in muscle (IMCLs) and liver (HCLs) predict insulin resistance (2).

Interventional studies emphasized that statin treatment leads to a reduction in cardiovascular events with benefits for patients with type 2 diabetes (3). Statins could also contribute to diabetes prevention owing to lipid-lowering and so-called pleiotropic action. Statin therapy was shown to improve endothelial function, inhibit smooth muscle cell proliferation, and reduce oxidative stress and inflammation (4). Retrospective analysis of the West of Scotland Coronary Prevention Study (WOSCOPS) revealed that 5 years of treatment with pravastatin reduced diabetes incidence by  $\sim 30\%$ . The authors suggested that although lowering of triglyceride levels could influence diabetes incidence, other mechanisms such as anti-inflammatory action may be involved (5). However, pravastatin did not decrease diabetes incidence in another trial including glucose-intolerant humans, suggesting that early inception of statin therapy may be required for effective diabetes prevention (6). Likewise, simvastatin did not affect diabetes incidence in patients with atherosclerosis in the Heart Protection Study (7). In contrast, atorvastatin marginally increased diabetes incidence in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT-LLA), which could be explained by statistical variation (8). Thus, the effect of statins on diabetes incidence is still uncertain.

The direct action of statins on insulin sensitivity remains controversial because beneficial (9) and indifferent and unfavorable (10) effects were reported. Statins not only decrease LDL cholesterol but may also interfere with fasting and postprandial metabolism of triglyceride-rich lipoproteins, resulting in altered substrate flux and accumulation of HCLs (11,12), which could subsequently affect muscle glucose metabolism and deposition of IMCLs.

Simvastatin is one of the most frequently prescribed statins because of its efficacy in reducing LDL lipoprotein cho-

From the <sup>1</sup>Department of Internal Medicine 3, Medical University of Vienna, Vienna, Austria; the <sup>2</sup>Karl-Landsteiner Institute of Endocrinology and Metabolism, Vienna, Austria; the <sup>3</sup>Department of Medicine/Metabolic Diseases, Institute for Clinical Diabetology, German Diabetes Center, Heinrich Heine University, Düsseldorf, Germany; the <sup>4</sup>Department of Radiology, Medical University of Vienna, Vienna, Austria; the <sup>5</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria; and the <sup>6</sup>Division of Special Gynecology, Medical University of Vienna, Vienna, Austria.

Corresponding author: Michael Roden, michael.roden@ddz.uni-duesseldorf.de.

Received 25 June 2008 and accepted 16 October 2008.

Published ahead of print at <http://care.diabetesjournals.org> on 28 October 2008. DOI: 10.2337/dc08-1123.

Clinical trial reg. no. NCT00704314, [clinicaltrials.gov](http://clinicaltrials.gov).

The funding bodies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

© 2009 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

lesterol levels, its tolerability, and its reduction of cardiovascular risk and mortality (7). Its effects on insulin action and metabolism at the maximal recommended dose of 80 mg/day are unclear. Thus, we examined the effects of 80 mg/day simvastatin therapy on 1) insulin sensitivity, 2) IMCLs and HCLs, 3) fasting and insulin-mediated suppression of plasma FFAs, and 4)  $\beta$ -cell function using euglycemic-hyperinsulinemic clamps combined with stable isotope dilution and nuclear magnetic resonance spectroscopy in hypercholesterolemic, normotriglyceridemic patients with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

Twenty patients with type 2 diabetes and hypercholesterolemia were included. Eligibility criteria were known duration of disease of 3–10 years, age 35–75 years, BMI <32 kg/m<sup>2</sup>, LDL cholesterol >4.16 mmol/l, triglycerides <2.75 mmol/l, A1C <9%, serum creatinine <1.8 mg/dl, liver transaminases <20% over the upper limit with no active liver disease and creatine kinase <50% above the upper limit, and no evidence of metabolic diseases other than type 2 diabetes. Patients were taking neither lipid-lowering drugs nor other drugs known to interfere with metabolism of statins. The only glucose-lowering drugs allowed were metformin, sulfonyleureas, and  $\alpha$ -glucosidase inhibitors. Ten age-, sex-, and BMI-matched healthy volunteers (control subjects) were examined only at baseline.

The study had a double-blind, randomized, placebo-controlled and parallel group design. The trial has been registered as a clinical trial. The sample size was calculated using data from our previous studies in diabetic patients who complied with the inclusion criteria of the present study and were examined with identical experimental methods. The false-positive and false-negative error rates tolerated were  $Z\alpha = 1.96$  for a two-tailed  $\alpha$  of 0.05 and  $Z\beta = 0.84$  for a  $\beta$  of 0.2. An increase or decrease of ~20% in the mean values for the primary target variables, insulin-stimulated whole-body glucose disposal ( $M$  value) and insulin-suppression of endogenous glucose production (EGP), was considered to be physiologically and clinically relevant. The respective mean  $\pm$  SD values were  $\sim 5 \pm 1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for  $M$  values ( $3 \pm 0.3$  [ref. 13],  $8 \pm 1$  [ref. 14]), and  $\sim 0.5 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for EGP

suppression (13,14). These considerations revealed a sample size of eight as the minimal number of patients receiving simvastatin. Expecting a dropout rate of ~15%, we included 10 participants for each study group.

