Treatment with high-dose simvastatin reduces secretion of apolipoprotein B-lipoproteins in patients with diabetic dyslipidemia

Merle Myerson,* Colleen Ngai,* Jeffrey Jones,* Steve Holleran,† Rajasekhar Ramakrishnan,† Lars Berglund,* and Henry N. Ginsberg[1],*

Department of Medicine* and Department of Pediatrics,† Columbia University College of Physicians and Surgeons, New York, NY 10032

Abstract HMG-CoA reductase inhibitors (statins) are effective lipid-altering drugs for the treatment of dyslipidemia in patients with type 2 diabetes mellitus. We conducted a randomized, double-blind, placebo-controlled, crossover design trial to determine the effects of simvastatin, 80 mg/day, on plasma lipid and lipoprotein levels and on the metabolism of apolipoprotein B (apoB) in VLDL, intermediate density lipoprotein (IDL), and LDL and of triglycerides (TGs) in VLDL. Simvastatin therapy decreased TG, cholesterol, and apoB significantly in VLDL, IDL, and LDL. These effects were associated with reduced production of LDL-apoB, mainly as a result of reduced secretion of apoB-lipoproteins directly into the LDL density range. Statin therapy also reduced hepatic production of VLDL-TG. There were no effects of simvastatin on the fractional catabolic rates of VLDL-apoB or -TG or LDL-apoB. The basis for decreased VLDL-TG secretion during simvastatin treatment is not clear, but recent studies suggest that statins may activate peroxisomal proliferator-activated receptor α (PPARα). Activation of PPARα could lead to increased hepatic oxidation of fatty acids and less synthesis of TG for VLDL assembly.—Myerson, M., C. Ngai, J. Jones, S. Holleran, R. Ramakrishnan, L. Berglund, and H. N. Ginsberg. **Treatment with high-dose simvastatin reduces secretion of apolipoprotein B-lipoproteins in patients with diabetic dyslipidemia. *J. Lipid Res.* 2005.** 46: **2735–2744.**

Supplementary key words very low density lipoproteins • low density lipoproteins • triglycerides • 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors • statins • diabetes mellitus • hypertriglyceridemia

Patients with type 2 diabetes mellitus (T2DM) are at significantly increased risk for the development of coronary artery disease (CAD) (1–4). Several complex and interrelated metabolic abnormalities in these patients are related directly to CAD risk. Among these is a diabetic dyslipidemia that includes increased blood levels of plasma VLDL-triglycerides (TGs), low levels of plasma high density lipoprotein-cholesterol (HDL-C), abnormalities in the composition of LDL, and increased levels of apolipoprotein B (apoB) (5–8). Although multiple abnormalities in lipid and lipoprotein metabolism likely play important roles in the pathophysiology of diabetic dyslipidemia, overproduction of apoB-lipoproteins appears to be a central component (9–12).

The HMG-CoA reductase inhibitors, or statins, are currently the most efficacious medications for reducing LDL-C levels. Statins have been shown to reduce morbidity and mortality from CAD in both primary and secondary prevention trials (13–15). Several studies have demonstrated that statins also decrease CAD events in patients with diabetes (16–19). Studies of the effect of statins on LDL-C have found that VLDL-TG and VLDL-C levels are also decreased, particularly when high doses of statins are used (20). Indeed, most studies have shown that levels of all apoB-lipoproteins are reduced by statin therapy. These findings have raised important questions regarding the mechanisms by which statins decrease plasma lipids. Inhibition of cholesterol synthesis leads to increased levels of LDL receptors, particularly in the liver (21, 22), and this is consistent with an increased fractional clearance rate (FCR; a measure of the efficiency with which a lipoprotein is cleared from plasma) of LDL-apoB observed during statin treatment of patients with familial hypercholesterolemia (23). However, the basis for lower VLDL levels is less clear (24). Although increased fractional clearance of VLDL by statin-induced hepatic LDL receptors might account for the lower plasma VLDL concentrations seen, reduced production rates (PRs) of VLDL-apoB and -TG (PR will be used to represent the secretion of apoB and TG from the liver under the conditions of our studies) have also been demonstrated in several studies of statin therapy in patients with increased plasma VLDL concentrations. In a study in which we treated patients with combined hyperlipidemia with lovastatin, we observed a decrease in total production of apoB-lipoproteins by the liver (25) and reductions in VLDL-TG

Manuscript received 1 August 2005 and in revised form 6 September 2005.

Published, JLR Papers in Press, September 14, 2005.
DOI 10.1194/jlr.M500335-JLR200

[1] To whom correspondence should be addressed.
e-mail: hng1@columbia.edu

TABLE 1. Subject characteristics

Subject	Gender	Age	Body Mass Index	Other Diagnoses	Diabetes Treatment	Hemoglobin A_{1c}
		years				%
1	Male	73	36.6	MI, CABG	Metformin	7.2
2	Male	59	30.2	CABG	Metformin	6.8
3	Female	66	25.8	Atrial fibrillation	Diet	5.6
4	Female	59	32.8	MI, percutaneous transluminal coronary angioplasty	Diet	6.9
5	Male	58	27.3	Cerebrovascular accident, MI	Metformin	8.5

CABG, coronary artery bypass graft; MI, myocardial infarction.

secretion (26). However, a review of the literature, with all but one study conducted in patients with dyslipidemia without T2DM, reveals inconsistent effects of statins on apoB-lipoprotein secretion. In the only study of the effects of statin treatment on apoB metabolism in patients with T2DM, Ouguerram et al. (27) observed both decreased VLDL-apoB secretion into plasma and normalization of a baseline low FCR of LDL in patients with T2DM. That study did not measure VLDL-TG metabolism. Because overproduction of apoB-lipoproteins is characteristic of the dyslipidemia of diabetes, we undertook a randomized, double-blind, crossover study of the effects of high-dose simvastatin therapy on the PRs and FCRs of VLDL-, intermediate density lipoprotein (IDL)-, and LDL-apoB and VLDL-TG in patients with T2DM.

METHODS

Patients

The clinical characteristics of the five patients who were studied are presented in **Table 1**. All subjects had diet- or oral agent-treated T2DM and had been referred to our lipid clinic for evaluation of hyperlipidemia. Lipid inclusion criteria included a TG of ⩾200 mg/dl but <600 mg/dl and/or a HDL-C of <40 mg/dl for men and <45 mg/dl for women. All patients were in stable health at the time of the study. None had an additional, secondary cause of dyslipidemia, such as renal, hepatic, or untreated thyroid disease. Three of five had a history of myocardial infarction, and all had multiple risk factors for CAD. All five patients had previously been treated with hypolipidemic drugs, but these were discontinued 1 month before beginning the study. Other medications were allowed if required by medical considerations (Table 1). Exclusion criteria included the use of other drugs that might interfere with the metabolism of simvastatin. Patients were also excluded if they had a hypersensitivity to HMG-CoA reductase inhibitors, drank >10 alcoholic beverages per week, had a serum creatinine of >1.8 mg/dl, increased liver transaminases (more than twice the upper limit of normal), unstable CAD, recent (<3 months) myocardial infarction, angioplasty or cardiac bypass surgery, and uncontrolled hypertension.

Protocol

We conducted a randomized, double-blind, crossover study. There were two 7 week testing periods, one on active drug and one on placebo. The testing periods were performed in random order, with a 1 week washout period between treatments. Studies of apoB and TG metabolism were performed in the General Clinical Research Center (GCRC) at the Columbia University Medical Center (CUMC) at the end of each treatment period. Informed consent was obtained before each study period. The project received approval by the CUMC Institutional Review Board.