After a run-in period of 3 weeks, the patients were randomly assigned to treatment with 80 mg daily simvastatin (Merck Sharp & Dohme, Hoddesdon, U.K.) or placebo for 2 months. Glucose metabolism, IMCLs, and HCLs were determined before and after treatment following overnight fasting for at least 12 h. According to previous studies, sulfonyleureas (three in the simvastatin group and nine in the placebo group), metformin (five in the simvastatin group and seven in the placebo group), and  $\alpha$ -glucosidase inhibitors (two in the simvastatin group and one in the placebo group) were withdrawn at 1 and 3 days before the clamps, respectively (13,14). The study was approved by the local ethics committee, and patients consented to participate.

### Glucose metabolism

At 7.00 A.M. patients were transferred to the metabolic unit. A primed infusion of D-[6,6-d<sub>2</sub>]glucose (3.6 mg/kg body weight  $\times$  [fasting plasma glucose/90]) followed by a continuous infusion (0.036 mg/min  $\times$  kg body weight) was started to determine EGP (15). At 9.00 A.M., a primed continuous infusion of 40 mU/min per m<sup>2</sup> body surface area was administered for 150 min to assess insulin sensitivity ( $M$ ) and the ratio of  $M$  to the prevailing plasma insulin concentration ( $M/I$ ) by hyperinsulinemic-isoglycemic (at baseline fasting plasma glucose [FPG]) clamps in control subjects and to standardize for increased FPG by a euglycemic-hyperinsulinemic (~100 mg/dl) clamp in type 2 diabetic patients. In type 2 diabetic patients, euglycemia was achieved by identical primed continuous insulin infusions as in control subjects, and no additional insulin infusion was required. A 20% dextrose infusion, 2% enriched with D-[6,6-d<sub>2</sub>]glucose was periodically adjusted to maintain euglycemia (15).

### Analytical procedures

Glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Atom percent <sup>2</sup>H enrichments in glucose were determined by gas chromatography–mass spectrometry (15). FFAs were assayed microfluorimetrically (Wako Chemicals

USA, Richmond, VA) in blood samples using orlistat to prevent in vitro lipolysis (15). Triglyceride levels were measured colorimetrically (Roche, Vienna, Austria). Insulin, C-peptide, and glucagon were determined by double-antibody radioimmunoassay (15). Retinol-binding protein (RBP)-4 was assayed nephelometrically using an antiserum to human plasma RBP (code OUVO; Dade Behring, Deerfield, IL) (16).

### <sup>1</sup>H nuclear magnetic resonance spectroscopy

Volunteers were lying supine inside a 1.5-T spectrometer (Magnetom; Siemens, Erlangen, Germany). HCLs were quantified using a breath-hold–triggered single voxel sequence without water suppression applied on the 27-cm<sup>3</sup> volume within the right lateral liver (2). IMCLs were determined in 1.73-cm<sup>3</sup> volumes within soleus and tibialis anterior muscles using water-suppressed PRESS and the AMARES algorithm as implemented in the jMRUI software package. After T<sub>2</sub> relaxation, IMCLs were quantified from the intensity of the (CH<sub>2</sub>)<sub>n</sub> = 1.25 ppm resonance, which was compared with the water resonance intensity obtained from spectra without water suppression.

### Calculations and statistics

The computer-solved homeostasis model assessment (HOMA2) was used to derive surrogate parameters of basal  $\beta$ -cell function (HOMA-B) and insulin sensitivity (HOMA-IR). EGP was calculated from the difference between rates of glucose appearance ( $R_a$ ) (15) and of mean glucose infusion. Statistical analyses were performed using SPSS software (version 6.0; SPSS, Chicago, IL). Data are presented as means  $\pm$  SD ( $\pm$ SEM in the figures). Comparisons between groups and drug-induced effects were assessed by ANOVA with or without repeated measurements with Tukey post hoc testing. Within-group differences were determined using two-tailed Student's  $t$  tests. Differences were considered significant at the 5% level for  $M$ , FFAs, and EGP and at 1% for other parameters to correct for interrelated comparison. Linear correlations are Pearson product-moment correlations and were considered to be significant at the 5% level for  $M$ , FFAs, and EGP and at 1% for all other relations.