At the start of each study period, blood was drawn after a 12 h fast for the measurement of plasma lipids, routine chemistries, glucose, and glycosylated hemoglobin. Body weight and blood pressure were documented. After 4 weeks of each period, blood was drawn, and both VLDL and LDL were isolated by sequential ultracentrifugation as described previously (25, 28). VLDL was radiolabeled with ^{125}I and LDL with ^{131}I by a modification of the

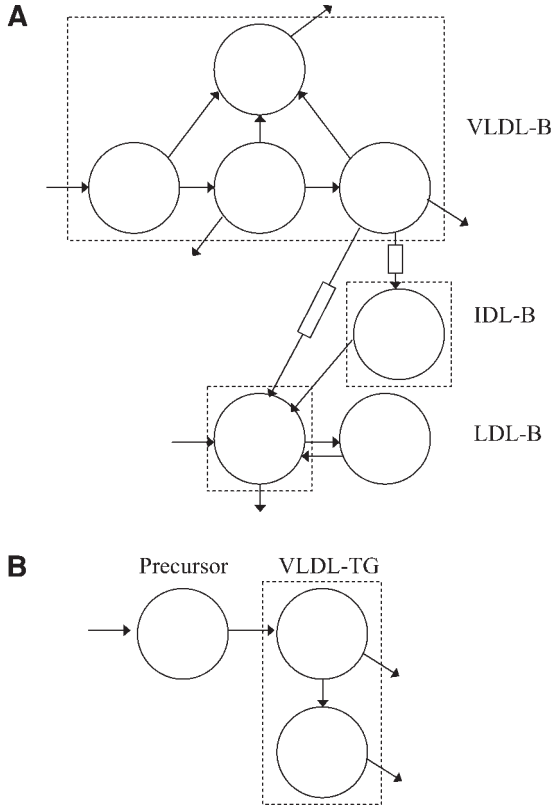

Fig. 1. Metabolism of apolipoprotein B (apoB) and triglyceride (TG). A: Compartmental models for the metabolism of apoB in VLDL, intermediate density lipoprotein (IDL), and LDL based on apoB specific activity in VLDL, IDL, and LDL after injection of ^{125}I-VLDL and in LDL after injection of ^{131}I-LDL. The model includes the secretion of apoB into plasma in the VLDL and LDL density ranges, a three-step delipidation cascade with a remnant pool in VLDL, direct conversion of VLDL to LDL, delays in the conversion of VLDL to IDL and VLDL to LDL, and direct removal of VLDL and LDL from plasma. B: Metabolism of TG in VLDL based on the specific activity of [^3H]TG-glycerol after injection of [^3H]glycerol. The model includes a precursor pool in the liver and a two-step lipolytic cascade in plasma with direct removal of TG from each step.

TABLE 2. Effects of simvastatin treatment on plasma lipid levels

	Total Cholesterol		TG		HDL-C	
Subject	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin
			mg/dl			
1	245	107	232	147	20.3	23.5
2	239	162	458	318	20.3	21.5
3	304	172	718	401	17.5	30.7
4	277	163	281	172	28.3	33.3
5	235	154	307	217	21.0	20.8
Mean ± SD	260 ± 30	152 ± 26	399 ± 197	251 ± 106	21.5 ± 4	25.9 ± 6
Change (%)	−42[a]		−37[a]		+21	

Triglyceride (TG) values were log-transformed for analysis.
[a] $P < 0.01$.

iodine monochloride method (29, 30). All procedures were carried out using sterile equipment, and the radiolabeled lipoproteins were passed through a 0.45 μm filter for VLDL and a 0.22 μm filter for LDL before injection into patients.

At 5 weeks, subjects were admitted to the GCRC for 72 h. Fasting blood was obtained for the measurement of lipids and glucose. Nine hours before the start of the study (at midnight of the first day of hospitalization), patients began consuming a liquid diet (75% carbohydrate and 25% protein, at 60% of their total calorie requirements). They continued on this diet every 3 h for the 48 h of the VLDL turnover study. This dietary regimen, used in numerous studies, has been shown to provide stable plasma levels of cholesterol and TG (31, 32). At 9 AM of the second day, subjects received an intravenous injection of 75 μCi of autologous ^{125}I-VLDL and 300 μCi of tritiated glycerol. The latter tracer was used to endogenously label VLDL-TG. Eighteen blood samples were collected over the next 48 h. After completion of the VLDL turnover study, 25 μCi of ^{131}I-LDL was injected intravenously, patients were switched back to their solid food diet, and several blood samples were obtained over the next 24 h. The patients were then discharged from the hospital, and blood samples were taken over the next 14 days while the subjects were outpatients. Potassium iodide supplements were started the evening before the VLDL injection and continued until 1 week after the last LDL sample was obtained.

Laboratory procedures

VLDL, IDL, and LDL were isolated from each of the 18 samples obtained after injection of ^{125}I-labeled VLDL by sequential ultracentrifugation in a 50.3 Ti fixed-angle rotor (25, 33). ApoB in each lipoprotein fraction from each time point was then isolated using 1,1′3,3′-tetramethylurea as described previously (34). ApoB specific activity in each sample was determined by measuring radioactivity and protein mass. LDL was isolated from the 16 samples obtained during the 2 week period after injection of ^{131}I-labeled LDL, and apoB specific activity was determined directly by γ counting and protein determination (25, 33). TG-glycerol, for analysis of VLDL-TG metabolism, was isolated using isopropyl alcohol and zeolite as described previously (26). Samples were stored at 4°C for 120 days to minimize contamination with ^{125}I. The tritium content of the samples was determined in a scintillation counter, correcting for any remaining ^{125}I.

Plasma cholesterol and TG levels were determined as the means of plasma concentration in nine time point specimens obtained during the turnover studies. Plasma HDL-C was determined after precipitation of apoB-containing lipoproteins with dextran sulfate magnesium chloride. VLDL-, IDL-, and LDL-lipid and -apoB concentrations were determined from samples obtained by ultracentrifugation during the turnover studies. ApoB mass was determined by specific radioimmunoassay (25, 33). Cholesterol and TG concentrations were determined using enzymatic methods on a Hitachi 704 automated spectrophotometer.

Compartmental analysis

The apoB tracer data were fitted as reported by Arad, Ramakrishnan, and Ginsberg (25). In each subject, the LDL-apoB specific activity data after the LDL-apoB tracer injection were fitted to a two-pool model. The VLDL-, IDL-, and LDL-apoB specific activity data from the VLDL-apoB tracer injection were then fitted, fixing the LDL kinetic parameters at the best estimates from the separate fit of the LDL-apoB tracer injection data. The model used is shown in **Fig. 1A**. The model was the simplest that could fit the data adequately; the deletion of any pool (e.g., a VLDL pool) or pathway (e.g., direct LDL synthesis) led to a sig-

TABLE 3. Effects of simvastatin on lipoprotein lipid levels

	VLDL-C		VLDL-TG		IDL-C		IDL-TG		LDL-C		LDL-TG	
Subject	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin
						mg/dl						
1	49	22	151	94	22.3	7.5	12.5	5.1	124	46	36	15
2	69	41	368	230	12.1	5.8	6.8	3.2	105	66	21	12
3	163	67	557	306	23.3	11.6	17.3	16.6	54	47	27	22
4	57	28	186	113	29.5	11.8	10.3	5.9	122	71	30	18
5	70	33	205	154	25.5	9.9	12.7	7.2	85	70	26	18
Mean ± SD	82 ± 46	38 ± 17	293 ± 170	179 ± 88	22.5 ± 6.5	9.3 ± 2.6	11.9 ± 3.8	7.6 ± 5.2	98 ± 29	60 ± 12	28 ± 6	17 ± 4
Change (%)	−53[a]		−39[b]		−59[b]		−36[a]		−39[a]		−39[a]	

IDL, intermediate density lipoprotein; VLDL-C, very low density lipoprotein-cholesterol; VLDL-TG values were log-transformed for analysis.
[a] $P < 0.05$.
[b] $P < 0.01$.