**Table 1—Baseline characteristics of type 2 diabetic patients and matched nondiabetic volunteers**

	Simvastatin (80 mg/day)	Placebo	Control subjects
n (women/men)	10 (3/7)	10 (5/5)	10 (5/5)
BMI (kg/m <sup>2</sup> )	28.9 ± 3.5	27.3 ± 3.7	27.4 ± 4
Age (years)	55 ± 6	58 ± 8	55 ± 7
A1C (%)	6.7 ± 0.6	6.7 ± 0.7	5.6 ± 0.2‡
FPG (mmol/l)	8.7 ± 1.3	8.5 ± 1.3	4.9 ± 0.4‡
HOMA-B	64 ± 23	69 ± 27	81 ± 17
HOMA-IR	2.7 ± 0.9	2.7 ± 0.8	0.8 ± 0.2
Fasting EGP (mg · kg <sup>-1</sup> · min <sup>-1</sup> )	1.7 ± 0.3	1.7 ± 0.4	1.4 ± 0.4**
TGs (mmol/l)	1.7 ± 0.5	1.9 ± 0.6	1.1 ± 0.4*†
FFAs (μmol/l)	503 ± 229	618 ± 206	613 ± 206
TC (mmol/l)	7.6 ± 2.5	6.6 ± 0.8	5.6 ± 0.9*
TG-to-HDL cholesterol ratio	2.9 ± 1.0	3.3 ± 1.2	1.8 ± 0.8¶
HDL cholesterol (mmol/l)	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.2
LDL cholesterol (mmol/l)	5.4 ± 2.3	4.3 ± 0.6	3.6 ± 0.8*
ALT (units/l)	37 ± 13	34 ± 11	26 ± 9
AST (units/l)	25 ± 7	21 ± 4	26 ± 7
GGT (units/l)	37 ± 13	34 ± 11	21 ± 12*

Data are mean ± SD anthropometric and laboratory characteristics of type 2 diabetic patients after allocation to either placebo or simvastatin therapy and healthy control subjects. BMI, FPG, surrogate parameters of basal  $\beta$ -cell function (HOMA-B) and basal insulin sensitivity (HOMA-IR), total triglycerides (TGs), FFAs, total cholesterol levels (TC), HDL cholesterol and calculated LDL cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and GGT were determined. \* $P < 0.05$ , control versus simvastatin; \*\* $P < 0.05$ , control versus simvastatin and placebo; † $P < 0.005$ , control versus placebo; ‡ $P < 0.0005$ , simvastatin and placebo versus control; § $P < 0.00001$ , simvastatin and placebo versus control; || $P < 0.00005$ , simvastatin and placebo versus control; ¶ $P < 0.01$ , placebo versus control.

## RESULTS

### Baseline characteristics

Baseline characteristics of patients with type 2 diabetes after allocation to either placebo or simvastatin therapy and control subjects are shown in Table 1. A1C, FPG, and triglycerides were increased in both diabetic groups, and total cholesterol and LDL cholesterol were slightly higher in the simvastatin group than in control subjects. In type 2 diabetic patients, HOMA-IR was ~3.4-fold higher than in control subjects, whereas HOMA-B was comparable.  $\gamma$ -Glutamyl transpeptidase (GGT) was ~76 and ~62% higher in type 2 diabetic patients than in control subjects ( $P = 0.020$  versus simvastatin;  $P = 0.062$  versus placebo). Basal EGP was ~21% higher in type 2 diabetic patients (simvastatin  $1.7 \pm 0.3$ , placebo  $1.7 \pm 0.4$ , and control  $1.4 \pm 0.4$  mg · kg<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.05$  versus type 2 diabetes). IMCLs in soleus and in tibialis anterior muscles in type 2 diabetic patients were comparable to IMCLs in control subjects (simvastatin  $1.4 \pm 0.5$  and  $0.2 \pm 0.2$ , placebo  $1.3 \pm 0.6$  and  $0.3 \pm 0.2$ , and control  $1.5 \pm 0.9$  and  $0.4 \pm 0.4$ ). In contrast, HCLs were ~3.6-fold higher in type 2 diabetic patients (simva-

statin  $14.2 \pm 8.6$ , placebo  $14.1 \pm 5.8$ , and control  $4 \pm 4$ ;  $P < 0.001$  versus type 2 diabetes) (Fig. 1B). Across the whole study population, HCLs tended to relate positively to FPG ( $r = 0.544$ ,  $P < 0.005$ ), A1C ( $r = 0.409$ ,  $P < 0.05$ ), and GGT ( $r = 0.442$ ,  $P < 0.05$ ) without reaching predefined statistical significance ( $P < 0.01$ ) but related negatively to  $M$  ( $r = -0.386$ ,  $P < 0.05$ ). IMCLs did not correlate with any other metabolic parameters.

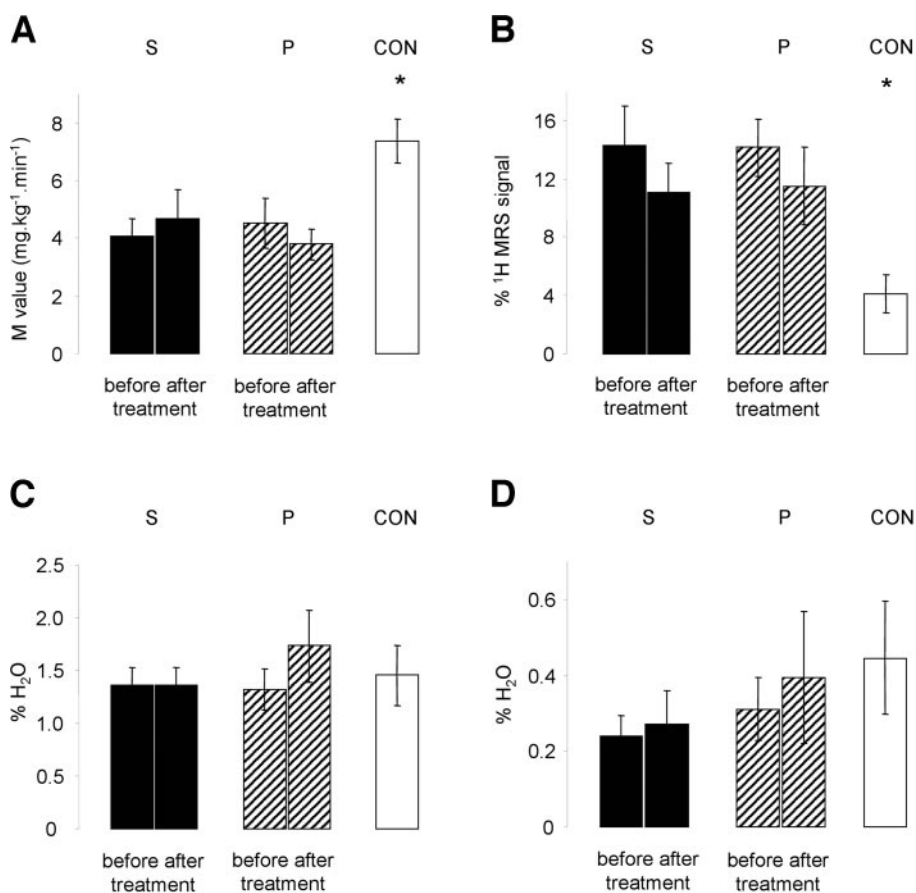
### Whole-body metabolism during the clamps

Within 60 min of the clamps, plasma glucose levels reached steady-state conditions before and after treatment (simvastatin  $5.7 \pm 0.3$  and  $5.7 \pm 0.3$ , placebo  $5.9 \pm 0.6$  and  $5.7 \pm 0.2$ , and control  $4.9 \pm 0.4$  mmol/l) and did not differ within or among the intervention groups. During the last 60 min of the clamps, plasma glucose levels before and after treatment were  $5.4 \pm 0.3$  and  $5.4 \pm 0.3$  mmol/l in the simvastatin group,  $5.5 \pm 0.3$  and  $5.4 \pm 0.3$  mmol/l in the placebo group, and  $4.9 \pm 0.3$  mmol/l in control subjects and did not differ within or among the intervention groups but was lower in control subjects than in type 2

diabetic patients ( $P < 0.005$ ). Plasma insulin concentrations were  $580 \pm 102$  and  $609 \pm 109$  pmol/l in the simvastatin group,  $537 \pm 80$  and  $551 \pm 94$  pmol/l in the placebo group, and  $515 \pm 58$  pmol/l in control subjects and did not differ within or among the intervention groups.  $M$  values were ~42% lower in type 2 diabetic patients and did not differ among the intervention groups (control  $7.4 \pm 2.4$ , simvastatin  $4.1 \pm 1.9$ , and placebo  $4.5 \pm 2.7$  mg · kg<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.005$ , type 2 diabetic patients versus control subjects) (Fig. 1A). Similarly, the  $M$ -to- $I$  ratio was lower in type 2 diabetic patients [control subjects  $0.01 \pm 0.005$  mg · kg<sup>-1</sup> · min<sup>-1</sup> · (pmol/l)<sup>-1</sup>;  $P < 0.01$ ] but not different among intervention groups (Table 2). Insulin-mediated suppression of EGP (Table 2) and FFAs (control  $94 \pm 5$ , simvastatin  $87 \pm 10$ , and placebo  $92 \pm 2$ ) was comparable in all groups. Plasma triglycerides related positively to HOMA-IR ( $r = 0.683$ ,  $P = 0.00003$ ) and negatively to  $M$  ( $r = -0.555$ ,  $P = 0.001$ ), A1C ( $r = -0.539$ ,  $P = 0.002$ ), and FPG ( $r = -0.497$ ,  $P = 0.005$ ).