TABLE 4. Plasma and lipoprotein apoB levels

	Plasma		VLDL		IDL		LDL	
Subject	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin
				mg/dl				
1	160	57	24	17	13.5	7.0	94	37
2	115	77	22	20	7.5	4.0	87	53
3	135	84	48	24	19.0	6.5	59	42
4	151	82	23	15	13.0	6.0	92	40
5	160	100	28	20	10.3	4.9	97	58
Mean ± SD	144 ± 19	80 ± 15	29 ± 11	19 ± 4	12.7 ± 4	5.7 ± 1	86 ± 16	46 ± 9
Change (%)	−44[a]		−34[b]		−55[a]		−47[a]	

apoB, apolipoprotein B.
[a] $P < 0.01$.
[b] $P = 0.05$.

nificant worsening of the fits, whereas the addition of a pool (e.g., a fourth VLDL pool in the cascade) or pathway (direct IDL synthesis, conversion of the first two VLDL pools or the remnant VLDL to IDL or directly to LDL) resulted in no improvement to the fits. The TG tracer data were fitted by a single precursor pool and a two-pool VLDL configuration, shown in Fig. 1B (26). The fitting was with POOLFIT, a pool-modeling program developed and used in our previous work.

Statistical analysis

Results are reported as means and standard deviations. Significance was tested at $P = 0.05$ by paired *t*-tests.

RESULTS

As noted in Methods, baseline subject characteristics are shown in Table 1. **Table 2** shows the effects of simvastatin on plasma lipid levels. Individual subject values and group means are listed for placebo and treatment with simvastatin, 80 mg/day. On placebo, the patients had plasma TG levels ranging from the upper limits of normal to significant hypertriglyceridemia. All had low levels of HDL-C. There were significant reductions in both plasma total cholesterol (−42%) and TG (−37%) on 80 mg/day simvastatin therapy versus placebo. HDL-C levels increased by 21%.

Table 3 presents the individual and group means for cholesterol and TG in each lipoprotein fraction isolated by sequential ultracentrifugation. There were dramatic and uniform reductions in all lipoprotein lipids in every subject during treatment with simvastatin compared with placebo. VLDL-C decreased by 53%, VLDL-TG by 39%, IDL-C by 59%, IDL-TG by 36%, LDL-C by 39%, and LDL-TG by 39%. Simvastatin treatment had similar effects on total plasma and lipoprotein-apoB levels (**Table 4**), with reductions in every subject. Plasma levels of total apoB declined by 44%, VLDL-apoB by 34%, IDL-apoB by 55%, and LDL-apoB by 47%.

FCRs for VLDL-, IDL-, and LDL-apoB (**Table 5**) were estimated by fitting the model shown in Fig. 1 to the specific activity data for each lipoprotein fraction. For VLDL-apoB, four subjects had increased FCRs and one had decreased FCRs, and there was a trend toward an increase in the mean FCR during simvastatin treatment for the group (2.0 ± 0.80 to 2.7 ± 1.3 h^{-1}; NS). The FCR for IDL-apoB increased in four of five subjects during simvastatin therapy, with a significant overall increase (0.82 ± 0.30 to 1.40 ± 0.16 h; $P < 0.05$). However, LDL-apoB FCR decreased in two subjects and increased in three subjects on simvastatin, with no significant change overall (0.75 ± 0.41 vs. 0.81 ± 0.40 h^{-1}; NS).

Rates of secretion into plasma for VLDL-apoB, IDL-apoB, and LDL-apoB are shown in **Table 6**. Secretion rates were calculated by multiplying fractional catabolic rates by the plasma pool size of apoB in each lipoprotein. For VLDL-apoB, treatment was associated with lower secretion rates in three subjects and increased rates in two subjects. Overall, there was no effect of simvastatin therapy on VLDL-

TABLE 5. Fractional catabolic rates of VLDL-, IDL-, and LDL-apoB

	VLDL		IDL		LDL	
Subject	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin
				day^{-1}		
1	2.2	2.4	0.9	1.3	0.47	0.46
2	2.9	1.6	1.3	1.2	1.24	1.15
3	0.9	1.5	0.7	1.4	1.16	1.31
4	2.6	4.7	0.7	1.6	0.44	0.48
5	1.4	3.1	0.5	1.5	0.44	0.63
Mean ± SD	2.0 ± 0.8	2.7 ± 1.3	0.82 ± 0.3	1.40 ± 0.16[a]	0.75 ± 0.41	0.81 ± 0.40

[a] $P < 0.05$ versus placebo.

TABLE 6. Production rates of VLDL-, IDL-, and LDL-apoB

Subject	VLDL		IDL		LDL	
	Placebo	Simvastatin	Placebo	Simvastatin	Placebo	Simvastatin
	mg/kg/day					
1	23.6	18.3	5.3	4.0	19.7	7.6
2	28.4	14.3	4.5	2.2	48.4	27.3
3	19.0	16.3	6.1	4.2	30.8	24.8
4	27.1	31.5	4.2	4.3	18.4	8.6
5	17.9	28.3	2.3	3.3	19.4	16.4
Mean ± SD	23.2 ± 5	21.7 ± 8	4.5 ± 1.4	3.6 ± 0.9	27.3 ± 13	16.9 ± 9[a]

[a] $P < 0.05$ versus placebo.

apoB secretion rates (23.2 ± 5 vs. 21.7 ± 8 mg/kg/h; NS). IDL-apoB production was also unaffected by simvastatin therapy (4.5 ± 1.4 vs. 3.6 ± 0.9 mg/kg/h; NS). However, the effect of simvastatin therapy on LDL-apoB production was consistent: all subjects had a decline in production, with the mean rate on placebo of 27.3 ± 13 mg/kg/day decreasing to 16.9 ± 9 mg/kg/day on simvastatin ($P < 0.05$). The effects of simvastatin, 80 mg/day, on FCR and PR of VLDL-, IDL-, and LDL-apoB are summarized graphically in **Fig. 2**.

The decrease in LDL-apoB production without a change in VLDL-apoB secretion into plasma suggested that less VLDL was converted to IDL and LDL during simvastatin treatment compared with placebo. This was not the case, however, as conversion rates of VLDL-apoB to LDL-apoB (**Table 7**) actually increased in three of the five subjects, with no overall change in conversion for the group: conversion of VLDL-apoB to LDL-apoB was 31% with placebo and 43% during simvastatin therapy.