### Effects of simvastatin on lipid and glucose metabolism

Intervention-related changes of plasma lipids and glucose metabolism are shown in Table 2. At 2 months, plasma total and LDL cholesterol decreased by ~33 and ~48% in the simvastatin group but remained unchanged in the placebo group. There were no significant changes in triglycerides, HDL cholesterol, and fasting FFAs after simvastatin therapy compared with baseline. Nevertheless, the simvastatin group had ~29 and ~35% lower triglycerides and FFAs than the placebo group. In the simvastatin group, the decreases in LDL cholesterol and FFAs were positively associated ( $r = 0.774$ ,  $P < 0.001$ ) but did not relate to changes in triglycerides. Despite no significant changes in  $M$  after simvastatin treatment, changes in FFAs were negatively correlated with the change in  $M$  in the simvastatin group ( $r = -0.840$ ,  $P = 0.002$ ), which was weakened by the exclusion of one subject with excessive changes in  $M$  and FFAs ( $r = -0.641$ ,  $P = 0.063$ ). The relationship between changes in  $M$  and LDL cholesterol ( $r = -0.796$ ,  $P = 0.006$ ) was completely lost by omission of this subject ( $r = 0.242$ ,  $P = 0.531$ ) (Fig. 2A). Adjustment for FFAs disrupted the relationship between the changes in LDL cholesterol and  $M$  ( $r = 0.424$ ,  $P = 0.256$ ), whereas the association between changes



**Figure 1**— Whole-body insulin sensitivity (M value) (A), ectopic lipid deposition in liver (B) soleus muscle (C), and anterior tibialis muscle (D) in patients with type 2 diabetes before and after treatment with 80 mg/day simvastatin (S, n = 10, ■) or placebo (P, n = 10, ▨), and healthy humans (CON, n = 10, □, P < 0.005 versus simvastatin and placebo groups).

**Table 2**—Effects of simvastatin on lipid profiles and glucose metabolism

	Simvastatin (80 mg/day)	Placebo
A1C (%)	6.7 ± 0.6 (−0.01 ± 0.3)	6.7 ± 0.6 (−0.01 ± 0.4)
HOMA-B	71 ± 31 (6.8 ± 16.6)	67 ± 29 (−1.3 ± 1)
HOMA-IR	2.7 ± 0.6 (−0.03 ± 0.6*)	3.3 ± 1.2† (0.6 ± 0.5)
M (mg · kg <sup>−1</sup> · min <sup>−1</sup> )	4.7 ± 3.3 (0.6 ± 2.1)	3.8 ± 1.6 (−0.3 ± 2.0)
M-to-I ratio (mg · kg <sup>−1</sup> · min <sup>−1</sup> ) · (pmol/l) <sup>−1</sup>	0.008 ± 0.005 (0.002 ± 0.01)	0.006 ± 0.003 (−0.001 ± 0.008)
Rate of glucose disappearance (mg · kg <sup>−1</sup> · min <sup>−1</sup> )	5.3 ± 3.1 (0.0 ± 2.9)	4.0 ± 1.3 (−1.2 ± 1.2)
EGP during clamp (mg · kg <sup>−1</sup> · min <sup>−1</sup> )	0.48 ± 0.32 (0.29 ± 0.95)	0.39 ± 0.33 (−0.01 ± 0.60)
EGP suppression (%)	72 ± 14 (−3 ± 13)	74 ± 12 (4 ± 16)
TGs (mmol/l)	1.5 ± 0.4* (−0.2 ± 0.5*)	2.1 ± 0.8 (0.3 ± 0.4)
FFAs (μmol/l)	392 ± 130* (−111 ± 205)	600 ± 234 (−18 ± 211)
TC (mmol/l)	5.1 ± 1.0†‡ (−2.5 ± 1.8‡)	6.6 ± 0.8 (0.0 ± 0.6)
TG-to-HDL cholesterol ratio	2.7 ± 1.2 (−0.1 ± 1.2)	3.7 ± 1.7 (0.4 ± 0.7)
HDL cholesterol (mmol/l)	1.4 ± 0.3 (2.9 ± 5.9)	1.4 ± 0.3 (−1.8 ± 7.1)
LDL cholesterol (mmol/l)	2.8 ± 0.9†‡ (−2.6 ± 1.6§)	4.2 ± 0.5 (−0.2 ± 0.4)
ALT (units/l)	40 ± 20 (6 ± 16)	29 ± 12 (2 ± 5)
AST (units/l)	31 ± 15 (6 ± 14)	22 ± 6 (1 ± 5)
GGT (units/l)	39 ± 23 (2 ± 15)	36 ± 8 (2 ± 6)
RBP-4 (mg/dl)	5.0 ± 1.1 (−0.4 ± 0.8)	5.8 ± 1.7 (0.7 ± 0.6)

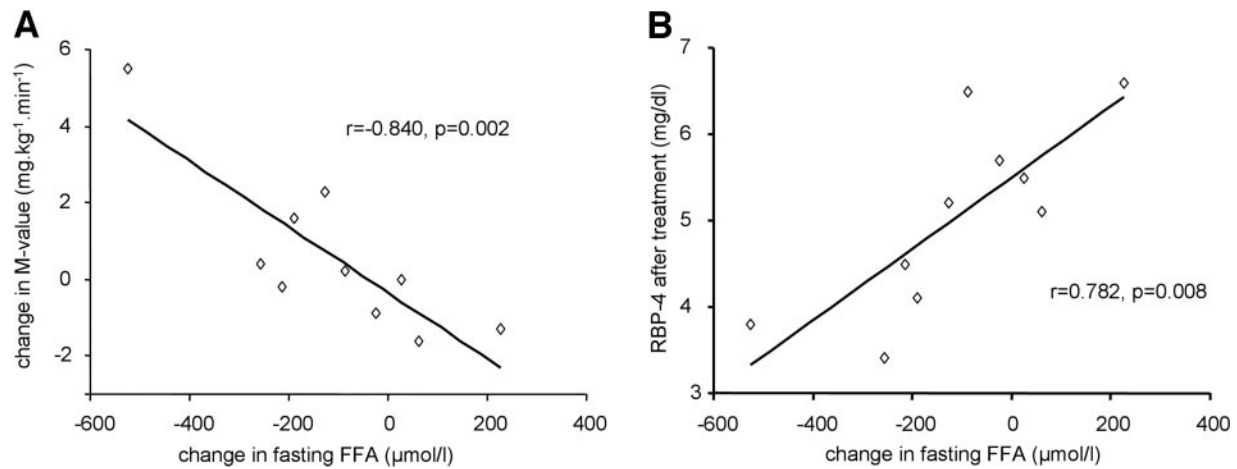
Data are mean ± SD laboratory characteristics of type 2 diabetic patients after treatment with 80 mg/day simvastatin for 8 weeks or application of placebo; changes compared with baseline are given in parentheses. Surrogate parameters of basal β-cell function (HOMA-B) and basal insulin sensitivity (HOMA-IR), total triglycerides (TGs), whole-body glucose disposal (M), FFAs, total cholesterol levels (TC), HDL cholesterol and calculated LDL cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT, and rate of glucose disappearance were determined. \*P < 0.05 simvastatin versus placebo; †P < 0.005 versus baseline; ‡P < 0.005, simvastatin versus placebo; §P < 0.0005, simvastatin versus placebo.

in FFAs and M remained robust after adjustment for LDL cholesterol ( $r = 0.584$ ,  $P = 0.099$ ). Changes in the M-to-I ratio after simvastatin treatment also related positively to changes in plasma FFAs ( $r = 0.674$ ,  $P = 0.033$ ). Plasma RBP-4 did not differ between the groups (simvastatin  $5.4 \pm 0.4$  and placebo  $5.0 \pm 0.5$  mg/dl) but tended to relate positively to HOMA-IR ( $r = 0.479$ ,  $P = 0.032$ ). After simvastatin treatment, plasma RBP-4 correlated with the change in FFAs ( $r = 0.782$ ,  $P = 0.008$ ) (Fig. 2B). IMCLs and HCLs remained unchanged (simvastatin  $1.4 \pm 0.6$ ,  $0.3 \pm 0.3$ , and  $11.0 \pm 6.5\%$  and placebo  $1.7 \pm 1.0$ ,  $0.4 \pm 0.5$ , and  $11.5 \pm 8.0\%$ ) (Fig. 1). Changes in insulin sensitivity did not relate to muscle and liver lipids. Also, basal EGP and EGP suppression were not affected by treatment (simvastatin  $1.7 \pm 0.2$  mg · kg<sup>−1</sup> · min<sup>−1</sup> [72 ± 14%] and placebo  $1.5 \pm 0.4$  mg · kg<sup>−1</sup> · min<sup>−1</sup> [74 ± 12%]).

## CONCLUSIONS

### Effects on serum lipids

High-dose simvastatin treatment reduced LDL cholesterol by ~48% in agreement with the maximum achievable LDL cholesterol reduction. Increases in HDL cholesterol and decreases in fasting triglycerides and FFAs were not observed in our patients with only slight hypertri-



**Figure 2**—Correlation of changes in fasting FFAs with the changes in whole-body insulin sensitivity (M value) (A) and RBP-4 (B) in patients with type 2 diabetes before and after treatment with 80 mg/day simvastatin (S, n = 10).

glyceridemia. Simvastatin might, therefore, exert larger effects on HDL cholesterol and triglycerides in more severe hypertriglyceridemia.