The PRs of LDL-apoB during both placebo and simvastatin administration, together with the calculated conversion rates, indicated that flux from VLDL to LDL was inadequate to account for LDL production in each study period. Indeed, our results demonstrated that a significant proportion of LDL-apoB flux was independent of VLDL secretion into plasma. Approximately 20 mg/kg/day, ~75% of the calculated apoB flux through LDL during the placebo period, appeared to enter the plasma directly as apoB secreted into the LDL density range. During simvastatin treatment, the rate of secretion of apoB appearing directly as LDL decreased in every subject, with the mean during therapy of ~9 mg/kg/day ($P < 0.01$ compared with placebo). However, this still constituted >50% of the flux of apoB through LDL (~65%). A summary of the effects of simvastatin on VLDL conversion to LDL, and the direct production of LDL, is presented graphically in **Fig. 3**.

Because of the significant secretion into plasma of apoB-lipoproteins that appear directly in the LDL density range, it is necessary to examine the effect of simvastatin on total apoB secretion (the sum of VLDL secretion and direct LDL secretion). Simvastatin treatment resulted in a decrease in total apoB secretion rates in four subjects and an increase in one. The mean total apoB secretion rate during placebo treatment was 43.5 ± 14 mg/kg/day, and this decreased to 30.7 ± 8 mg/kg/day during simvastatin therapy, but this change was not statistically significant (−12.8 ± 14; $P = 0.1$).

We also determined the effect of simvastatin on the rates of secretion of VLDL-TG; these results are shown in **Table 8** and **Figure 4**. VLDL-TG FCR decreased in four of five subjects, with a trend toward a lower FCR on simvastatin (0.44 ± 0.34 vs. 0.26 ± 0.10; NS). VLDL-TG PR decreased in all five subjects, with a mean reduction of ~67% on simvastatin (59.9 ± 62.8 vs. 19.6 ± 10.9 mg/kg/h; $P < 0.01$). The decrease in VLDL-TG PR was associated with a 39% reduction in VLDL-TG levels during simvastatin compared with placebo.

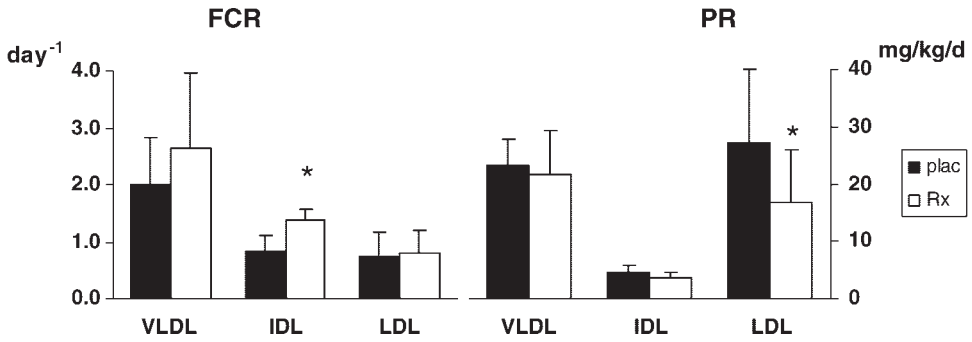

Fig. 2. ApoB fractional catabolic rates (FCRs) and production rates (PRs) in VLDL, IDL, and LDL during placebo (plac) and simvastatin (Rx) therapy. FCRs, in pools/day, were estimated by fitting the model shown in Fig. 1A to the apoB specific radioactivity data. PRs, in mg/kg/day, were calculated by multiplying the FCRs in VLDL, IDL, and LDL by the respective pool sizes of apoB. Values shown are means ± SD. * $P < 0.05$.

TABLE 7. Percentage conversion of VLDL- to LDL-apoB and direct production of LDL-apoB

Subject	Conversion		Direct Production	
	Placebo	Simvastatin	Placebo	Simvastatin
	%		*mg/kg/d*	
1	29	32	12.8	1.8
2	29	40	40.1	21.5
3	49	99	21.6	8.7
4	20	15	13.0	3.9
5	29	27	14.2	8.8
Mean ± SD	31.2 ± 11	42.6 ± 33	20.3 ± 11.6	8.9 ± 7.7[a]

[a] $P < 0.01$ versus placebo.

TABLE 8. Fractional catabolic rates and production rates of VLDL-TG

Subject	Fractional Clearance Rate		Production Rate	
	Placebo	Simvastatin	Placebo	Simvastatin
	h^{-1}		*mg/kg/h*	
1	0.37	0.19	25.1	7.8
2	1.02	0.35	169.6	36.1
3	0.21	0.16	51.5	21.5
4	0.19	0.24	15.9	12.2
5	0.41	0.38	37.4	20.5
Mean ± SD	0.44 ± 0.34	0.26 ± 0.10	59.9 ± 62.8	19.6 ± 10.9[a]

[a] $P < 0.01$, percentage change versus placebo.

DISCUSSION

In this randomized, placebo-controlled, crossover study, treatment with 80 mg/day simvastatin produced significant and clinically relevant reductions of plasma total cholesterol, TG, and TG and cholesterol in VLDL, IDL, and LDL in patients with T2DM and dyslipidemia. Plasma and lipoprotein-apoB levels decreased significantly as well. HDL-C levels increased significantly during simvastatin treatment. These effects of statins on plasma levels of lipids, lipoproteins, and apoB are consistent with several other studies in patients with dyslipidemia and T2DM (35–37).

Increased rates of secretion of VLDL-apoB and VLDL-TG have been demonstrated to be central features of the dyslipidemia present in individuals with insulin resistance and/or T2DM (10–12, 38, 39). Our subjects had a mean VLDL-apoB secretion rate of ~23 mg/kg/day, a mean LDL PR of ~27 mg/kg/day, and a total apoB secretion rate of ~44 mg/kg/day. Although we did not study normal subjects in this investigation, we have previously reported secretion rates of 15 and 13 mg/kg/day for VLDL- and LDL-apoB, respectively, in normal individuals (40), consistent with reports from other laboratories (41). Thus, the present subjects with T2DM had, as expected, significantly increased rates of production of VLDL- and LDL-apoB. We also determined that these patients had significant secretion of LDL density particles directly into plasma. This is consistent with findings from our laboratory in previous studies of obese hypertriglyceridemic subjects (33), in a patient with cholesteryl ester storage disease (42), and in patients with combined hyperlipidemia (25). The rates of VLDL-TG secretion in our subjects were also clearly increased compared with those in previous studies in normal subjects conducted by us (40) and others (43). Increased VLDL-TG secretion has been a consistent finding in patients with T2DM (9, 44).

The basis for the increased rates of apoB and TG secretion into plasma in subjects with T2DM appears to be a combination of factors related to insulin resistance and FA metabolism. First, several studies have shown that patients with T2DM have insensitivity to the insulin-mediated suppression of lipolysis (45, 46) and have, therefore, increased rates of FA flux in plasma and increased uptake of FA by the liver. Numerous studies with cultured liver cells indicate that FA availability in hepatocytes targets newly synthesized apoB for assembly with lipids and secretion, rather than degradation (47, 48). Lewis and coworkers (49) have demonstrated in humans that an acute increase in plasma FA (resulting from intravenous infusions of intralipid and heparin) stimulates VLDL-apoB secretion. Second, FA synthesis in the liver, by de novo lipogenesis, can also be an important source of substrate for VLDL-TG in patients with insulin resistance and T2DM (50–52). Recent studies from several laboratories using rodent models have suggested that hyperinsulinemia, a consequence of insulin resistance, can stimulate hepatic lipogenesis via increased expression of the gene for sterol response element binding protein 1c and subsequent upregulation of the expression of several lipogenic genes (53, 54), even in an insulin-resistant liver. Finally, insulin can acutely inhibit apoB secretion from cultured liver cells (55, 56), and this action is lost in hepatocytes from insulin-resistant animals (57, 58). In vivo studies of the effects of acute hyperinsulinemia in normal subjects suggest the inhibition of VLDL-apoB se-

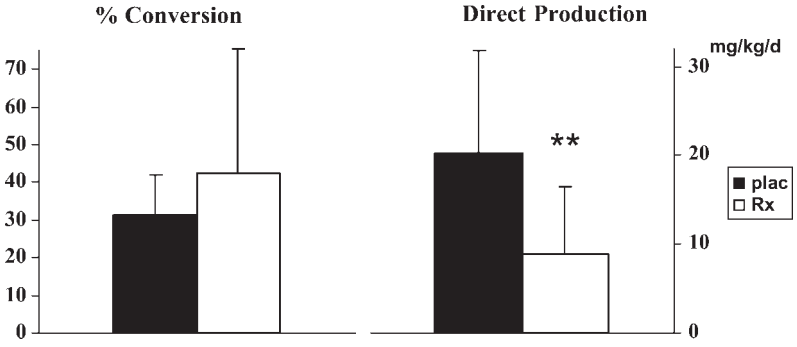

Fig. 3. Percentage conversion VLDL to LDL-apoB and direct production of LDL-apoB. The proportion of VLDL-apoB (indicating the number of particles) that was converted to LDL-apoB and the rate of secretion of apoB-lipoproteins into plasma as LDL were estimated from the specific activity in apoB and the model shown in Fig. 1A. plac, placebo; Rx, simvastatin therapy. Values shown are means ± SD. ** $P < 0.01$.

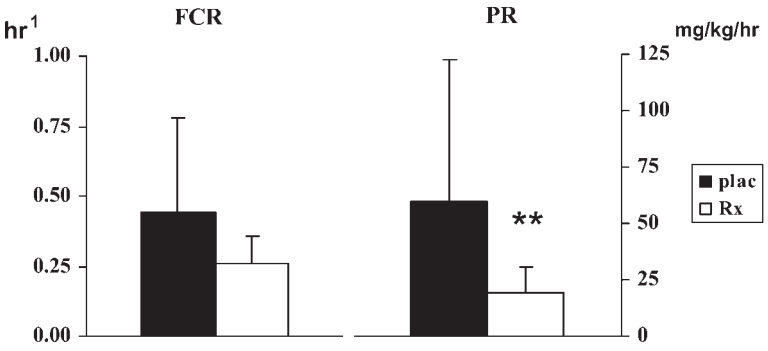

Fig. 4. VLDL-TG FCRs and PRs. The FCRs for VLDL-TG, in pools/h, were estimated from TG-glycerol specific activities using the model shown in Fig. 1B. The PRs of VLDL-TG, in mg/kg/h, were calculated by multiplying the FCRs by the VLDL-TG pool size. plac, placebo; Rx, simvastatin therapy. Values shown are means ± SD. ** $P < 0.01$.

cretion (59, 60), but this effect seems to be defective in obese individuals (59) and in subjects with T2DM (61).

The uniform reductions in VLDL-, IDL-, and LDL-lipid and -apoB concentrations we observed during simvastatin treatment were associated with complex changes in the metabolism of those lipoproteins and VLDL-TG. Reductions in VLDL-TG and VLDL-apoB levels on simvastatin were accompanied by reductions in VLDL-TG secretion and no change in VLDL-apoB secretion, respectively. There were trends toward a decreased FCR for VLDL-TG and an increased FCR for VLDL-apoB on simvastatin therapy. Together, these data indicate that similar numbers of smaller VLDL particles, with less TG per particle, were secreted during simvastatin treatment compared with placebo treatment. Furthermore, although the fraction of TG removed per hour tended to be smaller during simvastatin treatment, smaller nascent VLDL particles tended to be more efficiently catabolized either to IDL and LDL or removed directly from plasma. Indeed, the nonsignificant overall increase in VLDL-apoB FCR was quantitatively similar to the decrease in VLDL-apoB concentration.

The reductions in LDL-C and LDL-apoB were associated with a decrease in the production of LDL-apoB but, unexpectedly, no change in LDL-apoB FCR, respectively. The decrease in LDL production was not the result of a decrease in the production of its typical precursor, VLDL, nor could lower LDL production be explained by changes in the proportion of VLDL that was converted to LDL. We did find, however, that the majority of LDL was produced independently of VLDL secretion: LDL was being secreted directly into plasma. Importantly, simvastatin treatment reduced direct LDL production by >50%.

How do our data compare with other studies of statin effects on apoB and TG metabolism in individuals with dyslipidemia (or combined hyperlipidemia)? At approximately the same time that the earliest studies reported that statins increased the FCRs of LDL-apoB in patients with familial hypercholesterolemia (23, 62, 63), we demonstrated that lovastatin reduced the secretion of apoB-lipoproteins in a patient with cholesteryl ester storage disease and combined hyperlipidemia (42); LDL fractional clearance increased as well in that patient. We followed that report with a study of the effects of lovastatin in seven patients with dyslipidemia. In that study, secretion of apoB-lipoproteins into plasma was reduced significantly; VLDL-apoB secretion was not affected by lovastatin, but there was a significant reduction (44%) in the entry of LDL into plasma independent of VLDL secretion (25). LDL fractional clearance was not affected by lovastatin treatment in those patients with dyslipidemia. By contrast, Vega and Grundy (64) reported that reductions in LDL-C levels with lovastatin in patients with mixed hyperlipidemia were associated with increases in LDL fractional clearance and no change in LDL production; VLDL metabolism was not studied. Cortner et al. (65), however, observed reduced VLDL production during lovastatin therapy in patients with carefully defined familial combined hyperlipidemia, a disorder that is closely linked, in many families, with insulin resistance.

The studies described above used exogenously labeled VLDL and/or LDL. More recently, several groups have used stable isotopes of amino acids as precursors for studies of apoB-lipoprotein metabolism (66). When study subjects had combined hyperlipidemia and low FCRs for LDL at baseline, atorvastatin, simvastatin (67), and pravastatin (68) improved plasma lipid levels by increasing VLDL and LDL fractional clearance. In two studies in which baseline fractional clearance of LDL was similar to that in controls, patients with combined hyperlipidemia responded to atorvastatin (69) or lovastatin (70) with decreased production of apoB-lipoproteins with or without changes in the fractional clearance of apoB-lipoproteins from the circulation. In a third study of subjects with normal baseline LDL FCRs, pravastatin (71) increased the fractional clearance of apoB-lipoproteins. In recent studies by Watts and colleagues (72–74), improved fractional clearance was associated with reductions in the levels of apoB-lipoproteins when atorvastatin was used to treat subjects with obesity and insulin resistance. On the other hand, Ouguerram et al. (27), in the only study of patients with T2DM, reported that atorvastatin therapy was associated with both decreased VLDL-apoB secretion into plasma and normalization of a baseline low FCR of LDL in patients with T2DM. Overall, therefore, previous studies in patients with dyslipidemia, with or without T2DM, have produced variable results. An overview of these studies, however, suggests that when baseline FCRs of LDL were low, statins reduced plasma lipid levels by improving fractional clearance; when baseline PRs were high, irrespective of baseline FCRs, statins reduced the secretion of apoB-lipoproteins into plasma. LDL-apoB FCRs were normal or increased in our subjects during placebo treatment. The reason that statin therapy does not increase LDL-apoB FCR uniformly, as one might

expect, is unclear. Our group did provide evidence that statin treatment can alter the apparent in vivo affinity of LDL for its receptor (75), but we could not determine the molecular or biochemical basis for that observation.