#### Effects on insulin sensitivity

Simvastatin treatment slightly reduces insulin sensitivity using the quantitative insulin sensitivity check index (17) in line with findings in type 2 diabetes (10). Others reported that simvastatin does not change (18) or increases insulin sensitivity (HOMA-IR) in severely hypertriglyceridemic, hypercholesterolemic patients with type 2 diabetes (9). Only a few studies demonstrated changes in whole-body insulin sensitivity by statin therapy with the use of clamps (10,19). At a dose of 80 mg/day, we found no effect of simvastatin on whole-body insulin sensitivity in nonobese type 2 diabetes with good metabolic control. This finding does not exclude a specific simvastatin effect on hepatic insulin sensitivity. Our patients with type 2 diabetes exhibited marked hepatic insulin resistance indicated by only ~70% EGP suppression. However, simvastatin did not ameliorate EGP suppression in our patients with type 2 diabetes, a result that is in line with the only previous study on pravastatin treatment in familial hypercholesterolemia (20). Statins not only decrease LDL cholesterol but may also interfere with fasting and postprandial triglyceride-rich lipoprotein metabolism, resulting in altered substrate flux and accumulation of HCLs (11,12,21). Our patients exhibited a tight correlation between excessive HCL storage and M value similar to that in previous reports (2). Simvastatin did not affect either HCLs or IMCLs in two muscles with different

compositions. Also no relationship between changes in insulin sensitivity and ectopic lipids was found.

#### Effects on parameters influencing insulin sensitivity

According to current paradigms, mechanisms determining insulin sensitivity comprise 1) circulating FFAs arising from adipocyte lipolysis, lipoprotein secretion, or dietary fat intake, 2) cytokines from adipose tissue or liver, and 3) low-grade inflammation. Recently, simvastatin was found to improve FFA composition, fasting lipid fractions, and postprandial plasma triglycerides even in normotriglyceridemic patients (21). In the present study, a reduction in plasma FFAs during the clamp, reflecting insulin-mediated suppression of lipolysis, remained unchanged after therapy. Statins could affect insulin resistance via declining plasma triglycerides in type 2 diabetes. Triglyceride levels were negatively related to M at baseline and changes in fasting FFAs were found to induce considerable effects on insulin sensitivity. Accordingly, evidence is accumulating that intracellular long-chain fatty acyl CoA and diacylglycerol inhibit muscular insulin action by stimulating serine phosphorylation of insulin receptor substrate-1 rather than IMCLs (22).

Statins may further affect inflammatory markers (4), which could relate to changed adipocytokines. Circulating RBP-4, produced mainly by adipocytes, is related to whole-body insulin sensitivity and is elevated in insulin-resistant states (23), but its role remains controversial (16). Here we show that serum RBP-4 relates to a surrogate of fasting insulin sen-

sitivity and to changes in plasma FFAs upon simvastatin therapy. Nevertheless, serum RBP-4 did not relate to whole-body insulin sensitivity as assessed from the euglycemic clamp and simvastatin did not affect RBP-4.

#### Effects on fasting $\beta$ -cell function

High-dose lipophilic statins may induce unfavorable pleiotropic effects including impairment of insulin secretion (24,25). The proposed mechanism suggests that these statins inhibit the glucose-induced elevation of free  $[Ca^{2+}]$  in cytoplasm, thereby diminishing insulin secretion. However, other studies reported increased or unchanged fasting insulin (9,10). We found no changes in either fasting insulin or HOMA-B during simvastatin therapy.

Some limitations of this study need to be considered. First, the number of participants per treatment group is low but was based on a sample size calculation considering that increases of whole-body and hepatic insulin sensitivity by ~20% represent a clinically relevant treatment effect. Second, only patients with untreated hypercholesterolemia in need of cholesterol-lowering drug treatment according to current guidelines were included. Thus, this trial comprised a typical but preselected population, which does not allow extrapolation of the results to normolipidemic type 2 diabetic or nondiabetic populations. Third, the extensive metabolic characterization revealed a high number of parameters assessed so that the level of significance was adjusted to correct for interrelated comparison. Nevertheless, despite the extensive metabolic characterization by gold-standard

techniques, a number of anti-inflammatory and antioxidant mechanisms that potentially affect insulin action were not explored in the present study. As a result, the issue of whether a possible dissociation exists among different pleiotropic effects of statins cannot be completely resolved. Finally, different glucose-lowering drugs were used in both groups and withdrawn before the clamp. However, antidiabetic medication did not have any impact on whole-body and hepatic insulin sensitivity and patients taking thiazolidinediones or insulin were not included in this study.

Thus, this study shows that even high-dose simvastatin treatment that effectively reduces LDL cholesterol does not directly improve either whole-body or hepatic insulin sensitivity or intracellular lipid deposition in near normotriglyceridemic patients with type 2 diabetes.

**Acknowledgments**—This study was supported by grants from the Austrian Science Foundation (P15656) and European Foundation for the Study of Diabetes (GlaxoSmith-Kline Grant) to M.R. and by a grant from Merck Sharp & Dohme to W.W. The Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Study was supported by European Union Grant QLGI-CT-2001-01252.

No other potential conflicts of interest relevant to this article were reported.