A possible explanation for the complex results we observed is that during placebo treatment, because of hepatic insulin resistance, the targeting of apoB for secretion is no longer tightly linked to TG secretion, so that even though there is increased hepatic TG availability and increased VLDL secretion, there is also the assembly and secretion of TG-poor, LDL-density lipoproteins. During simvastatin treatment, a reduction in the availability of hepatic TG for lipoprotein assembly leads to both the assembly and secretion of smaller VLDLs and increased apoB degradation (despite persistent insulin resistance). The latter change results in reduced secretion of LDL. Relevant to this proposed scheme are reports that statins may stimulate hepatic expression of the gene for the peroxisomal proliferator-activated receptor α (PPARα) as well as its target genes, acyl-CoA oxidase and carnitine palmitoyltransferase (76). Martin et al. (77) suggested that statins activate PPARα by inhibiting Rho signaling. Whatever the mechanism, such activation could increase fatty acid oxidation in the liver, resulting in less TG synthesis. On the other hand, fibrates, which are PPARα agonists, are thought to act, at least in part, by reducing apoC-III production (78) and thereby improving the lipolysis of VLDL-TG (69, 74). In our study, however, we did not see improvement in the FCR of either VLDL-TG or VLDL-apoB. Further studies will be required to fully understand the effects of statins on VLDL-TG metabolism.

In this study, treatment of patients with T2DM and dyslipidemia with 80 mg of simvastatin per day resulted in marked decreases of plasma cholesterol, TG, and apoB. These changes were associated with significant reductions in LDL-apoB PRs without effects on fractional catabolic rates. The decrease in LDL-apoB production was almost completely accounted for by reductions in the production of LDL independent of VLDL entry into plasma. Total production of apoB-lipoproteins decreased in four of the five subjects during simvastatin treatment. VLDL-TG secretion rates were also reduced during simvastatin treatment. Treatment with high-dose simvastatin corrected the dyslipidemia in these patients with T2DM by reducing the assembly and secretion of apoB-containing lipoproteins.

The authors thank Minnie Myers, Wahida Karmally, and the staff of the Bionutrition Unit of the GCRC, and Media Berghout and the nursing GCRC staff, for their clinical, statistical, and technical support. This work was supported by National Heart, Lung, and Blood Institute Grants T32 HL-07343 and R01 HL-55638 and by an investigator-initiated grant from Merck, Inc.

REFERENCES

1. NCEP Expect Panel. 1988. Report of the National Cholesterol Education Program expert panel on detection, evaluation and treatment of high blood cholesterol in adults. *Arch. Intern. Med.* **148:** 36–69.
2. Adult Treatment Panel II. 1993. Summary of the second report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel II). *J. Am. Med. Assoc.* **269:** 3015–3023.
3. American Diabetes Association. 1989. Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. *Diabetes Care.* **12:** 573–579.
4. Wingard, D. L., and E. Barrett-Connor. 1995. Heart Disease in Diabetes. *In* Diabetes in America. (NIH) Publication No. 95-1468, National Institute of Diabetes and Digestive and Kidney Diseases. 429–448.
5. Ginsberg, H. N. 1991. Lipoprotein physiology in nondiabetic and diabetes states. *Diabetes Care.* **14:** 839–855.
6. Howard, B. V. 1987. Lipoprotein metabolism in diabetes mellitus. *J. Lipid Res.* **28:** 613–628.
7. Ginsberg, H. N. 2000. Insulin resistance and cardiovascular disease. *J. Clin. Invest.* **106:** 453–458.
8. Taskinen, M. R. 2002. Diabetic dyslipidemia. *Atheroscler. Suppl.* **3:** 47–51.
9. Reaven, G. M., and Y-D. Chen. 1988. Role of insulin in regulation of lipoprotein metabolism in diabetes. *Diabetes Metab. Rev.* **4:** 639–652.
10. Kissebah, A. H., S. Alfarsi, D. J. Evans, and P. W. Adams. 1982. Integrated regulation of very-low-density lipoprotein triglyceride and apolipoprotein-B kinetics in non-insulin-dependent diabetes mellitus. *Diabetes.* **31:** 217–225.
11. Ginsberg, H., and S. M. Grundy. 1982. Effect of caloric restriction on very low density lipoprotein triglyceride metabolism in subjects with diabetes mellitus. *Diabetologia.* **23:** 421–425.
12. Adiels, M., J. Boren, M. J. Caslake, P. Stewart, A. Soro, J. Westerbacka, B. Wennberg, S-O. Olofsson, C. Packard, and M. R. Taskinen. 2005. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1697–1703.
13. Shepherd, J., S. M. Cobbe, I. Ford, C. G. Isles, A. R. Lorimer, P. W. MacFarlane, J. H. McKillop, and C. J. Packard. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N. Engl. J. Med.* **333:** 1301–1307.
14. Pedersen, T. R., and the Simvastatin Survival Study Group. 1994. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet.* **344:** 1383–1389.
15. Sacks, F. M., M. A. Pfeffer, L. A. Moye, J. L. Rouleau, J. D. Rutherford, T. G. Cole, L. Brown, J. W. Warnica, J. M. Arnold, C. C. Wun, et al. 1996. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and recurrent events trial investigators. *N. Engl. J. Med.* **335:** 1001–1009.
16. Pyorala, K., T. R. Pedersen, J. Kjekshus, O. Faergeman, A. G. Olsson, and G. Thorgeirsson. 1997. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). *Diabetes Care.* **20:** 614–620.
17. Goldberg, R. B., M. J. Mellies, F. M. Sacks, L. A. Moye, B. V. Howard, W. J. Howard, B. R. Davis, T. G. Cole, M. A. Pfeffer, and E. Braunwald. 1998. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the Cholesterol And Recurrent Events (CARE) trial. The Care Investigators. *Circulation.* **23:** 2513–2519.
18. Heart Protection Study Collaborative Group. 2003. MRC/BHF heart protection study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet.* **361:** 2005–2016.
19. Colhoun, H. M., D. J. Betteridge, P. N. Durrington, G. A. Hitman, H. A. Neil, S. J. Livingstone, M. J. Thomason, M. I. Mackness, V. Charlton-Menys, J. H. Fuller, et al. 2004. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet.* **364:** 685–696.
20. Stein, E. A., M. Lane, and P. Laskarzewski. 1998. Comparison of statins in hypertriglyceridemia. *Am. J. Cardiol.* **81:** 66B–69B.
21. Kovanen, P. T., M. S. Brown, and J. L. Goldstein. 1979. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. *J. Biol. Chem.* **254:** 11367–11373.

22. Kovanen, P. T., D. W. Bilheimer, J. L. Goldstein, J. J. Jaramillo, and M. S. Brown. 1981. Regulatory role for hepatic low density lipoprotein receptors in vivo in the dog. *Proc. Natl. Acad. Sci. USA.* **78:** 1194–1198.
23. Bilheimer, D. W., S. M. Grundy, M. S. Brown, and J. L. Goldstein. 1983. Mevinolin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Proc. Natl. Acad. Sci. USA.* **80:** 4124–4128.
24. Ginsberg, H. N. 1998. Effects of statins on triglyceride metabolism. *Am. J. Cardiol.* **81:** 32B–35B.
25. Arad, Y., R. Ramakrishnan, and H. N. Ginsberg. 1990. Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J. Lipid Res.* **31:** 567–582.
26. Arad, Y., R. Ramakrishnan, and H. Ginsberg. 1992. Effects of lovastatin therapy on very-low-density lipoprotein triglyceride metabolism in subjects with combined hyperlipidemia: evidence for reduced assembly and secretion of triglyceride-rich lipoproteins. *Metabolism.* **41:** 487–493.
27. Ouguerram, K., T. Magot, Y. Zair, J. S. Marchini, B. Charbonnel, H. Laouenan, and M. Krempf. 2003. Effect of atorvastatin on apolipoprotein B100 containing lipoprotein metabolism in type-2 diabetes. *J. Pharmacol. Exp. Ther.* **306:** 332–337.
28. Ginsberg, H., N-A. Le, J. Melish, D. Steinberg, and W. V. Brown. 1981. Effect of a high carbohydrate diet on apoprotein-B catabolism in man. *Metabolism.* **30:** 347–353.
29. Bilheimer, D. W., J. Eisenberg, and R. L. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vivo and in vitro observations. *Biochim. Biophys. Acta.* **26:** 212–221.
30. McFarlane, A. S. 1958. Efficient trace labeling of proteins with iodine. *Nature.* **182:** 153.
31. Ginsberg, H. N., A. Jacobs, N-A. Le, and J. Sandler. 1982. Effect of somatostatin-induced suppression of postprandial insulin response upon the hypertriglyceridemia associated with a high carbohydrate diet. *J. Clin. Invest.* **70:** 1225–1233.
32. Grundy, S. M., H. Y. Mok, L. Zech, D. Steinberg, and M. Berman. 1979. Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. *J. Clin. Invest.* **63:** 1274–1283.
33. Ginsberg, H. N., N-A. Le, and J. C. Gibson. 1985. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. Effect of weight loss. *J. Clin. Invest.* **75:** 614–623.
34. Le, N-A., J. Melish, B. Roach, H. Ginsberg, and W. V. Brown. 1978. Direct measurement of apoprotein-B specific activity in ^{125}I-labeled lipoproteins. *J. Lipid Res.* **19:** 578–584.
35. Sweany, E. A., D. R. Shapiro, A. C. Tate, R. B. Goldberg, and E. A. Stein. 1995. Effects of simvastatin versus gemfibrozil on lipids and glucose control in patients with non-insulin-dependent diabetes mellitus. NIDDM Study Group. *Clin. Ther.* **17:** 186–203.
36. Cassader, M., G. Ruiu, R. Gambino, N. Alemanno, F. Veglia, and G. Pagano. 1993. Hypercholesterolemia in non-insulin-dependent diabetes mellitus: different effect of simvastatin on VLDL and LDL cholesterol levels. *Atherosclerosis.* **99:** 47–53.
37. Farrer, M., P. H. Winocour, K. Evans, H. A. Neil, M. F. Laker, P. Kesteven, and K. G. Alberti. 1994. Simvastatin in non-insulin-dependent diabetes mellitus: effect on serum lipids, lipoproteins and haemostatic measures. *Diabetes Res. Clin. Pract.* **23:** 111–119.
38. Cummings, M. H., G. F. Watts, A. M. Umpleby, T. R. Hennessy, R. Naoumova, B. M. Slavin, G. R. Thompson, and P. H. Sonksen. 1995. Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. *Diabetologia.* **38:** 959–967.
39. Duvillard, L., F. Pont, E. Florentin, C. Galland-Jos, P. Gambert, and B. Verges. 2000. Metabolic abnormalities of apolipoprotein B-containing lipoproteins in non-insulin-dependent diabetes: a stable isotope kinetic study. *Eur. J. Clin. Invest.* **8:** 685–694.
40. Ginsberg, H. N., N-A. Le, I. J. Goldberg, P. Wang-Iverson, J. C. Gibson, A. Rubinstein, R. A. Norum, and W. V. Brown. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoprotein C-III and A-I: evidence that apolipoprotein C-III inhibits lipoprotein lipase in vivo. *J. Clin. Invest.* **78:** 1287–1295.
41. Kesaniemi, Y. A., W. F. Beltz, and S. M. Grundy. 1985. Comparisons of metabolism of apolipoprotein B in normal subjects, obese patients, and patients with coronary heart disease. *J. Clin. Invest.* **76:** 586–595.
42. Ginsberg, H. N., N-A. Le, M. P. Short, R. Ramakrishnan, and R. J. Desnick. 1987. Suppression of apolipoprotein B production during treatment of cholesteryl ester storage disease with lovastatin: implications for regulation of apolipoprotein B synthesis. *J. Clin. Invest.* **80:** 1692–1697.
43. Zech, L. A., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides. Evidence for a slow production pathway and results for normolipidemic subjects. *J. Clin. Invest.* **63:** 1262–1273.
44. Ginsberg, H. N. 1987. Very low density lipoprotein metabolism in diabetes mellitus. *Diabetes Metab. Rev.* **3:** 571–589.
45. Laws, A., H. M. Hoen, J. V. Selby, M. F. Saad, S. M. Haffner, and B. V. Howard. 1997. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status. Relation to plasma triglyceride and apolipoprotein B concentrations. Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Arterioscler. Thromb. Vasc. Biol.* **17:** 64–71.
46. Arner, P. 2002. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab. Res. Rev.* **18 (Suppl. 2):** 5–9.
47. Fisher, E. A., and H. N. Ginsberg. 2002. Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. *J. Biol. Chem.* **277:** 17377–17380.
48. Zhang, Y-L., A. Hernandez-Ono, C. Ko, K. Yasunaga, L-S. Huang, and H. N. Ginsberg. 2004. Regulation of hepatic apolipoprotein B-lipoprotein assembly and secretion by the availability of fatty acids. I. Differential effects of delivering fatty acids via albumin or remnant-like emulsion particles. *J. Biol. Chem.* **279:** 19362–19374.
49. Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95:** 158–166.
50. Aarsland, A., D. Chinkes, and R. R. Wolfe. 1996. Contributions of de novo synthesis of fatty acids to total VLDL-triglyceride secretion during prolonged hyperglycemia/hyperinsulinemia in normal man. *J. Clin. Invest.* **98:** 2008–2017.
51. Hellerstein, M. K., M. Christiansen, S. Kaempfer, C. Kletke, K. Wu, J. S. Reid, K. Mulligan, N. S. Hellerstein, and C. H. L. Shackleton. 1991. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J. Clin Invest.* **87:** 1841–1852.
52. Diraison, F., and M. Beylot. 1998. Role of human liver lipogenesis and reesterification in triglycerides secretion and in FFA reesterification. *Am. J. Physiol.* **274:** E321–E327.
53. Shimomura, I., Y. Bashmakov, S. Ikemoto, J. D. Horton, M. S. Brown, and J. L. Goldstein. 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA.* **96:** 13656–13661.
54. Elam, M. B., H. G. Wilcox, L. M. Cagen, X. Deng, R. Raghow, P. Kumar, M. Heimberg, and J. C. Russell. 2001. Increased hepatic VLDL secretion, lipogenesis, and SREBP-1 expression in the corpulent JCR:LA-cp rat. *J. Lipid Res.* **42:** 2039–2048.
55. Sparks, J. D., and C. E. Sparks. 1994. Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochim. Biophys. Acta.* **1215:** 9–32.
56. Sparks, J. D., T. L. Phung, M. Bolognino, and C. E. Sparks. 1996. Insulin-mediated inhibition of apolipoprotein B secretion requires an intracellular trafficking event and phosphatidylinositol 3-kinase activation: studies with brefeldin A and wortmannin in primary cultures of rat hepatocytes. *Biochemistry.* **313:** 567–574.
57. Taghibiglou, C., A. Carpentier, S. C. Van Iderstine, B. Chen, D. Rudy, A. Aiton, G. F. Lewis, and K. Adeli. 2000. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular apoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *J. Biol. Chem.* **275:** 8416–8425.
58. Bourgeois, C. S., D. Wiggins, R. Hems, and G. F. Gibbons. 1995. VLDL output by hepatocytes from obese Zucker rats is resistant to the inhibitory effect of insulin. *Am. J. Physiol.* **269:** E208–E215.
59. Lewis, G. F., K. D. Uffelman, L. W. Szeto, and G. Steiner. 1993. Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes.* **42:** 833–842.
60. Malmstrom, R., C. J. Packard, M. Caslake, D. Bedford, P. Stewart, H. Yki-Jarvinen, J. Shepherd, and M. R. Taskinen. 1998. Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes.* **47:** 779–787.
61. Malmstrom, R., C. J. Packard, M. Caslake, D. Bedford, P. Stewart, H. Yki-Jarvinen, J. Shepherd, and M. R. Taskinen. 1997. Defective

regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia.* **40:** 454–462.
62. Malmendier, C. L., J. F. Lontie, C. Delcroix, and T. Magot. 1989. Effect of simvastatin on receptor-dependent low density lipoprotein catabolism on normocholesterolemic human volunteers. *Atherosclerosis.* **80:** 101–109.
63. Vega, G. L., C. East, and S. M. Grundy. 1989. Effects of combined therapy with lovastatin and colestipol in heterozygous familial hypercholesterolemia. Effects of kinetics of apolipoprotein B. *Arteriosclerosis.* **9:** I135–I144.
64. Vega, G. L., and S. M. Grundy. 1991. Influence of lovastatin therapy on metabolism of low density lipoprotein in mixed hyperlipidaemia. *J. Intern. Med.* **230:** 341–350.
65. Cortner, J. A., M. J. Bennett, N. A. Le, and P. M. Coates. 1993. The effect of lovastatin on very low-density lipoprotein apolipoprotein B production by the liver in familial combined hyperlipidaemia. *J. Inherit. Metab. Dis.* **16:** 127–134.
66. Marsh, J. B., F. K. Welty, A. H. Lichtenstein, S. Lamon-Fava, and E. J. Schaefer. 2002. Apolipoprotein B metabolism in humans: studies with stable isotope-labeled amino acid precursors. *Atherosclerosis.* **162:** 227–244.
67. Forster, L. F., G. Stewart, D. Bedford, J. P. Stewart, E. Rogers, J. Shepherd, C. J. Packard, and M. J. Caslake. 2002. Influence of atorvastatin and simvastatin on apolipoprotein B metabolism in moderate combined hyperlipidemic subjects with low VLDL and LDL fractional clearance rates. *Atherosclerosis.* **164:** 129–145.
68. Aguilar-Salinas, C. A., P. Hugh, R. Barrett, J. Pulai, X. L. Zhu, and G. Schonfeld. 1997. A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism. Kinetics of apolipoprotein B during placebo and pravastatin therapy. *Arterioscler. Thromb. Vasc. Biol.* **17:** 72–82.
69. Bilz, S., S. Wagner, M. Schmitz, A. Bedynek, U. Keller, and T. Demant. 2003. Effects of atorvastatin versus fenofibrate on apolipoprotein B-100 and apolipoprotein A-I kinetics in mixed hyperlipidemia. *J. Lipid Res.* **45:** 174–185.
70. Cuchel, M., E. J. Schaefer, J. S. Millar, P. J. H. Jones, G. G. Dolnikowski, C. Vergani, and A. H. Lichtenstein. 1997. Lovastatin decreases de novo cholesterol synthesis and LDL apo B-100 production rates in combined-hyperlipidemic males. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1910–1917.
71. Parhofer, K. G., P. H. Barrett, J. Dunn, and G. Schonfeld. 1993. Effect of pravastatin on metabolic parameters of apolipoprotein B in patients with mixed hyperlipoproteinemia. *Clin Investig.* **71:** 939–946.
72. Watts, G. F., D. C. Chan, P. H. Barrett, F. H. O'Neill, and G. R. Thompson. 2003. Effect of a statin on hepatic apolipoprotein B-100 secretion and plasma campesterol levels in the metabolic syndrome. *Int. J. Obes. Relat. Metab. Disord.* **27:** 862–865.
73. Chan, D. C., G. F. Watts, P. H. Barrett, L. J. Beilin, T. G. Redgrave, and T. A. Mori. 2002. Regulatory effects of HMG CoA reductase inhibitor and fish oils on apolipoprotein B-100 kinetics in insulin-resistant obese male subjects with dyslipidemia. *Diabetes.* **51:** 2377–2386.
74. Watts, G. F., P. H. R. Barrett, J. Ji, A. P. Serone, D. C. Chan, K. D. Croft, F. Loehrer, and A. G. Johnson. 2003. Differential regulation of lipoprotein kinetics by atorvastatin and fenofibrate in subjects with the metabolic syndrome. *Diabetes.* **52:** 803–811.
75. Berglund, L., J. L. Witztum, N. F. Galeano, A. S. Khouw, H. N. Ginsberg, and R. Ramakrishnan. 1998. Three-fold effect of lovastatin treatment on low density lipoprotein metabolism in subjects with hyperlipidemia: increase in receptor activity, decrease in apoB production, and decrease in particle affinity for the receptor. Results from a novel triple-tracer approach. *J. Lipid Res.* **39:** 913–924.
76. Roglans, N., E. Sanguino, C. Peris, M. Alegret, M. Vazquez, T. Adzet, C. Diaz, G. Hernandez, J. C. Laguna, and R. M. Sanchez. 2002. Atorvastatin treatment induced peroxisome proliferator-activated receptor alpha expression and decreased plasma nonesterified fatty acids and liver triglyceride in fructose-fed rats. *J. Pharmacol. Exp. Ther.* **302:** 232–239.
77. Martin, G., H. Duez, C. Blanquart, V. Berezowski, P. Poulain, J-C. Fruchart, J. Najib-Fruchart, C. Glineur, and B. Staels. 2001. Statin-induced inhibition of the Rho-signaling pathway activates PPARα and induces HDL apoA-I. *J. Clin. Invest.* **107:** 1423–1432.
78. Haubenwallner, S., A. D. Essenburg, B. C. Barnett, M. E. Pape, R. B. DeMattos, B. R. Krause, L. L. Minton, B. J. Aurbach, R. S. Newton, T. Leff, et al. 1995. Hypolipidemic activity of select fibrates correlates to changes in hepatic apolipoprotein C-III expression: a potential basis for their mode of action. *J. Lipid Res.* **36:** 2541–2551.