## References

- Kannel WB, Castelli WP, Gordon T, McNamara PM: Serum cholesterol, lipoproteins, and the risk of coronary heart disease: the Framingham study. *Ann Intern Med* 74:1–12, 1971
- Roden M: Mechanisms of disease: hepatic steatosis in type 2 diabetes—pathogenesis and clinical relevance. *Nat Clin Pract Endocrinol Metab* 2:335–348, 2006
- Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C: Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet* 371:117–125, 2008
- Forrester JS, Libby P: The inflammation hypothesis and its potential relevance to statin therapy. *Am J Cardiol* 99:732–738, 2007
- Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357–362, 2001
- Keech A, Colquhoun D, Best J, Kirby A, Simes RJ, Hunt D, Hague W, Beller E, Arulchelvam M, Baker J, Tonkin A: Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose: results from the LIPID trial. *Diabetes Care* 26:2713–2721, 2003
- Collins R, Armitage J, Parish S, Sleight P, Peto R: MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 361:2005–2016, 2003
- Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J: Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* 361:1149–1158, 2003
- Paolisso G, Barbagallo M, Petrella G, Ragno E, Barbieri M, Giordano M, Varricchio M: Effects of simvastatin and atorvastatin administration on insulin resistance and respiratory quotient in aged dyslipidemic non-insulin dependent diabetic patients. *Atherosclerosis* 150:121–127, 2000
- Ohrvall M, Lithell H, Johansson J, Vessby B: A comparison between the effects of gemfibrozil and simvastatin on insulin sensitivity in patients with non-insulin-dependent diabetes mellitus and hyperlipoproteinemia. *Metabolism* 44:212–217, 1995
- Isley WL, Harris WS, Miles JM: The effect of high-dose simvastatin on free fatty acid metabolism in patients with type 2 diabetes mellitus. *Metabolism* 55:758–762, 2006
- Twickler TB, Dallinga-Thie GM, de Valk HW, Schreuder PC, Jansen H, Cabezas MC, Erkelens DW: High dose of simvastatin normalizes postprandial remnant-like particle response in patients with heterozygous familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 20:2422–2427, 2000
- Anderwald C, Bernroider E, Krssak M, Stingl H, Brehm A, Bischof MG, Nowotny P, Roden M, Waldhausl W: Effects of insulin treatment in type 2 diabetic patients on intracellular lipid content in liver and skeletal muscle. *Diabetes* 51:3025–3032, 2002
- Krssak M, Brehm A, Bernroider E, Anderwald C, Nowotny P, Dalla Man C, Cobelli C, Cline GW, Shulman GI, Waldhausl W, Roden M: Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes* 53:3048–3056, 2004
- Krebs M, Krssak M, Nowotny P, Weghuber D, Gruber S, Mlynarik V, Bischof M, Stingl H, Fornsinn C, Waldhausl W, Roden M: Free fatty acids inhibit the glucose-stimulated increase of intramuscular glucose-6-phosphate concentration in humans. *J Clin Endocrinol Metab* 86:2153–2160, 2001
- Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P, Wagner O, Esterbauer H, Anderwald C: Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. *J Clin Endocrinol Metab* 92:4306–4312, 2007
- Koh KK, Quon MJ, Han SH, Lee Y, Ahn JY, Kim SJ, Koh Y, Shin EK: Simvastatin improves flow-mediated dilation, but reduces adiponectin levels and insulin sensitivity in hypercholesterolemic patients. *Diabetes Care* 31:776–782, 2008
- Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, Kang MH, Ahn TH, Choi IS, Shin EK: Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation* 110:3687–3692, 2004
- Paniagua JA, Lopez-Miranda J, Escribano A, Berral FJ, Marin C, Bravo D, Paz-Rojas E, Gomez P, Barcos M, Moreno JA, Perez-Jimenez F: Cerivastatin improves insulin sensitivity and insulin secretion in early-stage obese type 2 diabetes. *Diabetes* 51:2596–2603, 2002
- Galvan AQ, Natali A, Baldi S, Frascerra S, Sampietro T, Galetta F, Seghieri G, Ferrannini E: Effect of a reduced-fat diet with or without pravastatin on glucose tolerance and insulin sensitivity in patients with primary hypercholesterolemia. *J Cardiovasc Pharmacol* 28:595–602, 1996
- van Wijk JP, Buirma R, van Tol A, Halkes CJ, De Jaegere PP, Plokker HW, van der Helm YJ, Castro Cabezas M: Effects of increasing doses of simvastatin on fasting lipoprotein subfractions, and the effect of high-dose simvastatin on postprandial chylomicron remnant clearance in normotriglyceridemic patients with premature coronary sclerosis. *Atherosclerosis* 178:147–155, 2005
- Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97:2859–2865, 1996
- Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB: Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 354:2552–2563, 2006
- Yada T, Nakata M, Shiraishi T, Kakei M: Inhibition by simvastatin, but not pravastatin, of glucose-induced cytosolic Ca<sup>2+</sup> signalling and insulin secretion due to blockade of L-type Ca<sup>2+</sup> channels in rat islet β-cells. *Br J Pharmacol* 126:1205–1213, 1999
- Chamberlain LH: Inhibition of isoprenoid biosynthesis causes insulin resistance in 3T3-L1 adipocytes. *FEBS Lett* 507:357–361, 2001